



Comparative landscape genetics reveals differential effects of environment on host and pathogen genetic structure in Tasmanian devils (*Sarcophilus harrisii*) and their transmissible tumour

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Abstract

Genetic structure in host species is often used to predict disease spread. However, host and pathogen genetic variation may be incongruent. Understanding landscape factors that have either concordant or divergent influence on host and pathogen genetic structure is crucial for wildlife disease management. Devil facial tumour disease (DFTD) was first observed in 1996 and has spread throughout almost the entire Tasmanian devil geographic range, causing dramatic population declines. Whereas DFTD is predominantly spread via biting among adults, devils typically disperse as juveniles, which experience low DFTD prevalence. Thus, we predicted little association between devil and tumour population structure and that environmental factors influencing gene flow differ between devils and tumours. We employed a comparative landscape genetics framework to test the influence of environmental factors on patterns of isolation by resistance (IBR) and isolation by environment (IBE) in devils and DFTD. Although we found evidence for broad-scale costructuring between devils and tumours, we found no relationship between host and tumour individual genetic distances. Further, the factors driving the spatial distribution of genetic variation differed for each. Devils exhibited a strong IBR pattern driven by major roads, with no evidence of IBE. By contrast, tumours showed little evidence for IBR and a weak IBE pattern with respect to elevation in one of two tumour clusters we identify herein. Our results warrant caution when inferring pathogen spread using host population genetic structure and suggest that reliance on environmental barriers to host connectivity may be ineffective for managing the spread of wildlife diseases. Our findings demonstrate the utility of comparative landscape genetics for identifying differential factors driving host dispersal and pathogen transmission.

KEYWORDS

conservation genetics, disease biology, ecological genetics, host–parasite interactions, landscape genetics, population genetics—empirical

1 | INTRODUCTION

Infectious diseases are a major driver of wildlife population dynamics and can contribute to extinction (De Castro & Bolker, 2005). Thus, considerable effort is devoted to detecting diseases and identifying the processes influencing their transmission and spread. Traditional epidemiological approaches rely on direct estimates of disease prevalence, host movement and host contact rates for model parameterization, but these estimates are logistically challenging to obtain from wildlife populations (Craft, 2015; Craft, Volz, Packer, & Meyers, 2009; Hamede, Bashford, McCallum, & Jones, 2009). Further, there is often uncertainty as to whether observed contacts and movements reflect actual pathogen transmission and spread (Craft, 2015). In contrast, the spatial distribution of genetic variation contains signatures of past dispersal (in the case of the host) or spread (in the case of the pathogen) and often can be linked with environmental or ecological factors at fine spatial scales (Archie, Luikart, & Ezenwa, 2009; Biek & Real, 2010; Blanchong, Robinson, Samuel, & Foster, 2016; Hemming-Schroeder, Lo, Salazar, Puente, & Yan, 2018; Kozakiewicz et al., 2018). Knowledge of these relationships is critical to predicting the spread of wildlife diseases and can inform management strategies aimed at mitigating their impact.

Spatial patterns of genetic variation are routinely used to understand patterns of connectivity and movement in wildlife, and a number of studies have extended this framework for predicting the spread of wildlife diseases resulting from host movement. For example, estimates of host genetic variation have been used to explain and forecast the prevalence and distribution of pathogens (e.g. Blanchong et al., 2008; Guivier et al., 2011; Robinson, Samuel, Rolley, & Shelton, 2013), and the transmission potential of different host species (e.g. Paquette, Talbot, Garant, Mainguy, & Pelletier, 2014; Vander Wal et al., 2013) or of different sexes within species (e.g. Cote, Garant, Robert, Mainguy, & Pelletier, 2012; Talbot, Garant, Paquette, Mainguy, & Pelletier, 2012), and to improve the predictive power of models of disease spread (e.g. Davy, Martinez-Nunez, Willis, & Good, 2015; Kozakiewicz et al., 2018; Robinson et al., 2013; Wilder, Kunz, & Sorenson, 2015). However, pathogen genetic structure does not necessarily reflect that of the host, and pathogen transmission may be disconnected from patterns of host gene flow. Such a disconnect may be due to factors including multiple host/vector species, pathogen persistence in environmental reservoirs, transmission via nonreproducing hosts or host life history characteristics resulting in reduced susceptibility at dispersal age (Mazé-Guilmo, Blanchet, McCoy, & Loot, 2016; Talbot, Vonhof, Broders, Fenton, & Keyghobadi, 2017). Therefore, comparative approaches, ideally incorporating the relative influence of environmental or ecological factors, are necessary for understanding the extent to which host and pathogen genetic structure are related and to disentangle

the factors influencing each. One approach that is ideally suited for the comparative study of host and pathogen genetic variation is landscape genetics.

Landscape genetics is an analytical framework for testing the influence of environmental heterogeneity on patterns of gene flow and population genetic structure (Manel & Holderegger, 2013; Manel, Schwartz, Luikart, & Taberlet, 2003; Storfer et al., 2007). Most landscape genetics studies have focused on single species, but there are a growing number of comparative, multispecies studies (e.g. Cleary, Waits, & Finegan, 2017; Goldberg & Waits, 2010; Petren, Grant, Grant, & Keller, 2005; Trumbo, Spear, Baumsteiger, & Storfer, 2013; Zancolli, Rödel, Steffan-Dewenter, & Storfer, 2014). Even fewer multispecies studies have employed landscape genetics methods to study the dynamics of infectious diseases in wildlife systems (Biek & Real, 2010; Hemming-Schroeder et al., 2018; Kozakiewicz et al., 2018). Such comparative landscape genetics frameworks can provide valuable insights into how host–pathogen interactions shape patterns of disease transmission and spread across heterogeneous landscapes (Leo, Gonzalez, Millien, & Cristescu, 2016; Schwabl et al., 2017; Talbot et al., 2017).

Tasmanian devils (*Sarcophilus harrisii*) and their transmissible cancer provide a highly appropriate study system to test for host–pathogen costructuring in a comparative landscape genetics framework. In 1996, devil facial tumour disease (DFTD) was discovered in north-eastern Tasmania, Australia. DFTD is one of only a few documented transmissible cancers (Metzger & Goff, 2016; Ostrander, Davis, & Ostrander, 2016; Storfer et al., 2017) and, since its emergence, has spread across almost the entire geographic range of the Tasmanian devil (Save the Tasmanian Devil Programme, 2019) (Figure 1). With a nearly 100% mortality rate, DFTD has caused an estimated 80% population decline across the species range, with localized declines exceeding 90% (Lazenby et al., 2018; McCallum et al., 2007).

Devil facial tumours are transmitted as an allograft through biting, a common occurrence during social interactions among devils (Hamede, McCallum, & Jones, 2013; Hamilton et al., 2019). Direct transmission of DFTD among devils means that its spatial spread is inextricably tied to the movements of devils—a relationship that leads to the assumption of genetic costructuring among host and pathogen (Criscione, 2008; Jarne & Théron, 2001; Mazé-Guilmo et al., 2016). Yet, for costructuring to occur, pathogen dispersal must be synchronized with host dispersal and subsequent reproduction. In other words, individuals must disperse while infected and reproduce thereafter. This requirement has been identified previously but is often overlooked in studies that use host movements to predict disease spread. In devils, gene flow typically occurs through individuals that dispersed away from their natal sites as juveniles, whereas DFTD transmission occurs primarily during the adult life stage. Juvenile dispersal is likely to take place over greater

distances than the typical movements of adult devils because adults maintain high fidelity to their established home ranges, which are typically 5–30 km² (Lachish, Miller, Storfer, Goldizen, & Jones, 2011; Pemberton, 1990). Yet, genetic spatial autocorrelation distances are up to 100 km in eastern Tasmania (Lachish et al., 2011) and 60 km in western Tasmania (Storfer et al., 2017). On this basis, we would hypothesize that costructuring among devils and tumours is weak and that devil gene flow should be less geographically constrained than the spread of DFTD.

An alternative hypothesis is that DFTD disperses more rapidly and shows less genetic structure than devils because opportunities for transmission occur more frequently than devil reproduction. That is, whereas the biting contacts required for DFTD transmission are a common social behaviour occurring throughout the year (albeit more frequently during the mating season), devils generally mate between February and May (Hamede et al., 2009, 2013; Hamilton et al., 2019). Furthermore, the potential effect of a single dispersing individual on genetic structure is less in devils than in tumours. This is because a single tumour may proliferate into a large, clonal lineage that dramatically shifts the overall genetic structure of tumours in its new population. High mobility of DFTD tumours is supported by the fact that DFTD has spread from the northeastern corner of Tasmania to the west coast of Tasmania in just over 20 years (Epstein et al., 2016; Storfer et al., 2017; Figure 1). It is unclear which of these

competing hypotheses is best supported, exemplifying the need to understand the extent to which host and pathogen movements are linked and whether they are subject to the same environmental constraints.

Herein, we employ a comparative landscape genetics framework to test these competing hypotheses. Specifically, we test whether Tasmanian devil population structure predicts DFTD tumour population structure and whether host gene flow and pathogen spread are dictated by distinct environmental factors. We use 6,478 SNPs in devils and 1,595 SNPs in tumours genotyped using RAD capture (Ali et al., 2016; Margres et al., 2018) to reveal broad-scale population structure and fine-scale patterns of genetic variation. Individual-level estimates of genetic variation were analysed using complementary landscape genetics approaches to investigate the relative roles of isolation by environment (IBE) and isolation by resistance (IBR) in influencing genetic structure in both devils and DFTD. In short, IBE occurs due to the environment *at* sample locations, whereas IBR occurs due to the environment *intervening* sample locations. IBR approaches allow us to quantify how environmental heterogeneity across entire landscapes can affect functional connectivity (McRae, 2006), and distinguish these effects from the classical isolation-by-distance model (IBD; Wright, 1943). In contrast, IBE describes the effect of divergent environments on genetic differentiation (Wang & Bradburd, 2014) and can occur for various reasons, including population-specific adaptation to local environmental conditions (and thus maladaptation of and selection against migrants) or natal habitat preference induction leading to habitat-biased dispersal (Wang & Bradburd, 2014).

2 | METHODS

2.1 | Study system

Tasmanian devils are carnivorous marsupials endemic to the island of Tasmania, Australia, where they are apex predators. Tasmania comprises a total land mass of 68,401 km² and encompasses a dramatic east-to-west climatic gradient and a high degree of topographic variability. Devils prefer eucalypt and sclerophyll forests and coastal scrub lands, but they can also be found near to human developments and agricultural land (Guiler, 1970; Hawkins et al., 2006; James et al., 2019). Previous work indicates up to six genetic populations island-wide, including a clear distinction between individuals sampled from north-western Tasmania and those from elsewhere on the island (Brüniche-Olsen, Jones, Austin, Burridge, & Holland, 2014; Fraik et al., 2020; Hendricks et al., 2017; Miller et al., 2011; Storfer et al., 2017). The cause of this east-to-west spatial genetic heterogeneity remains unclear, with previous landscape genetics work implementing least-cost path modelling and microsatellite loci unable to identify any landscape factors driving this variation (Storfer et al., 2017).

The transmissible tumour first identified in 1996 is now present across nearly the entire devil geographic range, with all extant

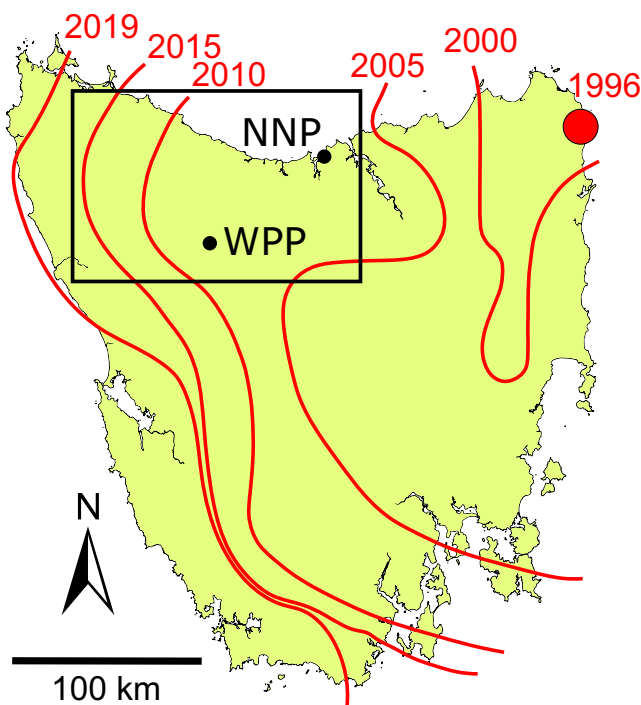


FIGURE 1 The spread of DFTD across the island of Tasmania, with the approximate disease front over time depicted as red lines labelled by year. The site of the first documented case of DFTD is identified by the red circle. The 12,000-km² study area is shown within the box, with Narawntapu National Park (NNP) and West Pencil Pine (WPP) indicated

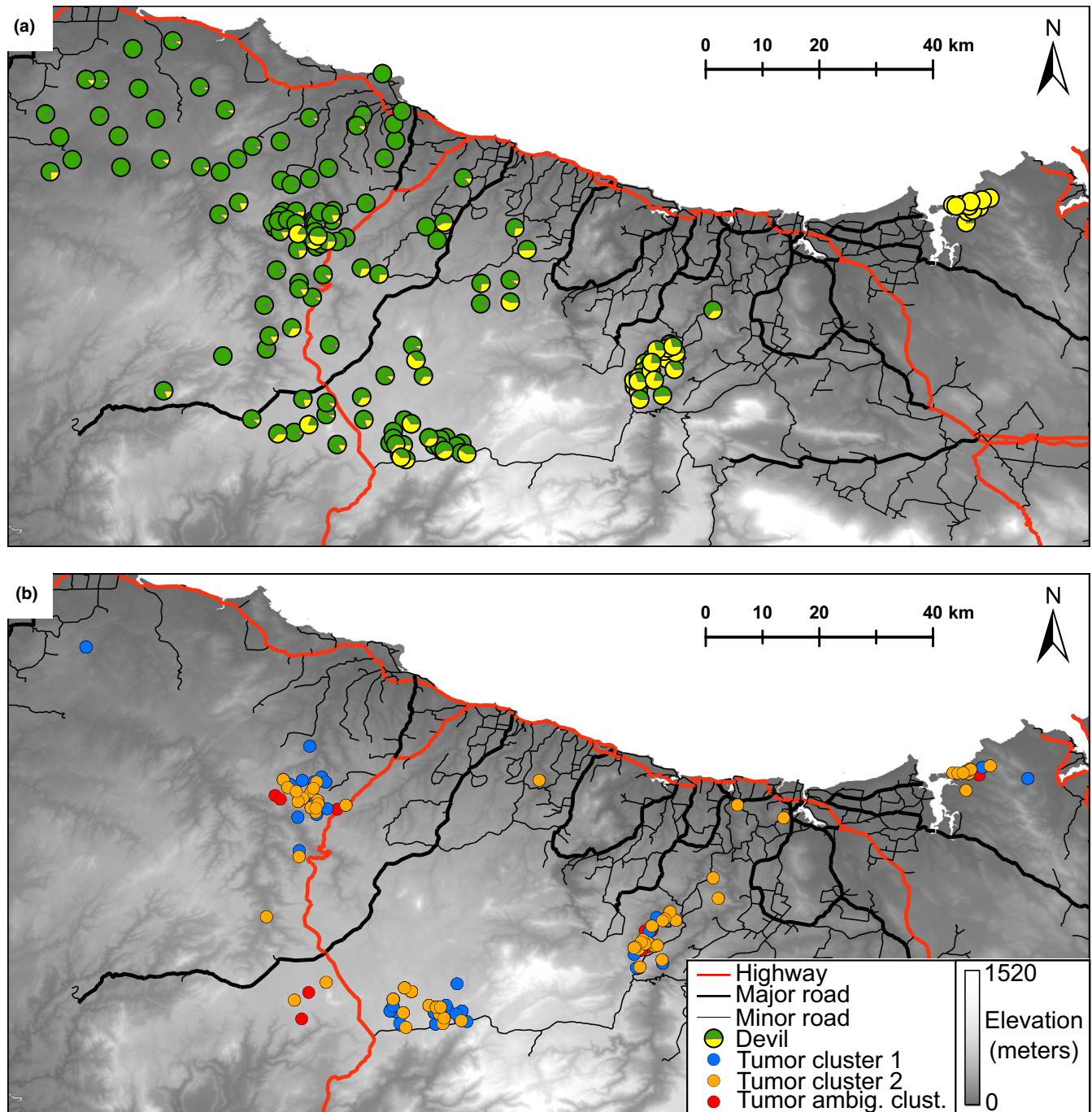


FIGURE 2 Spatial distribution of population genetic structure in (A) Tasmanian devils and (B) DFTD tumours. For devils, relative STRUCTURE assignment probabilities for $K = 2$ genetic clusters are depicted as pie charts. For tumours, genetic clusters as determined by combined STRUCTURE analysis and DAPC are depicted, with samples for which analyses were incongruent shown as being of ambiguous cluster assignment

cases having a common origin. However, in 2014, a second transmissible cancer of devils, devil facial tumour disease 2 (DFT2), was discovered in southern Tasmania, independent in origin from the first (Pye et al., 2016). DFT2 remains geographically restricted; yet, insights gained into the transmission of the first tumour may help inform the management of DFT2 and any subsequent transmissible tumours, as well as other directly transmitted diseases. Herein, we focus on the first tumour, to which we refer exclusively

in this study as “DFTD” or “tumours,” with any references to the second tumour, DFT2, specified as such. No cases of DFT2 were included in this study.

DFTD infection is typically observed in adult devils, most likely due to limited injurious biting contact until adulthood (Hamede et al., 2013) and changes in immune system function at sexual maturity (Cheng et al., 2017). Nonetheless, tumours are occasionally observed in juveniles, with a long latent period likely biasing

detectability towards adults (Hamede et al., 2013, 2015; Lazenby et al., 2018). DFTD replicates clonally, with no evidence of recombination among tumours. However, cancer lineages accumulate somatic mutations over time, thus generating genetic differences among locations that reflect how a given lineage has spread spatially (Murchison et al., 2012; Schwartz & Schäffer, 2017; Stammnitz et al., 2018). Therefore, characterization of DFTD genetic structure enables us to infer how it has spread among devil populations with respect to geographic and environmental factors.

2.2 | Sample collection

We collected georeferenced Tasmanian devil ear and tumour tissue samples using a 3-mm biopsy punch from wild devils over a 12-year period. A detailed description of field trapping protocols can be found in Hawkins et al. (2006) and Hamede et al. (2015). Two hundred and seventeen devil samples and 177 tumour samples, of which 87 were paired samples with both tumour and host tissues taken from the same individual, were collected from an approximately 12,000-km² area in northwest Tasmania between 2004 and 2016 (Figures 1 and 2). We focused on this area because it contains a high degree of environmental and topographic variation and overlaps with a broad-scale genetic discontinuity among devil populations identified in previous studies (Brüniche-Olsen et al., 2014; Hendricks et al., 2017; Miller et al., 2011; Storer et al., 2017), suggesting potential environmental/landscape constraints on devil movements. Sampling across this region was relatively consistent throughout the sample period and coincided with the arrival and spread of DFTD in this region (Figure 1).

2.3 | RAD-capture array

We used a restriction site-associated DNA (RAD)-capture (i.e. "Rapture") array (Ali et al., 2016) to target loci across the devil and tumour genome. The capture array was developed from RAD sequencing of 360 devils (Epstein et al., 2016); 15,898 of the 90,000 RAD loci from this earlier study were used to make a targeted array using the myBaits for high-throughput population genomics studies kit (Arbor Biosciences, Ann Arbor, MI) as described in Margres et al. (2018). Targeted loci met one or more of the following criteria: (a) genotyped in $\geq 50\%$ of individuals, contained ≤ 3 nonsingleton SNPs with a minor allele frequency (MAF) ≥ 0.05 and was ≥ 20 kb away from other targeted loci to increase genome coverage (7,108 loci); (b) located within 50 kb of an immune-related gene, with ≤ 4 nonsingleton SNPs and genotyped in $\geq 67\%$ of the individuals (6,315 loci); and (c) showing some preliminary evidence of association with DFTD susceptibility and having ≤ 5 nonsingleton SNPs (3,316 loci).

Because the myBaits array was developed for devils and not DFTD, we tested whether the array could successfully capture RAD loci from tumour samples by aligning whole-genome tumour samples (sequenced at 90x coverage; from Margres et al., 2020) to

the devil reference genome (downloaded from Ensembl June 2014; Murchison et al., 2012) using Burrows–Wheeler Aligner v0.7.12 (option MEM; Li & Durbin, 2009). We measured tumour coverage across each Rapture region using Bedtools v2.27.0 (Quinlan & Hall, 2010). Only 10 genomic regions covered on the capture array showed low coverage ($< 10\times$), illustrating that 99.9% of the baits should capture tumour DNA.

2.4 | Sequencing and data processing

We extracted DNA from tissue biopsies using the Qiagen DNeasy Blood & Tissue Kit, doubling the recommended amount of proteinase K to maximize lysis efficiency. DNA was digested using the *Pst*I restriction enzyme, and the RAD-capture libraries were sequenced on an Illumina HiSeq 4,000 at the Genomics Sequencing Laboratory at the University of California, Berkeley. We processed the raw data as previously described (Margres et al., 2018). Briefly, reads were demultiplexed, and low-quality reads and potential PCR duplicates were removed using Stacks v1.21 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). Reads were then aligned to the reference genome using bowtie2 v2.3.4 (Langmead & Salzberg, 2012) with the --sensitive, --end-to-end and -X 900 settings.

2.5 | Variant calling

To identify variants, we used HaplotypeCaller in GATK v3.8 (DePristo et al., 2011; McKenna et al., 2010), with devils and tumours genotyped separately. For each, we removed SNPs and indels matching any of the following criteria: quality by depth < 2.0 , strand bias Phred-scaled p-value > 60.0 , root mean square of the mapping quality < 40.0 , mapping quality rank sum test approximation of 12.5 and a read position rank sum test approximation of eight.

We removed nontargeted regions from the data set using Bcftools isec (Li, 2011), followed by removal of SNPs with a minimum depth < 5 , minimum genotype quality < 25 , missing data $> 50\%$ and MAF < 0.01 using VCFtools v0.1.15 (Danecek et al., 2011). Then, to identify tumour-specific somatic SNPs and account for possible host contamination during tumour biopsy, we again used Bcftools isec to remove any SNP in the tumour data set that was also identified in the host samples. Following filtering, we retained 6,478 devil and 1,595 tumour SNPs for analysis.

2.6 | Population structure

We investigated both host and tumour population genetic structure using two complementary approaches, following best practices recommended by Janes et al. (2017). First, we performed a discriminant analysis of principal components (DAPC) using *adegenet* (Jombart, 2008) in R version 3.6.3 (R Development Core Team, 2013). Briefly, we used the find.clusters function to perform

k-means estimation of the best-fit number of genetic clusters (K) as determined by the Bayesian information criterion (BIC), followed by the DAPC function to estimate probabilities of membership to each cluster for each tumour sample. Second, we evaluated population structure using STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000). We tested all values of K between 1 and 5, performing ten replicate runs per K. Each run comprised 1,000,000 Markov chain Monte Carlo iterations following a burn-in of 50,000 iterations. Although STRUCTURE is inappropriate for clonal populations, we performed this analysis for tumours to maintain consistency with the host analysis and to complement the more suitable DAPC. Because there are no recorded observations of recombination among tumours, we specified the admixture-free model in STRUCTURE. The most likely K was determined using ΔK according to the Evanno method (Evanno, Regnaut, & Goudet, 2005), implemented in STRUCTURE HARVESTER (Earl & VonHoldt, 2012; Van Rossum & Drake Jr, 1995), and assessment of mean estimated natural logarithm of the probability of the data $[\text{LnPr}(X|K)]$ values. To identify possible hierarchical population structure, we repeated analyses of population structure on individual genetic clusters identified in initial runs until no further additional genetic clusters were identified.

Due to lack of recombination among tumours, we interpreted genetically distinct clusters of tumour samples as discrete, nonrecombining groups. To ensure confidence in the identified clusters, we assigned tumours to a genetic cluster only where DAPC and STRUCTURE assignments were concordant. We excluded tumour samples with ambiguous assignment from any analysis pertaining to a specific cluster. Once tumour clusters had been identified, we performed an analysis of molecular variance (AMOVA; Excoffier, Smouse, & Quattro, 1992) to quantify the proportion of overall genetic variance explained by differentiation between clusters relative to that explained by variation among and within individual tumours. AMOVA was performed using the *poppr* package in R (Kamvar, Tabima, & Grünwald, 2014) and significance determined using a randomization test with 100 permutations, which was performed using *ade4* (Dray & Dufour, 2007).

We tested for genetic costructuring among paired devil–tumour samples using two approaches. First, we conducted a Mantel test comparing host genetic distances and tumour genetic distances. Individual genetic distances were calculated as $1 - D_{ps}$, where D_{ps} is the proportion of shared alleles between paired samples, using *ade-genet*. Second, we fitted a logistic regression to determine whether STRUCTURE assignment probabilities for hosts were predictive of the genetic cluster to which a given host's tumour was assigned. We also used a logistic regression to determine whether sample date was predictive of the genetic cluster of each tumour.

2.7 | Landscape genetics

We conducted landscape genetics analyses to identify how environmental factors influence patterns of devil and tumour genetic

structure within both IBR and IBE frameworks. All landscape genetics analyses were conducted separately for host and tumour. In addition to analysing all tumours together, we also conducted landscape genetics analyses separately for each of the identified tumour clusters (as identified by the above-described population genetic approaches) to account for differences in environmental associations among clusters.

We selected six continuous and two categorical variables to test in our landscape genetics analyses based on habitat preferences observed through prior devil mark-recapture studies (Guiler, 1970; Hawkins et al., 2006), radio-collaring studies (M.E. Jones, unpublished data) and a previous landscape genetics study of Tasmanian devils (Storfer et al., 2017). The continuous variables comprised elevation, elevation relief ratio (a measure of relative altitudinal relief between two points; Pike & Wilson, 1971), annual mean temperature, temperature annual range (the difference between the average 24-hr maximum temperature of the hottest month and the average 24-hr minimum temperature of the coldest month), annual precipitation and precipitation seasonality (the coefficient of precipitation variation; Feng, Porporato, & Rodriguez-Iturbe, 2013). Categorical variables were land cover type and roads. Land cover types were derived from the TASVEG 3.0 vegetation communities data set (DPIPWE, 2013) and partitioned according to the ten broad vegetation categories defined by TASVEG 3.0, with agriculture and urban areas/exotic vegetation forming a further two categories. Roads data were downloaded from Geoscience Australia (data.gov.au) and classified as principal, secondary and minor roads, to which we refer as highways, major roads and minor roads, respectively. Elevation data were downloaded from Geoscience Australia, and elevation relief ratio was calculated from the elevation data using the raster calculator in ArcGIS v10.7 (ESRI, 2011). Climatic data were downloaded from WorldClim v2 (Fick & Hijmans, 2017). We assessed multicollinearity among environmental rasters using variance inflation factors (VIF), retaining only variables with $VIF < 10$ (Table S1). Annual mean temperature had a VIF score exceeding this threshold and was subsequently removed from all analyses.

Pairwise individual genetic distances were interpreted for devils and tumours as a relative proxy for genetic connectivity and used as a response variable for landscape genetics analyses.

2.7.1 | Isolation by resistance: effects of landscape heterogeneity intervening sites

We tested for effects of landscape variables on genetic connectivity in an IBR framework using the R package *ResistanceGA* (Peterman, 2018). *ResistanceGA* optimizes the correlation between genetic distances and resistance surface cost values by using a genetic algorithm to explore potential resistance surface parameterizations for each landscape variable. Resistance surface optimization approaches avoid the need to a priori assign costs to environmental variables through expert opinion or species distribution and habitat suitability models, which can be difficult to

translate to numerical values of resistance to movement (Elliot, Cushman, Macdonald, & Loveridge, 2014; Spear, Balkenhol, Fortin, McRae, & Scribner, 2010). In contrast to least-cost path modelling, which considers only a single optimal dispersal pathway with respect to a heterogeneous landscape factor of interest (thus assuming that individuals have complete knowledge of the landscape), IBR analytical frameworks that implement circuit theory can account for all possible dispersal pathways in predicting rates of gene flow (McRae, 2006).

We optimized resistance surfaces singly for each environmental variable based on random-walk commute time between locations (van Etten, 2017). In brief, the genetic algorithm used for optimization comprised a randomly generated population of individuals possessing parameters with randomly chosen values that determine which of a variety of transformations is applied to the resistance surface, the shape of the transformation and the maximum resistance value (Peterman, 2018). Thus, each simulated individual represents a uniquely parameterized resistance surface. Across each resistance surface (i.e. per simulated individual), pairwise commute times are calculated and evaluated against the true pairwise genetic distances using a linear mixed-effect model with maximum-likelihood population effects (MLPE), which accounts for nonindependence among pairwise samples (Clarke, Rothery, & Raybould, 2002; Van Strien, Keller, & Holderegger, 2012). Model support or “fitness” of each simulated individual is determined using log-likelihood, with those achieving the highest log-likelihood allowed to “reproduce” to form a population comprising the next generation of the algorithm. This new population inherits the parameters from the previous generation (while allowing for random mutation and recombination of the parameters), and the process repeats itself through a number of subsequent generations. For each landscape variable, we specified that 25 generations must pass with no improvement to the MLPE model log-likelihood for a resistance surface to be identified as optimal. Following optimization, to test the relative support of each environmental variable as a predictor of genetic distance, we used the final optimized surfaces to again generate commute times and fit MLPE models, calculated without restricted maximum likelihood (REML) and bootstrapped for 1,000 iterations with 90% subsampling. We ranked environmental variables by average AIC_C scores across all bootstrap iterations.

2.7.2 | Isolation by environment: effects of environmental differentiation on genetic differentiation

To investigate the extent to which genetic differentiation in hosts and tumours is influenced by patterns of IBE, we used generalized dissimilarity modelling (GDM) as implemented in the R package *gdm* (Fitzpatrick & Keller, 2015). Originally designed for community-level modelling of species turnover, GDM performs linear regressions to test associations between dissimilarity and distance matrices, but fits i-spline functions to allow for nonlinear responses and controls

for geographic distance among sample locations (Ferrier, Manion, Elith, & Richardson, 2007). GDM assumes ordered categorical or continuous predictor variables (Ferrier et al., 2007), so we included only elevation, elevation relief ratio and our climatic variables in this analysis. Environmental variables were measured as the mean value within a 15-km² buffer around each sample location, approximating a typical devil home range (M.E. Jones, unpublished data). We used matrix permutation (500 permutations with 90% subsampling of both sites and site-pairs) with backward elimination to evaluate model and variable significance and estimate variable importance (Fitzpatrick & Keller, 2015). During each iteration of the backward elimination procedure, the least important variable was removed, and variable importance and significance recalculated for the new model. As nonexplanatory (i.e. the least important) variables were removed, the per cent deviance explained by each successive model relative to the null did not change. Only as explanatory variables were removed did the per cent deviance explained drop. Thus, the top model was identified as the model with the highest deviance explained and containing the fewest variables. Variable importance was calculated as the per cent change in model deviance upon permutation of the given variable. Geographic distance was included as a control in all models and was not subject to backward elimination.

In addition to the host, all-tumour and tumour cluster-specific tumour analyses, to compare the relative effects of host genetic variation and environmental variables on tumour genetic differentiation, we performed a separate GDM analysis of tumours for which the corresponding host devils were genotyped. For this analysis, the same environmental variables were included as above, with the addition of host genetic distances.

3 | RESULTS

3.1 | Host and pathogen population structure

Analysis of devil population genetic structure using DAPC suggested the most likely number of genetic clusters was $K = 2$, according to the BIC support. However, ΔBIC did not exceed 2 for either $K = 1$ or $K = 2$ (Figure S1), suggesting that patterns of population structure were difficult to resolve with our data (Burnham & Anderson, 2002). Similarly, STRUCTURE provided the most support for $K = 2$ as determined by ΔK calculated using the Evanno method. Although the Evanno method is unable to evaluate $K = 1$ and thus cannot exclude it as a potential solution (Janes et al., 2017), $K = 1$ was the least supported solution according to mean $\text{LnPr}(X|K)$, supporting the existence of multiple genetic clusters. Further, mean $\text{LnPr}(X|K)$ values supported successively higher values of K (Table 1), indicating a potential genetic cline. However, there was also greater variation in $\text{LnPr}(X|K)$ among iterations at higher values of K (Table 1), suggesting some uncertainty. Further analysis of hierarchical structure within the initial $K = 2$ clusters did not provide clear evidence for any further genetic clusters for DAPC, yet STRUCTURE again supported successively higher values of K according to $\text{LnPr}(X|K)$. Thus,

TABLE 1 Results from the Evanno method showing relative support for STRUCTURE models of varying numbers of genetic clusters (K) in Tasmanian devils and DFTD. Optimal K was determined according to the model with the highest ΔK , together with assessment of mean natural logarithm of the probability of the data ($\ln \Pr(X|K)$), and is shown in bold typeface

	K	Reps	Mean $\ln \Pr(X K)$	Stdev $\ln P(K)$	ΔK
Devil					
	1	10	-1,025,607.12	4.90	-
	2	10	-1,005,918.67	4.55	3,339.52
	3	10	-1,001,419.28	9.87	180.47
	4	10	-998,701.25	37.17	30.84
	5	10	-997,129.52	2,514.96	-
Tumour					
	1	10	-93,561.09	0.31	-
	2	10	-91,383.05	22.36	271.29
	3	10	-95,270.47	6,058.33	1.40
	4	10	-90,660.12	503.25	24.24
	5	10	-98251.00	23,635.46	-

for devils, we settled on $K = 2$ as representing opposite ends of a genetic cline. Accordingly, these clusters were not geographically discrete, with individual assignment probabilities indicating a continuous longitudinal admixture gradient (Figures 2 and 3).

In tumours, DAPC and STRUCTURE both supported two genetic clusters (Table 1, Figure S1). However, $\ln \Pr(X|K)$ was higher for STRUCTURE runs at $K = 4$ but, similar to devils, with greater variation among iterations. Subsequent DAPC and STRUCTURE runs showed no evidence of hierarchical population structure in tumours. Of the 177 tumours, 147 had cluster assignments that were supported by both DAPC and STRUCTURE at $K = 2$, with 30 tumours ambiguously assigned. Hereafter, we refer to the two identified tumour clusters as tumour cluster 1 ($n = 74$) and tumour cluster 2 ($n = 73$). There was no clear geographic structure among these clusters, with almost complete spatial overlap. However, we did observe a slight predominance of tumour cluster 2 towards the eastern end of the study area (Figures 2, 3). AMOVA revealed significant genetic variance attributable to the identified tumour clusters, which explained 6.48% of variation ($p = .01$) compared to minimal (1.58%; $p = .23$) variation attributable to among-tumour variation within clusters (Table S2). Variation within individual tumours comprised 91.94% of overall genetic variation ($p = .04$). This is due to somatic mutation in cancers producing high rates of heterozygosity.

3.2 | Tasmanian devils and DFTD are weakly costructured

Our STRUCTURE results provide some evidence for host–tumour costructuring along a longitudinal gradient (Figures 2, 3). Logistic regression revealed that tumour cluster identity was predicted by devil

population structure ($LRT = 5.81$; $p = .016$; Figure 4a) but not sampling date ($LRT = 1.74$; $p = .19$), suggesting cocirculating tumour clusters that broadly costructure with host populations. However, when comparing host and tumour genetic distances directly, we found no correlation (Mantel $r = -0.10$, $p = .92$; Figure 4b). Further, host genetic distances performed worse than environmental differences in predicting tumour genetic differentiation (see below landscape genetics analyses). Mean genetic distances were greater among devils (0.30 , $SD = 0.025$) than among tumours (0.14 , $SD = 0.035$).

3.3 | Landscape factors more strongly affect spatial genetic variation in devils than DFTD

3.3.1 | Isolation by resistance: effects of landscape heterogeneity intervening sites

In devils, landscape heterogeneity intervening sites explained substantial variation in gene flow. Although geographic distance was most frequently the top model (52.7% of bootstrap iterations) and explained 24.9% of genetic variation among devils, it had relatively low mean ΔAIC_c support across bootstraps (mean $\Delta AIC_c = 5.97$; Table 2). Roads as a barrier to gene flow had the highest average support of all models and was the top model in 38.4% of bootstrap iterations, explaining 37.9% of genetic variation among devils. Optimization of the roads resistance surface assigned the greatest costs to devil movement to highways and major roads, with minor roads and nonroad cells having relatively low costs to devil movement. Although two other variables—precipitation seasonality and elevation relief ratio—had greater mean ΔAIC_c support than geographic distance, they were poorly supported overall, being the top models in less than 7% of bootstrap iterations and having a mean $\Delta AIC_c > 2$. No other landscape resistance model had mean $\Delta AIC_c < 2$.

Among DFTD tumours, genetic variation was poorly explained by between-site landscape variables. Elevation and precipitation seasonality both had mean $\Delta AIC_c < 2$ and were the most frequent top models among bootstrap iterations. However, these models explained minimal genetic variation among tumours. Elevation was most the supported model (mean $\Delta AIC_c = 0$; top model in 67.7% of bootstrap iterations) but explained only 1.5% of genetic variation among tumours. Precipitation seasonality was less supported (mean $\Delta AIC_c = 1.70$; top model in 30.5% of bootstrap iterations) but explained slightly more genetic variation among tumours (mean $mR^2 = 0.03$).

When analysing each tumour cluster separately, the top landscape resistance models differed among clusters but still explained relatively little genetic variation for each. For tumour cluster 1, elevation relief ratio was the top model in 77.2% of bootstrap iterations, with annual precipitation being the top model in 11.5% of bootstrap iterations (mean $\Delta AIC_c = 1.48$). However, all but two of the models for cluster 1 had a mean $\Delta AIC_c < 4$, suggesting relatively weak support for the top models over the others. For tumour cluster 2, elevation was the top model in 77.6% of bootstrap iterations (mean $\Delta AIC_c = 0$), with all other models poorly supported.

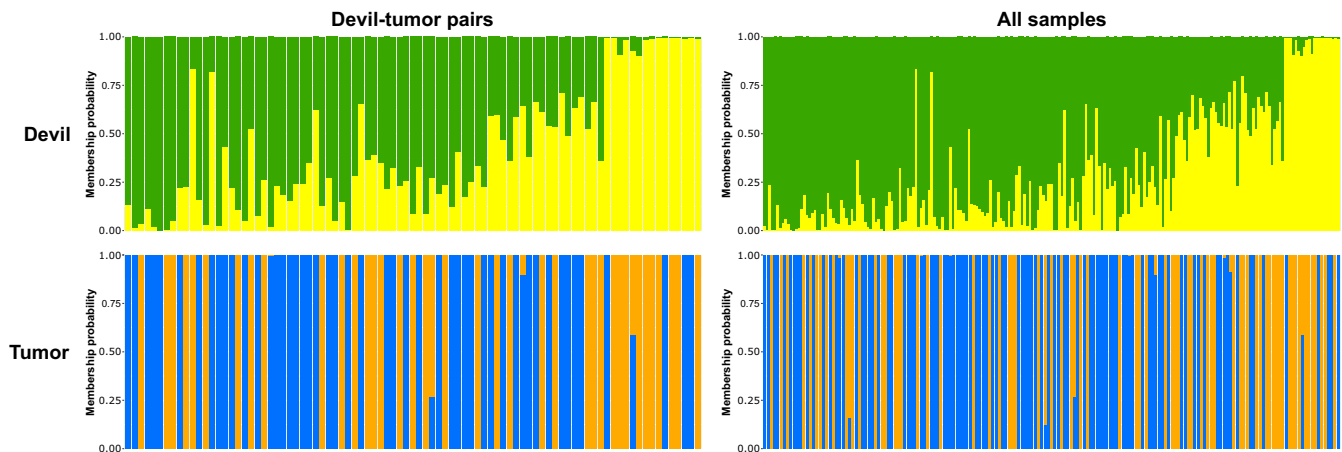


FIGURE 3 Population structure is evident in both Tasmanian devils (top) and DFTD (bottom). STRUCTURE genetic assignment probabilities are shown for $K = 2$, showing both full (right) and paired host–tumour (left) sets. Each column represents an individual devil or tumour sample, with genetic clusters indicated by colour, and the relative proportions of each colour represent a sample's relative probability of membership of each genetic cluster. Tumour cluster 1 is indicated in blue, and tumour cluster 2 is indicated in orange. Samples are arranged along the x-axis from west to east

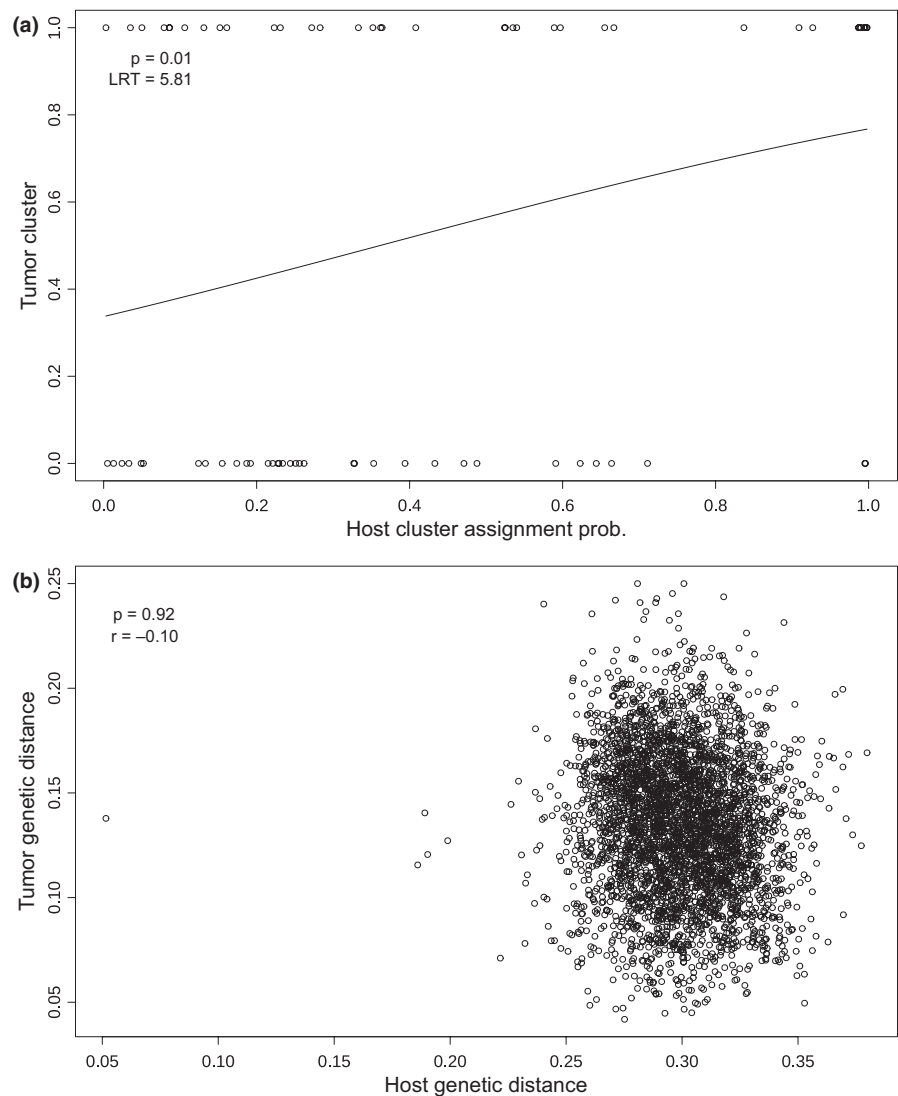


FIGURE 4 Tests of Tasmanian devil and DFTD tumour costructuring, with logistic regression (A), suggesting that host genetic cluster assignment broadly predicts tumour genetic cluster, but with a Mantel test (B) showing no correlation between devil and tumour individual genetic distances ($1 - D_{PS}$). Removal of left-tail outlier in panel B Mantel test produced negligible change in result

TABLE 2 Linear mixed-effect models with maximum-likelihood population effects testing the influence of landscape resistance variables on genetic differentiation in devils, tumours and tumour clusters 1 and 2. Model performance was evaluated by AICc averaged over 1,000 bootstrap iterations, with models with $\Delta\text{AICc} < 2$ highlighted. Marginal R^2 (mR^2) is the proportion of overall variation explained by the model fixed effects, and % top model is the percentage of times the model was the top-performing model over 1,000 bootstraps, as determined by AICc support

	Variable	K	AICc	ΔAICc	mR^2	% Top Model
Devils	Roads	5	-7,364.67	0.00	0.379	38.4
	Precip. seasonality	4	-7,359.56	4.66	0.269	1.7
	Elevation relief ratio	4	-7,358.72	5.49	0.253	6.8
	Temp. annual range	4	-7,357.35	6.87	0.255	0.0
	Distance	2	-7,357.64	5.97	0.249	52.7
	Elevation	4	-7,357.35	6.86	0.253	0.1
	Annual precip.	4	-7,355.11	9.11	0.255	0.0
	Land cover	13	-7,322.04	50.90	0.319	0.3
All tumours	Elevation	4	-7,083.89	0.00	0.015	67.7
	Precip. seasonality	4	-7,082.19	1.70	0.030	30.5
	Annual precip.	4	-7,080.23	3.67	0.020	1.4
	Elevation relief ratio	4	-7,072.25	11.64	0.012	0.3
	Distance	2	-7,070.41	13.48	0.009	0.1
	Temp. annual range	4	-7,069.27	14.62	0.006	0.0
	Land cover	13	-7,067.75	16.14	0.024	0.0
	Roads	5	-7,065.33	18.57	0.009	0.0
Tumour cluster 1	Elevation relief ratio	4	-7,627.07	0.00	0.026	77.2
	Annual precip.	4	-7,625.59	1.48	0.019	11.5
	Precip. seasonality	4	-7,624.56	2.51	0.016	4.6
	Distance	2	-7,624.19	2.88	0.009	6.4
	Elevation	4	-7,623.73	3.35	0.021	0.2
	Temp. annual range	4	-7,623.67	3.40	0.009	0.0
	Roads	5	-7,622.07	5.01	0.020	0.1
	Land cover	13	-7,616.24	10.83	0.016	0.0
Tumour cluster 2	Elevation	4	-7,972.78	0.00	0.025	77.6
	Annual precip.	4	-7,969.02	3.76	0.043	2.1
	Elevation relief ratio	4	-7,967.41	5.37	0.044	10.3
	Precip. seasonality	4	-7,965.74	7.04	0.024	8.6
	Distance	2	-7,959.39	13.39	0.007	0.5
	Roads	5	-7,958.98	13.80	0.047	0.9
	Land cover	13	-7,957.66	15.12	0.053	0.0
	Temp. annual range	4	-7,958.77	14.01	0.006	0.0

3.3.2 | Isolation by environment: effects of environmental differentiation on genetic differentiation

Analysis of isolation-by-environment patterns using GDM did not identify any environmental differences among sample locations that were explanatory of genetic differentiation among devils (Table 3). The top model explained a reduction in deviance relative to the null model of 11.87%, but contained only a significant effect of geographic distance,

which explained just 1.84% of this reduction in model deviance. This suggests a weak effect of isolation by distance among devils.

Among all tumours, the top model explained a reduction in model deviance relative to the null model of 6.67% and contained significant effects of geographic distance and differences in elevation. Elevation predominated, explaining 99.95% of this reduction in model deviance, while the effect of geographic distance was non-existent. This lack of an effect of geographic distance despite statistical significance was likely due to geographically distant animals

TABLE 3 Summary of top generalized dissimilarity models explaining genetic distances in devils, all DFTD tumours and DFTD clusters separately. % deviance explained refers to the reduction in model deviance relative to the null. % model deviance explained refers to the per cent change in model deviance upon permutation of a given variable

	% deviance explained	Variable	Variable significance (P)	% model deviance explained
Devils	11.87	Geographic distance	0.00	1.84
		Elevation relief ratio	—	—
		Elevation	—	—
		Precip. seasonality	0.47	0.90
		Annual precip.	0.08	7.64
		Temp. annual range	0.25	3.17
All tumours	6.67	Geographic distance	0.00	0.00
		Elevation relief ratio	0.99	0.00
		Elevation	0.00	99.95
		Precip. seasonality	—	—
		Annual precip.	—	—
		Temp. annual range	—	—
Tumours with matched host samples	5.50	Geographic distance	0.01	0.00
		Elevation relief ratio	0.07	38.96
		Elevation	0.03	44.81
		Precip. seasonality	—	—
		Annual precip.	—	—
		Temp. annual range	—	—
		Host genetic distance	—	—
Tumour cluster 1	No significant model			
Tumour cluster 2	4.66	Geographic distance	0.11	0.00
		Elevation relief ratio	0.47	7.07
		Elevation	0.02	93.36
		Precip. seasonality	—	—
		Annual precip.	—	—
		Temp. annual range	—	—

being located in areas that differ in elevation, resulting in elevation explaining a high proportion of model deviance that would otherwise be partly explained by geographic distance.

When analysing tumour clusters separately, the effect of elevation differences on genetic differentiation among tumours persisted only for tumour cluster 2, whose top model explained a 4.66% reduction in deviance relative to the null model. Elevation explained 93.36% of this reduction in model deviance, whereas the effect of geographic distance was nonsignificant. None of the models for tumour cluster 1 were significant upon permutation of environmental dissimilarity matrices, suggesting that none of the tested landscape variables, nor geographic distance, were influencing genetic differentiation among tumours within this cluster.

Host genetic distances did not perform better than environmental differences in explaining tumour genetic differentiation. In tumours for which the host devil was genotyped, host genetic distance was absent from the top model, which explained a reduction in model deviance relative to the top model of 5.50%. Elevation was the most important variable, explaining 44.81% of this reduction in model deviance, consistent with the all-tumour analysis.

4 | DISCUSSION

We conducted a comparative landscape genetics study of Tasmanian devils and DFTD to identify environmental factors driving IBR and IBE patterns in both host and pathogen. Our results warrant caution when inferring pathogen spread using host population genetic structure. Although we found evidence for broad-scale costructuring between devils and tumours, the primary landscape processes influencing genetic variation appeared to differ between host and pathogen. In devils, we found two genetic clusters, consistent with previous studies (Storfer et al., 2017). Further, a relatively strong IBR pattern was present, whereby genetic variation was driven largely by major roads and highways acting as barriers to gene flow. However, evidence of IBE was absent in devils. Surprisingly, we found no geographic or temporal structure among two identified tumour clusters, suggesting coexistence of distinct tumour lineages throughout the study area for the entire sampling period. IBE in tumour cluster 2, although not particularly strong, was largely attributable to differences in elevation. Despite almost complete spatial overlap with cluster 2, tumour cluster 1 exhibited no evidence of IBE.

4.1 | Costructuring among Tasmanian devils and DFTD

In devils, we observed a clinal pattern of admixture between two genetic clusters from east to west across our study area. Previous broader-scale studies of devils have identified relatively discrete genetic clusters, with a genetic discontinuity approximately overlapping our study area that distinguishes northwest Tasmanian devils from other populations (Brüniche-Olsen et al., 2014; Hendricks et al., 2017; Jones, Paetkau, Geffen, & Moritz, 2004; Miller et al., 2011). Our study encompasses a relatively smaller geographic area at a higher sampling density than previous studies, and it is likely that the observed clinal pattern reflects admixture between the previously identified northwestern population and those further to the east.

We observed broad-scale genetic costructuring between infected devils and their tumours, whereby devil genetic cluster assignment probabilities were predictive of the tumour cluster to which a given individual was host. However, there was no correlation between host and tumour genetic distances, suggesting that individual-level tumour variation (i.e. within clusters) is not influenced by host gene flow and a lack of costructuring at a fine scale. Our inferred DFTD clusters had almost complete spatial overlap with one another, but we observed a higher prevalence of cluster 2 within Narawntapu National Park, our eastern-most collection site. Devils from this area showed almost complete assignment to a genetic cluster that was only present at admixture levels among other samples in this study (Figure 2), suggesting at least partial isolation of the Narawntapu population. Geographic isolation of Narawntapu, a coastal site surrounded by large water bodies and mountains, is further supported by documentation of a genetic distinct group of bare-nosed wombats (*Vombatus ursinus*) in this area (Martin, Carver, et al., 2019). Thus, we believe the observed broad-scale costructuring is driven predominantly by geographic isolation of Narawntapu from the rest of our study area, rather than concordant patterns of gene flow.

Previous work has shown evidence of DFTD lineage replacement based on karyotype (Hamede et al., 2015), which may affect the spatial structuring of tumours. For example, a tetraploid DFTD strain first arrived at West Pencil Pine (a portion of our study area) in 2006, and initially resulted in lower than typical prevalence and higher than typical survival rates among infected individuals (Hamede et al., 2012, 2015). Devil populations subsequently began to decline with the arrival of a diploid tumour strain, which outcompeted and replaced the tetraploid strain (Hamede et al., 2015). In contrast, we did not observe lineage replacement but rather cocirculation. However, we had insufficient data to test for differences in karyotype or virulence among our observed tumour strains, and the spatial scale of our study was considerably broader than that at which karyotypic partitioning was observed by Hamede et al. (2015) (within a 25-km² area). As such, our results do not preclude the occurrence of lineage replacement at highly localized spatial scales.

4.2 | Isolation by resistance affects devils but not DFTD

Overall, IBR had a strong influence on genetic structure of devils but not tumours, supporting our hypothesis that tumour transmission among adult devils is less constrained by landscape than gene flow among juvenile dispersers. A significant negative effect of roads (predominantly highways and major roads) on gene flow was observed in devils, explaining 38% of genetic variation among our samples, compared to 25% of variation attributable to isolation by distance alone. Roads are a source of wildlife mortality due to vehicle collision, including in devils (Grueber et al., 2017; Jones, 2000). Coupled with the loss of habitat associated with road construction, and fences and other structures, roads reduce structural and functional landscape connectivity and often lead to decreased gene flow. Such responses to roads are well-documented and have been observed in both small, sedentary species (Arens et al., 2007; Holzman et al., 2009) and large, wide-ranging species (Coulon et al., 2006; Epps et al., 2005), including carnivores (Kozakiewicz et al., 2019; Riley et al., 2006). However, use of minor roads as movement corridors has been observed in devils, likely due to greater ease of movement through cleared vegetation and an abundance of road-killed carcasses for scavenging (Andersen, Johnson, Barmuta, & Jones, 2017). We found neither a positive nor a negative effect of minor roads on devil gene flow. However, our study was conducted over a much larger area than Andersen et al. (2017), with road use potentially occurring over only small distances without a significant effect on longer distance dispersal events.

Roads that form barriers to wildlife do not necessarily act similarly as barriers to their pathogens—even those relying on direct transmission. For example, a major highway was found to produce strong population genetic structure in bobcats (*Lynx rufus*) but not in their directly transmitted viruses (Lee et al., 2012). Similarly, our results suggest that roads do not significantly influence DFTD transmission. Roads are known to disproportionately affect juvenile devils, which exhibit higher mortality rates from vehicle collision than adults (Jones, 2000). Thus, roads likely present a greater barrier to dispersing juveniles, via which devil gene flow is primarily mediated, than to adults, via which DFTD transmission predominantly occurs. Overall, differentiation among tumours was not governed by any variation in connectivity due to landscape or environmental heterogeneity, with IBR patterns explaining barely more than 3% of tumour genetic variation. DFTD has spread across Tasmania very rapidly (Lazenby et al., 2018; McCallum et al., 2007), so it is not surprising that tumour movement has been largely unconstrained by geography. Tumours can proliferate rapidly if even a single infected individual reaches a naive population after crossing a challenging landscape. By contrast, the same challenging landscape may facilitate only occasional dispersal by juveniles, which may not even reproduce subsequently. Thus, although major roads constrain devil movements to the extent that devil population genetic structure is increased, even infrequent crossing of roads by DFTD-infected individuals is sufficient to sustain rapid DFTD spread.

4.3 | Isolation by environment affects DFTD but not devils

Despite substantial topographic variation and a large rainfall gradient from east to west that produces dramatic variation in vegetation types and structure across Tasmania, we found no detectable IBE effect. This result is somewhat surprising because devil population densities vary throughout Tasmania concordant with habitat preference for low-elevation dry eucalypt forest, with lower densities at high elevations and in areas of cool temperate rainforest (Jones & Barmuta, 2000). However, the relatively narrow geographic focus of our study likely did not capture a sufficient proportion of these environmental gradients (relative to range-wide variation) for IBE patterns to be evident, with devils throughout our study area occupying (and preferring) relatively similar habitats.

In contrast to devils, we did detect evidence of IBE in tumours. Specifically, we found a significant positive correlation between elevation difference and genetic distance of tumours. When analysing tumour clusters separately, we found that this effect was entirely driven by cluster 2, with genetic differentiation among cluster 1 lacking any significant association with any tested environmental factor, including geographic distance. We believe this effect of elevation on genetic differentiation among cluster 2 is most likely a result of population structure that is coincident with an elevation gradient. As we discussed above, a large proportion of cluster 2 tumours were sampled from Narawntapu National Park. Narawntapu is effectively at sea level, whereas the majority of tumours were sampled from devil populations in higher elevation areas. Thus, genetic differentiation among these groups owing to geographic isolation of Narawntapu tumours would be expected to produce an isolation-by-elevation effect. Although it is possible that some innate biological characteristic of the tumour may instead be driving this effect, the mechanism by which this would occur is unclear. Potentially, climatic differences (e.g. temperature, moisture) among high- and low-elevation areas could influence the ability of tumour cells to successfully implant in uninfected devils, driving local adaptation of tumours in these regions, although this has yet to be demonstrated. Alternatively, differences in local landscape characteristics and thus devil densities may change the frequency of biting interactions among devils that in turn alters the DFTD transmission rate. However, existing studies of devil interactions provide no support for this explanation, with biting behaviours tied to interactions that devils seek out regardless of landscape structure (Hamede et al., 2009, 2013; Hamilton et al., 2019).

5 | CONCLUSIONS

Comparative landscape genetics studies facilitate identification of patterns of connectivity that are common to multiple species. However, use of host gene flow estimates as a proxy for pathogen transmission and spread can lead to erroneous conclusions in cases of incongruent host and pathogen genetic structure (Kozakiewicz et al., 2018; Mazé-Guilmo et al., 2016). We have shown that host

population structure, and the landscape features that influence it, is decoupled from that of their pathogens. Roads, which were found to constrain devil gene flow significantly, were not associated with DFTD transmission, suggesting that genetic studies of devils are insufficient to infer or predict the spatial spread of tumours. This decoupling of host and pathogen likely occurred due to a mismatch between dispersal life stage and the stage at which devils typically carry and transmit DFTD. In other systems, external ecological factors such as multiple host/vector species or transmission via the environment are also known to mediate host-pathogen interactions (Näpflin et al., 2019; Witsenburg et al., 2015). Such ecological factors are amenable to inclusion in a comparative landscape genetics framework, emphasizing the value of comparative landscape genetics studies in host-pathogen systems where the dynamics of host dispersal and pathogen transmission may differ. Examples include sarcoptic mange, which infects various mammal species and can be transmitted environmentally (Martin, Ricardo, et al., 2019; Niedringhaus, Brown, Sweeley, & Yabsley, 2019), or pathogens requiring arthropod vectors, such as *Plasmodium* spp. (malaria; Lo et al., 2017), whereby disease spread relies on multiple species and is strongly mediated by the environment (Hemming-Schroeder et al., 2018; Schwabl et al., 2017).

Despite the apparent decoupling of host and pathogen gene flow herein, host connectivity generally plays a significant role in wildlife disease dynamics. Higher connectivity among habitat patches and increased host movements increase rates of pathogen spread, prevalence and persistence in the landscape (Becker, Snedden, Altizer, & Hall, 2018; Wilber, Johnson, & Briggs, 2020). However, wildlife populations themselves benefit similarly from increased connectivity, which is critical for maintaining genetic diversity and facilitating demographic rescue (Brown & Kodric-Brown, 1977; Keyghobadi, 2007; Whiteley, Fitzpatrick, Funk, & Tallmon, 2015). Thus, management of the landscape to isolate and constrain the spread of disease must be balanced against the need to maintain genetic and demographic exchange among wildlife populations (McCallum & Dobson, 2002). In the light of increasing threats owing to habitat loss and wildlife disease globally (Haddad et al., 2015; Jones et al., 2008), this trade-off has become a major conundrum for wildlife managers. Any interventions should therefore proceed with caution and can benefit from comparative landscape genetics studies to help consider the impact of alternative management strategies.

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AUTHOR CONTRIBUTIONS

CPK conducted all statistical and spatial analyses, prepared figures and wrote the manuscript with contributions from all authors. LR led the initial study design and conducted preliminary analyses. LR,

ARS and SAH performed laboratory work. MJM performed all bioinformatics. AHP provided conceptual advice and contributed to the study design. MRA, DGH and RH collected samples. HM, MEJ, PAH and AS conceived of and oversaw the project.

DATA AVAILABILITY STATEMENT

Devil and tumour sequence data have been deposited under NCBI BioProjects PRJNA306495, PRJNA634071 and PRJNA641648. Sample Accession nos and sample locations are provided in the Supporting Information.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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