

## ORIGINAL ARTICLE

## Evidence that disease-induced population decline changes genetic structure and alters dispersal patterns in the Tasmanian devil

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Infectious disease has been shown to be a major cause of population declines in wild animals. However, there remains little empirical evidence on the genetic consequences of disease-mediated population declines, or how such perturbations might affect demographic processes such as dispersal. Devil facial tumour disease (DFTD) has resulted in the rapid decline of the Tasmanian devil, *Sarcophilus harrisii*, and threatens to cause extinction. Using 10 microsatellite DNA markers, we compared genetic diversity and structure before and after DFTD outbreaks in three Tasmanian devil populations to assess the genetic consequences of disease-induced population decline. We also used both genetic and demographic data to investigate dispersal patterns in Tasmanian devils along the east coast of Tasmania. We observed a significant increase in inbreeding ( $F_{IS}$  pre/post-disease  $-0.030/0.012$ ,  $P < 0.05$ ; relatedness pre/post-disease  $0.011/0.038$ ,  $P = 0.06$ ) in devil populations after just 2–3 generations of disease arrival,

but no detectable change in genetic diversity. Furthermore, although there was no subdivision apparent among pre-disease populations ( $\theta = 0.005$ , 95% confidence interval (CI)  $-0.003$  to  $0.017$ ), we found significant genetic differentiation among populations post-disease ( $\theta = 0.020$ ,  $0.010$ – $0.027$ ), apparently driven by a combination of selection and altered dispersal patterns of females in disease-affected populations. We also show that dispersal is male-biased in devils and that dispersal distances follow a typical leptokurtic distribution. Our results show that disease can result in genetic and demographic changes in host populations over few generations and short time scales. Ongoing management of Tasmanian devils must now attempt to maintain genetic variability in this species through actions designed to reverse the detrimental effects of inbreeding and subdivision in disease-affected populations.

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## Introduction

Infectious diseases are increasingly recognized as important drivers of population declines, and even extinctions (de Castro and Bolker, 2005; Smith *et al.*, 2006; Pedersen *et al.*, 2007). Rapid declines in population size can lead to a loss of genetic diversity and elevated levels of inbreeding, resulting in lowered fitness, reduced adaptive potential and increased probability of extinction (Gilpin and Soulé, 1986). However, despite the rise in emerging infectious diseases worldwide (Jones *et al.*, 2008a) and their potential to negatively impact the genetic viability of host populations, there are still few studies investigating the genetic consequences of epizootics for host populations (Trudeau *et al.*, 2004; Valsecchi *et al.*, 2004; Teacher *et al.*, 2009).

Populations in decline are predicted to lose genetic diversity by both a loss of allelic diversity and a reduction in heterozygosity. The former is predicted to

occur first, because rare alleles that contribute little to heterozygosity are more easily lost during population size reductions (Nei *et al.*, 1975; Cornuet and Luikart, 1996). The few empirical studies investigating the genetic consequences of disease-induced bottlenecks reveal that although some populations do lose genetic diversity following disease impacts (Trudeau *et al.*, 2004; Foster *et al.*, 2007), others do not (Queney *et al.*, 2000; Teacher *et al.*, 2009); this result has also been found for populations that have undergone bottlenecks not associated with disease (Carson, 1990; Flagstad *et al.*, 2003; Hailer *et al.*, 2006). Indeed, the impact of a population size reduction on genetic diversity depends on the intensity of the perturbation, the length of time before recovery and the rate of recovery to original population numbers (England *et al.*, 2003). Formulating generalized predictions regarding the consequences of infectious disease epidemics on host genetic diversity is therefore difficult, and further studies are needed to elucidate the extent to which they negatively impact host genetic diversity.

Another likely consequence of population decline is a change in dispersal patterns, because behavioural mechanisms driving dispersal decisions are influenced

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by population density (Greenwood, 1980; Travis *et al.*, 1999). As population density declines, decreased dispersal may result, thereby decreasing gene flow among sub-populations, leading to loss of genetic diversity through genetic drift (Bohonak, 1999; Busch *et al.*, 2009). Alternatively, an increase in dispersal rates at low population densities may not only counteract the effects of genetic drift but can also swamp local genetic adaptation (Slatkin, 1987; Pope *et al.*, 2007). Because dispersing individuals can be a significant factor in the spread of infectious diseases (Hosseini *et al.*, 2006), and because disease management strategies often involve population perturbation (for example, culling regimes; Wobeser, 2002), understanding the scale of dispersal and the relationship of dispersal patterns to population density is important for conserving species threatened by disease (Donnelly *et al.*, 2003). However, inherent difficulties in obtaining temporally stratified genetic samples both before and after disease have resulted in a dearth of studies of the consequences of disease epidemics for dispersal patterns (Nunn *et al.*, 2008).

The Tasmanian devil (*Sarcophilus harrisii*), the largest carnivorous marsupial, is threatened with extinction as a result of an epidemic of an infectious cancer, devil facial tumour disease (DFTD) (McCallum *et al.*, 2007). The tumour is an infectious cell line, thought to be transmitted between individuals by biting, that is able to elude the immune system because of very low genetic diversity at the functional major histocompatibility complex gene complex associated with tumour recognition (Siddle *et al.*, 2007). This consistently fatal disease leads to altered population age structures and major declines in population size (Hawkins *et al.*, 2006; Lachish *et al.*, 2007; Jones *et al.*, 2008b; McCallum *et al.*, 2009). Thus, DFTD has the potential to jeopardize severely the genetic viability of affected populations.

In this study, we used microsatellite DNA data to estimate the impact of an infectious disease epidemic on genetic diversity, genetic structure and dispersal patterns in Tasmanian devils. We examined whether DFTD-driven population decline has reduced genetic diversity in Tasmanian devil populations. In addition, we investigated whether altered dispersal rates of devils in low-density, disease-affected populations have resulted in changes to gene flow and population genetic structure. We also used a combination of individual-based genetic analyses and available demographic records of dispersal events to investigate dispersal patterns and estimate average dispersal distance in Tasmanian devils along the east coast of Tasmania.

## Methods

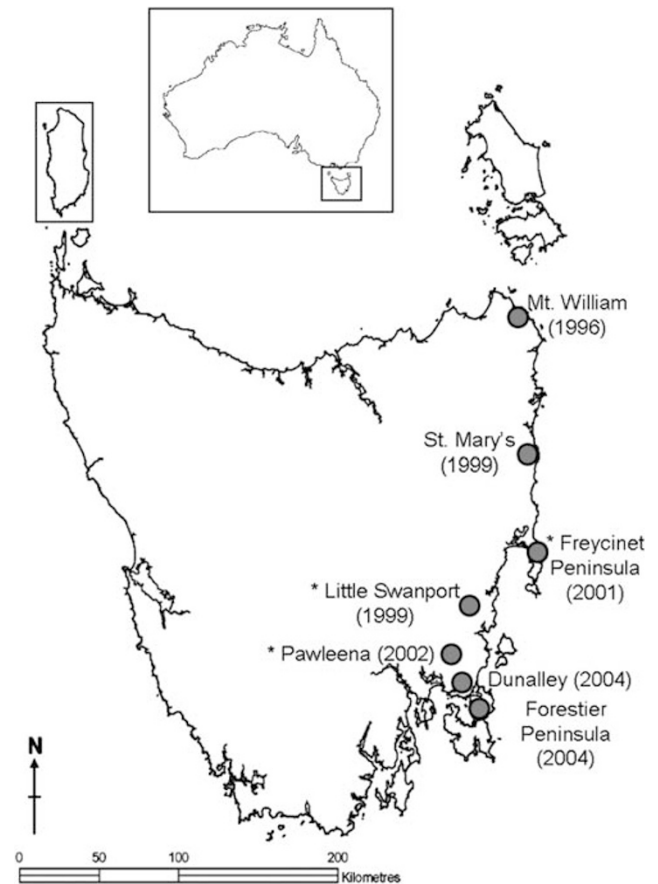
### Study sites and sample collection

From October 2005 to July 2007, we sampled a total of 309 adult devils (devils aged 2 years and older) from seven sites ( $n = 22\text{--}69$  per site; Table 1) spanning 230 km along the east coast of Tasmania (Figure 1). Ear tissue biopsies were taken from trapped animals (see Hawkins *et al.*, 2006 for trapping protocols) and the location of each trapped animal recorded using a GPS. The coastal area sampled is bounded to the west by the Eastern Tiers range (800 m) and dominated by dry sclerophyll forests and coastal heath interspersed with grazing/agricultural

**Table 1** Genetic differentiation ( $F_{ST}$ ) among populations of Tasmanian devils on the east coast of Tasmania

Locus	Pre-disease	Post-disease	East Coast
Sh2i	<i>(0.205)</i>	-0.006	0.019
Sh2g	0.006	0.027	0.078
Sh2v	-0.003	0.029	0.034
Sh2p	0.006	<i>(0.096)</i>	<i>(0.122)</i>
Sh3a	-0.009	0.018	0.041
Sh3o	0.002	0.036	0.052
Sh6l	0.015	0.011	0.002
Sh2l	-0.005	-0.007	0.009
Sh5c	-0.007	0.018	0.022
Sh6e	0.039	-0.002	0.002
$F_{ST}$	0.005	0.020	0.033
	(-0.003-0.017)	(0.010-0.027)**	(0.015-0.048)**

Loci found to be under selection are shown italicized and in parentheses.  $F_{ST}$  gives the overall  $F_{ST}$  (with 95% confidence interval) calculated with both the loci identified as being under selection excluded (see Methods and Results sections). \*\*Denotes significant genetic differentiation at  $P < 0.001$ .



**Figure 1** Map showing the island of Tasmania in relation to Australia and the location of the genetic sampling sites. The years shown in parentheses next to each site indicate approximate arrival year of devil facial tumour disease at each site. The three populations marked by an asterisk were used in the pre- and post-disease analyses.

land. It represents a linear stretch of continuous habitat for devils with no major barriers to movement (no large rivers, mountain ranges, major roads or large cities).

DFTD was present in all seven locations during our study, although the estimated time of disease arrival at each site varied from 2 to 10 years before sampling (Figure 1; Hawkins *et al.*, 2006). We refer to all these seven east coast sites that we sampled post-disease as 'East Coast'.

For three of these seven East Coast sites (Freyrcinet, FNP; Little Swanport, LS; and Pawleena, PA; Figure 1), we had existing genetic data from samples collected in 1999 (Jones *et al.*, 2004), which we used to directly compare genetic diversity before and after the onset of the disease outbreak. Substantial trapping throughout 1999–2000 at FNP and PA confirmed that they were disease-free at this time (Hawkins *et al.*, 2006; Lachish *et al.*, 2007). Although disease was likely present at very low prevalence in 1999 at LS (first unconfirmed DFTD case in late 1999, Hawkins *et al.*, 2006), population density was high and the population age structure (which changes markedly after disease arrival, Lachish *et al.*, 2009) was intact (MEJ, unpublished data). The FNP population of devils has declined >70% since disease arrival (Lachish *et al.*, 2007) with similar, or greater, declines recorded state-wide in all disease-affected devil populations, including LS and PA (Hawkins *et al.*, 2006; McCallum *et al.*, 2007; MEJ, unpublished data). For simplicity, we refer to the 1999 data for these three sites as 'pre-disease' and the 2005–2007 data for these three sites as 'post-disease', even though disease was still present at all three sites in 2005–2007. Pre-disease and post-disease samples constitute different generations because devils live a maximum of 6 years.

#### Laboratory methods

Genomic DNA was extracted from ear tissue biopsies stored at  $-20^{\circ}\text{C}$  in 70% ethanol by the Hot-SHOT method (Truett *et al.*, 2000). Genetic analyses were conducted using 10 microsatellite loci (Jones *et al.*, 2003; Sh6b excluded). PCR amplifications contained 2.5 mM  $\text{MgCl}_2$  (Promega, Sydney, Australia), 1.5  $\mu\text{M}$  forward primer, 3.75 nM reverse primer, 0.2 mM of each dNTP, PCR buffer (670 mM Tris-HCL, pH8.8, 4.5% Triton X-100, 166 mM  $(\text{NH}_4)_2\text{SO}_4$ , 2 mg  $\text{ml}^{-1}$  gelatin), 0.5 U Promega *Taq* and 1  $\mu\text{l}$  ( $\sim 50$  ng) of DNA. Reaction conditions were as described by Jones *et al.* (2003). PCR products were screened concurrently in three groups as follows: (a) Sh3o/Sh3a/Sh6l, (b) Sh6e/Sh5c/Sh2l, (c) Sh2g/Sh2v/Sh2i/Sh2p. Forward primers were labelled with WellRED fluorescent dyes (D2, D3 or D4, Sigma-Proligo, Sydney, Australia) and genotypes determined by comparing products against a size standard (D1 dye) using a Beckman-Coulter CEQ8000XL sequencer (Galesville, Australia). We tested for the presence of null alleles at all loci using MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.*, 2004).

To compare our data directly with that obtained by Jones *et al.* (2004) using polyacrylamide-gel technology (ABI-373, Applied Biosystems, Mulgrave, Australia), 39 of the samples collected in 1999 (representing at least three replicates of all previously recorded alleles) were re-amplified and genotyped on the Beckman sequencer (Farmer, 2006). All replicate alleles amplified consistently and allele spacings were identical between the two systems. On the basis of these comparisons, we adjusted our allele scoring to match the original data set by calling

allele sizes as follows: Sh2i/-6 bases; Sh2g/-6 bases; Sh2v/-8 bases; Sh3o/-6 bases; Sh5c/-7 bases; Sh6e/-7 bases; Sh6l/-4 bases; Sh2l/-6 bases; Sh2p/-5 bases; Sh3a/-5 bases.

#### Data analysis

Tests for linkage disequilibrium and Hardy–Weinberg equilibrium (HWE) were conducted on data for all East Coast sites and for all loci using exact tests (Guo and Thompson, 1992) implemented in the program GENEPOP 4.0 (Raymond and Rousset, 1995), using the sequential Bonferroni correction to account for multiple-related tests (Rice, 1989). Single- and multi-locus  $F_{IS}$  (Wright's fixation index,  $f$ ) values were calculated using FSTAT (available from <http://www2.unil.ch/popgen/softwares/fstat.htm>) (Goudet, 1995) to determine if departures from HWE represented heterozygote deficiency or excess. Genetic diversity measures for all sites including the mean number of alleles per locus, allelic richness (the number of alleles per locus standardized for differences in sample size), observed and expected heterozygosity were calculated using FSTAT (Goudet, 1995). We assessed the possibility that microsatellite markers were sex-linked by comparing allele frequencies between males and females.

#### Disease impacts on population subdivision

To understand changes in genetic structuring following disease impacts, we estimated the level of differentiation among the three pre-disease and post-disease sites and among all seven East Coast sites using estimates of  $F_{ST}$  (which was calculated as  $\theta$ , an unbiased estimator of  $F_{ST}$ ; Weir and Cockerham, 1984). Because  $F_{ST}$  values are correlated with heterozygosity levels, outliers from this relationship can indicate either directional ( $F_{ST}$  higher than expected) or balancing selection ( $F_{ST}$  lower than expected; Beaumont and Nichols, 1996). We tested this relationship for each locus in all three data sets (pre-disease, post-disease and East Coast sites) using  $F_{dist}$  (Beaumont and Nichols, 1996) as implemented through LOSITAN (with 20 000 simulations, 95% confidence interval (CI), and the neutral and forced-mean  $F_{ST}$  options, Antao *et al.*, 2008). Mean overall  $F_{ST}$  was calculated by jack-knifing over loci with departure from panmixia tested using bootstrap 95% CI in FSTAT (Goudet, 1995). The inclusion of non-neutral loci can bias the calculation of  $F_{ST}$  values, genetic diversity measures and effective population sizes. Accordingly, the two loci found to show signs of being under selection (see Results) were omitted from analyses of population differentiation, comparisons between groups, M-ratios and the temporal method (for  $N_e$  estimation, see below). Estimates of dispersal patterns (spatial autocorrelation analyses and isolation-by-distance (IBD) regression analyses; see below) were not affected whether the two loci potentially under selection were included or excluded.

#### Disease impacts on genetic diversity

Changes in genetic diversity pre- and post-disease at FNP, LS and PA were assessed using the FSTAT permutation analysis for comparison between groups (Goudet, 1995, with 10 000 permutations) on measures of allelic richness, observed heterozygosity ( $H_O$ ), unbiased



expected heterozygosity ( $H_E$ ),  $F_{IS}$  and relatedness (Rel). These measures of genetic diversity were also used to assess genetic diversity among East Coast sites (using analysis of variance, performed in R version 2.7.1) with significance of  $F_{IS}$  within each site determined by permutation in FSTAT.

We tested for reductions in population size and loss of genetic diversity by examining changes to the ratios of allelic number to allelic size range (M-ratios; Garza and Williamson, 2001) in the pre- and post-disease populations. The value of M decreases when a population is reduced in size because the number of alleles should decline faster than the allelic size range during a population reduction (Garza and Williamson, 2001). An M-value  $< 0.68$  indicates a bottleneck (for a data set using  $> 7$  loci; Garza and Williamson, 2001). We further tested for a genetic signature of population decline in the three populations sampled pre- and post-disease by the 'temporal method', which tests for small  $N_e$  based on the changes in the variance of allele frequencies between two samples (Waples, 1989; Luikart *et al.*, 1998). As the generation time of devils is 2 years (Lachish *et al.*, 2009), we calculated  $N_e$  assuming that either two or three generations had passed between disease arrival and post-disease sampling. We considered the population to have undergone a bottleneck if the estimated  $N_e$  was  $< 28$ , which was the critical value when using  $> 5$  microsatellite markers and sampling 30 individuals before and after the bottleneck (Luikart and Cornuet, 1998; Luikart *et al.*, 1998).

#### Disease impacts on dispersal patterns

Changes to dispersal patterns following disease impacts were inferred from patterns of spatial genetic structure in two ways. First, we performed multiple-distance class spatial autocorrelation analyses (with 10 km distance classes) for all individuals and for females and males separately in the pre- and post-disease populations, using GENALEX 6.2 (see Peakall *et al.*, 2003; Double *et al.*, 2005; Peakall and Smouse, 2005 for full description). Second, because changes to dispersal patterns will affect patterns of IBD (Leblois *et al.*, 2004), we examined changes in the strength of IBD across the three populations based on all individuals, and on males and females separately, by regressing  $\hat{a}$  on pairwise inter-individual log-transformed geographical distances and retaining the slope parameter,  $b$ , from each regression (performed in Excel, as described by Knight *et al.*, 1999; Stow *et al.*, 2001). A randomization test (10000 iterations) was used to determine if there was a significant difference in the mean  $b$  (strength of IBD) of each group pre- and post-disease (Hood, 2006).

#### Dispersal patterns and genetic estimate of dispersal distance

To estimate the spatial scale of dispersal and neighbourhood size across East Coast sites, we used a global spatial autocorrelation analysis with variable distance classes (5 km classes to 110 km; 20 km classes beyond that) to account for distances of between 0 and 238 km, using GENALEX6.2 (Peakall and Smouse, 2005). Significance of the global correlogram was determined with a non-parametric heterogeneity test (Smouse *et al.*, 2008). As detection of significant structure is influenced by the

range of distances over which autocorrelation is calculated (Peakall *et al.*, 2003), we also performed a multiple-distance class analysis for all individuals across the seven sites. Fine-scale genetic structuring was assessed by conducting spatial autocorrelation analyses within each of the seven East Coast sites, with distance intervals of 3 km.

Because the spatial scale over which IBD develops is proportional to the scale of gene flow (Rousset, 1997), the relationship (linear regression) between pairwise genetic and geographical distances of individuals may be used to infer information on individual dispersal distance ( $\sigma$ ; see Rousset, 2000). In general, the inverse of the slope ( $b$ ) of this regression provides an estimate of the product  $4\pi D\sigma^2$ , where  $D$  is the effective population density (Broquet *et al.*, 2006). GENEPOP (Raymond and Rousset, 1995) was used to calculate  $b$  from the regression of  $\hat{a}$  against pairwise log-transformed geographical distance for all individuals and to compute a 95% CI for  $b$  using the ABC bootstrap procedure. This analysis was repeated using the statistic  $\hat{e}$ , to obtain a more robust upper bound to our estimate of neighbourhood size, as recommended by Watts and Rousset (2007). To estimate effective density ( $D$ ), the area of each site was calculated as the total area within the polygon demarcated by the most extreme trap locations plus a 2 km buffer (approximate radius of a devil home range) added to all land boundaries. Population size estimates were obtained for each sampling trip made to each site within the period of genetic sampling using closed population mark-recapture models in program CAPTURE (see Hawkins *et al.*, 2006 for trapping protocols and statistical details; Model Mth, Otis *et al.*, 1978). To obtain estimates of effective population size, we multiplied our mark-recapture population estimates by 0.11; the average ratio of  $N_e/N$  for wild populations reported by Frankham (1995) in his survey of more than 100 species. Effective population densities (effective population size/area) for each trip at each site were averaged to obtain an estimate of effective population density across all seven East Coast sites.

Sex-biased dispersal should result in stronger spatial autocorrelation (Peakall *et al.*, 2003), greater genetic distances, as well as stronger IBD for the more philopatric sex (Handley and Perrin, 2007). We tested for evidence of sex-biased dispersal in Tasmanian devils in three ways, on the basis of the data from the East Coast sites. First, global spatial autocorrelation analyses were performed as described above, but for males and females separately. Second, Mantel tests (10000 simulations) were used to test for congruence between matrices of pairwise genetic distance and pairwise log-transformed geographical distance for male-male and female-female pairs. Third, the slope parameter,  $b$ , was obtained from separate regressions of  $\hat{a}$  on pairwise inter-individual log-transformed geographical distances calculated per individual for all female-female pairs and all male-male pairs (see description above and Knight *et al.*, 1999; Stow *et al.*, 2001). Randomization (10000 iterations) was used to test the assumption of no difference between mean  $b$  for females and males (Hood, 2006).

#### Direct estimate of dispersal

As part of the Tasmanian government's extensive disease monitoring program, intensive surveys have been con-

ducted across the entirety of the devil's range (60 sites surveyed an average of 3.2 times each). To obtain a direct estimate of the dispersal distance of devils, we searched this database of live devil captures for all individuals captured at multiple survey sites. We determined the distance 'moved' by these individuals as the straightline distances between their furthest two capture locations. These movements were considered dispersal events if the individual was a juvenile or sub-adult at its first capture.

## Results

There was no evidence for genotyping errors or null alleles at any locus, nor was there evidence of linkage disequilibrium between any pair of loci. All sites appeared to be largely in HWE. Only three of the 70 locus  $\times$  site tests for departures from HWE were significant following Bonferroni correction: two due to heterozygote excesses (Sh2v;  $f = -0.301$  and Sh2p;  $f = -0.601$  at Mt William) and one due to heterozygote deficiency (Sh2p;  $f = 0.320$  at LS). No loci showed evidence of sex linkage (allele frequencies for all loci differed by less than 10% between males and females).

### Population subdivision pre- and post-disease

Two loci were identified as candidates for directional selection ( $F_{ST}$  values greater than the upper 0.025% confidence limit of the simulated null distribution) and were omitted from analyses. These were locus Sh2i in the pre-disease populations ( $P = 0.014$ ) and locus Sh2p in both the post-disease populations ( $P = 0.061$ ) and in all seven East Coast sites ( $P = 0.015$ ) (Table 1). Investigating genetic differentiation pre- and post-disease revealed that, before DFTD outbreak and population decline, there was no evidence of genetic subdivision among the FNP, LS and PA sites ( $F_{ST} = 0.005$ , 95% CI  $-0.003$  to  $0.017$ ,  $P = 0.328$ , Table 1). However, in as few as 4 years following disease arrival, significant subdivision has developed among the three sites ( $F_{ST} = 0.020$ , 95% CI

$0.010$ – $0.027$ ,  $P = 0.0001$ , Table 1). Significant overall genetic differentiation was also found across all seven East Coast sites (Table 1).

### Disease impacts on genetic diversity

Measures of genetic diversity were low for all sites (Table 2, see Supplementary material for allele frequencies and genetic diversity measures per locus). We found a significant increase in  $F_{IS}$  post-disease and a trend towards increased relatedness between individuals post-disease, consistent with higher levels of inbreeding following disease-induced demographic decline (Table 3). There were no significant differences in allelic richness, observed heterozygosity or expected heterozygosity between the pre- and post-disease populations (Table 3). Nevertheless, all  $M$ -ratios pre- and post-disease were below the critical value of 0.68 and all declined post-disease (LS pre-/post =  $0.600/0.579$ ; FNP  $0.648/0.639$ ; PA  $0.593/0.532$ ), suggesting that disease-driven population decline may be contributing to the loss of genetic diversity in these three populations. Low initial values of  $M$ -ratios in pre-disease devil populations are consistent with founder effects or past population bottlenecks (Jones *et al.*, 2004). The temporal method revealed no

**Table 3** Results of FSTAT permutation analysis examining changes in genetic diversity measures at three Tasmanian devil populations sampled pre- and post-disease

Genetic diversity measure <sup>a</sup>	Pre-disease	Post-disease	P (10 000 permutations)
AR	3.185	3.139	0.830
H <sub>O</sub>	0.478	0.497	0.897
H <sub>E</sub>	0.464	0.463	0.999
F <sub>IS</sub>	-0.030	0.012	0.044
Rel	0.011	0.038	0.064

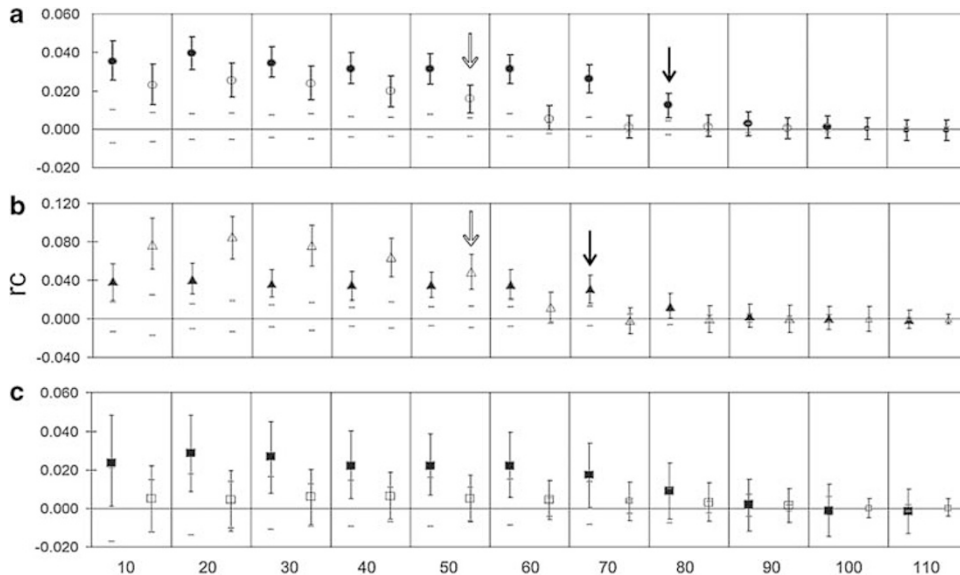
Abbreviations: AR, allelic richness; F<sub>IS</sub>, inbreeding coefficient; H<sub>O</sub>, observed heterozygosity; H<sub>E</sub>, unbiased expected heterozygosity; Rel, relatedness.

<sup>a</sup>Non-neutral loci removed (see Methods and Results sections).

**Table 2** Genetic diversity of Tasmanian devils at three pre-disease locations and the seven post-disease East Coast sites calculated using 10 microsatellite markers

	Years	N	# M/F	A	AR	H <sub>O</sub>	H <sub>E</sub>	F <sub>IS</sub>
<i>Pre-disease sites</i>								
Little Swanport (LS)	1999	41	19/22	3.3	3.231	0.428	0.427	-0.006
Freycinet Peninsula (FNP)	1999	70	32/38	3.5	3.022	0.443	0.427	-0.034
Pawleena (PA)	1999	35	17/18	3.3	3.301	0.421	0.425	0.009
<i>All three pre-disease sites</i>		146	68/78	3.4	3.243	0.433	0.426	-0.018
<i>East coast sites (post-disease)</i>								
Little Swanport (LS)	2006/7	55	32/23	3.3	3.031	0.436	0.446	0.035
Freycinet Peninsula (FNP)	2006/7	69	36/33	3.7	3.228	0.404	0.420	0.031
Pawleena (PA)	2006	22	13/9	3.0	3.010	0.405	0.415	0.026
<i>All three post-disease sites</i>		146	81/65	3.3	3.086	0.414	0.428	0.031
Dunalley (DY)	2006/7	35	15/20	3.2	2.981	0.400	0.394	-0.028
Forestier Peninsula (FO)	2006/7	57	25/32	2.9	2.673	0.334	0.343	0.029
Mt William (MW)	2005/6	38	20/18	2.7	2.623	0.482	0.431	-0.119*
St Marys (SM)	2006	33	18/15	2.9	2.773	0.361	0.388	0.073
<i>All seven East Coast Sites</i>		309	159/150	3.8	2.901	0.403	0.405	0.011

Parameters shown are the years when genetic samples were collected, the number of individuals genotyped (N), the number of males and females genotyped (# M/F), the mean number of alleles per locus (A), allelic richness (AR), observed heterozygosity (H<sub>O</sub>), unbiased expected heterozygosity (H<sub>E</sub>) and multi-locus F<sub>IS</sub> (\* $P < 0.01$ ).



**Figure 2** Correlogram plots of spatial genetic autocorrelation in Tasmanian devils sampled at three sites on the east coast of Tasmania showing the combined correlation coefficient ( $r_c$ ) as a function of increasing distance for (a) all individuals pre- (●) and post- (○) disease; (b) all females pre- (▲) and post- (△) disease; and (c) all males pre- (■) and post- (□) disease. Error bars indicate 95% CI as determined by bootstrapping, and dashed lines show the permuted 95% CI for the null hypothesis of no genetic structure. Arrows indicate the extent of detectable (significant) positive genetic structure in the pre-disease (solid arrow) and post-disease (open arrow) populations.

indication of recent population bottlenecks having occurred at any of the three populations in response to disease-induced population decline, assuming either two or three generations between samples (two generations: LS  $N_e = 46.68$ ; FNP  $N_e = 79.432$ ; PA  $N_e = 45.15$ , three generations: LS  $N_e = 96.08$ ; FNP  $N_e = 119.01$ ; PA  $N_e = 67.28$ ). There were no significant differences in genetic diversity among the seven East Coast sites (mean number alleles,  $F_{(6,63)} = 0.57$ ,  $P = 0.75$ ; allelic richness,  $F_{(6,63)} = 0.36$ ,  $P = 0.90$ ; observed heterozygosity,  $F_{(6,63)} = 0.32$ ,  $P = 0.91$ ; expected heterozygosity,  $F_{(6,63)} = 0.28$ ,  $P = 0.94$ ).

#### Changes to dispersal patterns following disease impacts

The establishment of DFTD appears to have had a marked effect on dispersal of individuals. We observed a 37% reduction in the spatial extent of positive (non-random) genetic structure following disease (from 80 to 50 km, Figure 2a). This appeared to be largely driven by changes in female dispersal. The genetic similarity of females within 30 km of each other increased significantly post-disease, and the spatial extent of detectable positive genetic structure for females was significantly reduced post-disease (Figure 2b). No such pattern was seen for males (Figure 2c). A reduction in dispersal rate/distance of females post-disease was also supported by the significantly steeper regression slope parameters for females post-disease (Table 4). Although this parameter did not change significantly for males post-disease, the trend was in the same direction as observed for females (Table 4).

#### Dispersal patterns and genetic estimate of dispersal distance in Tasmanian devils

The global spatial autocorrelation analysis revealed significant positive genetic structure along the East Coast (Heterogeneity test;  $\omega = 237.08$ ,  $P < 0.001$ , Figure 3a). Individuals within 20 km of each other were more

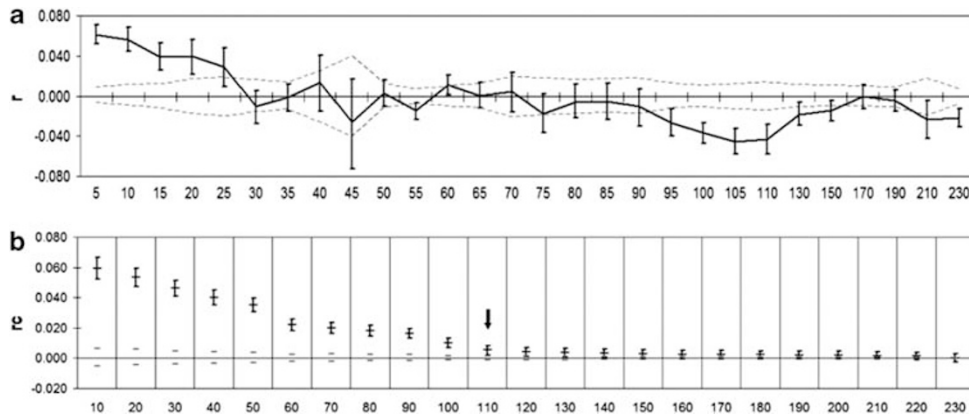
**Table 4** Results of two-sample randomization tests showing differences in the strength of isolation-by-distance among Tasmanian devils pre- and post-disease

	Pre-disease		Post-disease		Diff	P
	b	s.d.	b	s.d.		
All individuals	0.010	0.013	0.009	0.019	0.001	0.166
Females	0.012	0.017	0.023	0.036	0.012	0.007
Males	0.006	0.013	0.007	0.014	0.001	0.186

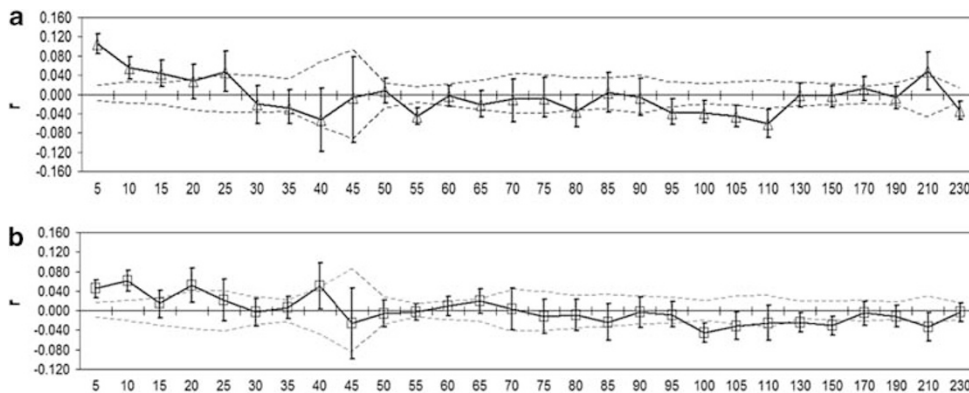
Abbreviations: *b*, the mean slope parameter estimated from all individual regressions of pairwise genetic distance on pairwise geographic distance; *diff*, absolute difference between means.

genetically similar than any two random individuals, with the first x-intercept of the correlogram indicating a genetic patch size of 28.7 km (Figure 3a). The true spatial extent of genetic structuring in Tasmanian devils extends to 110 km (interestingly, also the furthest dispersal distance recorded from trapping, see below) with the genetic similarity between individuals decreasing with increasing distance, indicating geographically restricted non-random dispersal (Figure 3b). Spatial autocorrelation analyses within East Coast sites revealed no evidence of fine-scale genetic structuring in any of the East Coast sites (results not shown).

We detected a distinct genetic signature of male-biased dispersal in this study. Although males and females both showed evidence of spatial genetic structuring ( $\omega_{\text{females}} = 217.20$ ,  $P < 0.001$ ;  $\omega_{\text{males}} = 147.94$ ,  $P < 0.001$ ), females within 5 km of each other were genetically more similar than were males within this distance (Figures 4a and b). Furthermore, IBD patterns were stronger in females than males, as revealed by Mantel tests (Table 5a) and slope parameters (Table 5b). IBD was also apparent among individuals across the seven East Coast sites ( $r = 0.72$ ,  $P = 0.022$ ). The slope, *b*, of the regression of  $\hat{a}$



**Figure 3** Spatial genetic structure in Tasmanian devils sampled at seven sites on the east coast of Tasmania showing (a) correlogram plot of the correlation coefficient for all individuals along the East Coast as a function of distance; (b) the combined correlation coefficient of all individuals as a function of increasing distance. Error bars indicate 95% CI for  $r$  or  $rc$  as determined by bootstrapping, and dashed lines show the permuted 95% CI for the null hypothesis of no genetic structure. The arrow indicates the extent of detectable (significant) positive genetic structure.



**Figure 4** Correlogram plots of spatial genetic autocorrelation in Tasmanian devils sampled at seven sites on the east coast of Tasmania showing the correlation coefficient for (a) all females ( $\Delta$ ) and (b) all males ( $\square$ ) along the East Coast as a function of distance. Error bars indicate 95% CI as determined by bootstrapping, and dashed lines show the permuted 95% CI for the null hypothesis of no genetic structure.

**Table 5** Results of tests to infer sex-biased dispersal patterns in Tasmanian devils: (a) Mantel tests of the correlation ( $r$ ) between pairwise genetic and (logarithmic) geographical distances for females and males; (b) two-sample randomization test of the difference in strength of isolation-by-distance (IBD) in females and males

	$r$	$P$			
<i>(a) Mantel tests</i>					
Females	0.112	<0.001			
Males	0.058	0.004			
	$b$	$s.d.$	$Diff$	$P$	
<i>(b) Strength of IBD test</i>					
Females	0.014	0.021			
Males	0.008	0.017	0.005	0.015	

Abbreviations:  $b$ , the mean slope parameter estimated from all individual regressions of pairwise genetic distance on pairwise geographic distance;  $diff$ , absolute difference between means.

versus log geographic distance was estimated as 0.01 (95% CI 0.005–0.019). This translates to a neighbourhood size ( $4\pi D\sigma^2$ ) of 97 individuals (95% CI 53–184 indi-

viduals). The estimate of effective density across the East Coast was 0.036 individuals  $\text{km}^{-2}$  (range: 0.007–0.113 individual  $\text{km}^{-2}$ ) giving an indirect estimate of mean dispersal distance ( $\sigma$ ) in devils of 14.18 km (95% CI 10.80–20.16 km).

#### Direct estimate of dispersal in Tasmanian devils

Interrogation of the statewide disease-monitoring database revealed only 15 records of dispersal events (sub-adult or juvenile individuals moving between survey sites) in more than 76 000 trap-nights involving 3155 individuals (only two records of adults moving between sites were found). These seven females and eight males dispersed on average 30.28 km (mean distance males =  $20.27 \pm 7.37$  km (95% CI); females =  $42.03 \pm 28.08$  km), with a broad range of dispersal distances recorded (12.5–109.3 km). This direct demographic dispersal estimate is approximately twice that obtained by the indirect genetic method, although the median dispersal distance (18.98 km, which places less emphasis on the single long-distance dispersal event recorded) does lie within the 95% CI of the indirect estimate.



## Discussion

Using temporally replicated spatial genetic data, we report that population decline following an epidemic of an emerging infectious disease is affecting population genetic structure in Tasmanian devils. Despite the relatively few generations since the establishment of DFTD in Tasmanian devil populations, there is evidence of increased inbreeding, greater population genetic differentiation, changing selection regimes and reduction in the dispersal movements of females. Recent studies of disease-induced genetic changes in wildlife populations (Teacher *et al.*, 2009; Trudeau *et al.*, 2004) have based inferences on spatially replicated diseased/disease-free sites, potentially confounding site differences as an explanatory factor. Our paper represents one of few studies to have directly investigated the population genetic impacts of a wildlife disease epidemic for the host population from a baseline of pre-disease genetic data. Our results highlight that wildlife epizootics can have detectable effects on host population dynamics and genetic structure over only a few generations and across short time scales.

### The consequences of DFTD-induced population decline for genetic diversity

Several processes could cause the elevated  $F_{IS}$  observed post-disease relative to pre-disease in this study: population subdivision (the Wahlund effect), selection and inbreeding (or assortative mating) (Frankham *et al.*, 2002). Neither population subdivision nor selection is a likely candidate in this study, as there were no departures from HWE and analyses were conducted with purportedly neutral markers. The trend towards increased relatedness between individuals post-disease suggests that the elevated  $F_{IS}$  observed in this study indicates increased inbreeding occurring in the decimated post-disease populations.

Despite substantial population declines occurring at all three populations (Hawkins *et al.*, 2006; Lachish *et al.*, 2007; McCallum *et al.*, 2007), we did not find strong evidence for a reduction in genetic diversity 4–7 years following disease outbreak (though lower M-ratios post-disease suggest that some loss of genetic diversity is occurring). In addition, no population bottlenecks were inferred from the temporal method, which is a powerful method for detecting recent population bottlenecks, when sampling >30 individuals before and after a bottleneck and utilizing more than five polymorphic loci (Luikart *et al.*, 1998). We think it unlikely that inadequate sampling has limited our power to detect bottlenecks and changes in genetic diversity because both sample sizes and the number of polymorphic loci used in this study are robust (see also discussion by Jones *et al.*, 2004) and because we were able to detect significant changes in other parameters measured pre- and post-disease arrival ( $F_{IS}$ , IBD and genetic correlation measures).

There are two plausible explanations for the retention of genetic diversity in Tasmanian devils following DFTD outbreaks. First, genetic diversity in post-disease populations may have been maintained by dispersal. Although we found evidence for reduced dispersal post-disease among females and significantly greater genetic differentiation between post-disease populations (see discus-

sion below), the value for  $F_{ST}$  post-disease (0.02) suggests some migration between sites. Immigration between populations was deemed the reason that genetic diversity in common frogs was retained following *Ranavirus* epidemics (Teacher *et al.*, 2009). Second, it is likely that despite ongoing population reductions of >70% at the time of sampling, simply too little time had passed since disease arrival (a maximum of 7 years and three generations) for substantial reductions in  $N_e$  and consequent changes to genetic diversity to have occurred. A similar conclusion was reached in a study that found no change in genetic diversity two generations after a disease-induced population crash in rabbits (Queney *et al.*, 2000). In fact, simulations show that even the more immediate effect of bottlenecks, reduced allelic diversity, may not be severe in the first few generations following a bottleneck (assuming  $N_e = 10$  and 10 equally frequent alleles in the pre-bottleneck population Allendorf, 1986). For this reason, the evidence of increased inbreeding and suggestion of a decline in genetic diversity from the M-ratio analysis after only two to three generations is of concern.

Infectious diseases, by virtue of causing high mortality rates and the well-established relationship between genetic diversity and disease resistance, may be strong selective agents in populations (Hudson *et al.*, 2002). Hence, selection may affect the impact of disease on the genetic makeup of host populations. Our analyses revealed that different loci were under directional selection in the pre-disease (Sh2i) and post-disease (Sh2p) populations. This change suggests that some aspect of DFTD arrival and impact in populations altered the selective environment for Tasmanian devils. Although selection for differential disease susceptibility in DFTD-affected devil populations remains to be tested, we found that one allele at one locus (allele 226 at locus Sh2i) present at high frequencies (>0.340) in pre-disease populations had been virtually lost in the post-disease populations (<0.015). This may indicate a disease-related selective sweep against individuals possessing one or more genes linked to this allele.

### Effects of disease on population genetic structure and dispersal

A key finding of this study is that demographic decline in devil populations following a disease epidemic has increased genetic subdivision among populations. In addition to the differential effects of selection operating pre- and post-disease, the observed increase in  $F_{ST}$  could have been driven either by the non-random loss of alleles from populations or by changes to the dispersal patterns of individuals within the populations. Although additional temporal genetic samples are needed to discern the roles of these factors in driving population divergence, our results showing altered dispersal patterns in females post-disease in the absence of sex-linked loci suggest that increased  $F_{ST}$  was at least in part driven by altered dispersal patterns. We found higher levels of genetic structure and reduced levels of gene flow among females post-disease, which indicate that females did not disperse either as far, or as often, in disease-affected populations. As is typical of most polygynous mammals, reproductive success in female devils is limited by their ability to secure resources needed for reproduction (food and den sites; M Jones, unpublished data; Handley and



Perrin, 2007). It follows then, that when populations are greatly diminished in size (following DFTD outbreak), and resources become abundant, females do not need to disperse as far or as frequently.

It is unlikely that this result is an artefact of different sampling scales or sampling intensities as there was only 12 km difference between the furthest two individuals (females or males) sampled pre- (112 km) and post-disease (100 km), and sample sizes were identical pre- and post-disease, although the ratio of males to females varied (male/female pre-disease = 68/78, post-disease = 81/65). Decreased dispersal rates following population decline and leading to changes in genetic structure have been reported in other mammal species (Bohonak, 1999; Richardson *et al.*, 2002; Sommer, 2003; Matthysen, 2005), consistent with our findings in female devils.

This study provides the first evidence for male-biased dispersal in Tasmanian devils, although demographic records and genetic analyses show that both sexes do disperse from their natal site. Devils display characteristic female-defence polygyny (M Jones, unpublished, data; Pemberton, 1990), in which local mate competition in males is expected to exceed local resource competition among females leading to male-biased dispersal (Pusey, 1987; Handley and Perrin, 2007). Although dispersal patterns in male devils were not significantly altered in disease-affected populations, there was a nonsignificant increase in the strength of IBD, suggesting some influence of disease-induced population decline on male movements. Our ability to detect a change in dispersal patterns in males may have been limited by the spatial scale of the pre- and post-disease study (110 km), as gene flow occurs at greater spatial scales in males. We did find significant positive genetic structure in males across the larger area of the post-disease East Coast (230 km), suggesting this may be the case.

#### Dispersal patterns in Tasmanian devils: genetic versus demographic estimates

The dispersal distances obtained by direct and indirect methods were qualitatively similar and corresponded well with the estimates of genetic patch size and the limit of positive genetic structure revealed by spatial autocorrelation analyses. Tasmanian devils have traditionally been thought to disperse large distances (Pemberton, 1990). Here we have shown that, while long-distance dispersal (to at least 110 km) does occur, dispersal is characterized by much shorter range distances (14–30 km), suggesting that exchange of individuals among populations will be common only between geographically proximate locations. Such leptokurtic distributions of dispersal distances are a commonly observed feature of natural populations (Koenig *et al.*, 1996).

Discrepancy between estimates of mean dispersal distances (demographic estimate = 30.28 km; indirect estimate = 14.2 km) most likely reflects the fact that the indirect estimate was determined across uniformly low-density, disease-affected populations, whereas the demographic estimate was determined from both healthy and diseased populations, which varied greatly in density. Population density is known to influence dispersal distances (Lambin *et al.*, 2001; Matthysen, 2005). In this study, the longest recorded dispersal movement (110 km) was undertaken by a female from a very high-density

population. Moreover, our analyses provided evidence for reduced dispersal distances in low-density diseased populations. In addition, the estimation of dispersal distance ( $\sigma$ ) depends enormously on the reliability of the estimate of effective population density. We currently lack the demographic data (age and sex ratios and variance in reproductive success) required to accurately estimate effective population density and so assumed the value for devils to be similar to that found in other species. This approach seems appropriate, or even conservative, given that carnivores are known to have intrinsically small  $N_e$  (Creel, 1998) and that DFTD impacts devil populations primarily by removing reproductively active adults (Lachish *et al.*, 2007; Jones *et al.*, 2008b).

#### Conclusions

High disease-induced mortality rates and massive reductions in population size as a result of the impacts of DFTD on Tasmanian devils have, within just 2–3 generations of disease arrival, significantly altered population genetic structure and resulted in an apparent increase in inbreeding but not, as yet, significantly reduced heterozygosity or allelic diversity. We suggest that the short period of time (three generations) that has elapsed since disease arrival precluded the detection of significant changes to genetic diversity. Nevertheless, evidence for both increased inbreeding and increased population differentiation post-disease suggest that processes capable of driving the loss of genetic diversity are operating in DFTD-affected populations. With DFTD-driven population declines ongoing in all DFTD-affected populations (McCallum *et al.*, 2009), any loss of genetic diversity, when combined with other detrimental stochastic processes that can affect small populations (Allee effects, increased genetic drift), may have serious consequences for the viability of devil populations in the longer term. The possibility of restricted dispersal and increased population differentiation following disease impacts is also of concern for the persistence of these populations, as these behavioural and genetic changes can lead to even more pronounced negative impacts from inbreeding and genetic drift (Frankham *et al.*, 2002).

The conservation management of devil populations in the wild is now a priority for the Tasmanian government (Jones *et al.*, 2007). This study confirms that there is still time to conserve the genetic diversity in this species if management can reverse the effects of inbreeding and subdivision in disease-affected populations. Our results suggest that any action undertaken must be implemented over linear distances greater than 30 km to enable gene flow and some within-site dispersal. Moreover, given the already low genetic diversity in devil populations before the emergence of DFTD (Jones *et al.*, 2004) and the elevated levels of inbreeding and potential for loss of genetic diversity associated with DFTD that we have found, it is important that any refuge populations be large enough to limit further genetic complications resulting from bottlenecks and inbreeding. Nonetheless, proposed translocations should also consider whether populations are locally adapted to different conditions (Storfer, 1999).

The detrimental impacts of infectious diseases on host demography and population dynamics are well known (Hudson *et al.*, 2002), but to date, the genetic

consequences of disease epidemics for host populations are little explored. The results of this study showing changes in population genetic structure and dispersal patterns of a wild carnivore following a disease epidemic highlight that infectious disease can rapidly change the genetic composition of host populations. The extent to which depleted genetic diversity and more restricted dispersal following disease epidemics affects the long-term resilience and viability of populations is of profound concern for species conservation. Incorporating genetic monitoring and conservation genetic tools into wildlife disease ecology is necessary to ensure a better understanding of host–pathogen dynamics and to more effectively manage species threatened by disease.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

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