1 Title: Historical and contemporary signatures of selection in response to transmissible cancer in
2 the Tasmanian Devil (*Sarcophilus harrisii*)
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14
15 Abstract
16 Tasmanian devils (*Sarcophilus harrisii)* are evolving in response to a unique transmissible
17 cancer, devil facial tumour disease (DFTD), first described in 1996. Persistence of wild
18 populations and the recent emergence of a second independently evolved transmissible cancer
19 suggest that transmissible cancers may be a recurrent feature in devils. We used a targeted
20 sequencing approach, RAD-capture, to identify genomic regions subject to rapid evolution in
21 approximately 2,500 devils as DFTD spread across the species range. We found evidence for
22 genome-wide contemporary evolution, including 186 candidate genes related to cell cycling
23 and immune response. We then searched for signatures of recurrent selection with a molecular
24 evolution approach and found widespread evidence of historical positive selection in devils
25 relative to other marsupials. We identified both contemporary and historical selection in 19
26 genes and enrichment for contemporary and historical selection independently in 22 gene sets.
27 Nonetheless, the overlap between candidates for historical selection and for contemporary
28 response to DFTD was lower than expected, supporting novelty in the evolutionary response of
29 devils to DFTD. Our results can inform management actions to conserve adaptive capacity of
30 devils by identifying high priority targets for genetic monitoring and maintenance of functional
31 diversity in managed populations.
32
33 Keywords: Rapid evolution, molecular evolution, gene set overlap, wildlife disease,
34 conservation genomics, RAD-capture
35

Introduction

Species are subject to selection by pathogens throughout their evolutionary history, shaping lineage diversification and leading to complex cellular and molecular defensive mechanisms (1). Still, emerging infectious diseases (EIDs) can cause mass mortality and, given sufficient survival and genetic variation, initiate rapid adaptive evolution in a naïve host population (2). Although the prevalence and severity of EIDs in wildlife populations is now well-recognized (3-6), we are just beginning to understand the evolutionary impacts of disease in wildlife. We have a relatively short recorded history of infectious disease in wildlife, and therefore a limited ability to predict outcomes or intervene when warranted (7, 8).

High-throughput DNA-sequencing techniques and the generation of high-quality annotated reference genomes have revolutionized our ability to monitor and identify mechanisms of evolutionary responses to pathogens (8-10). Inter-specific comparisons of non-synonymous and synonymous variation (dN/dS) within protein-coding regions have long been used to identify positive selection at immune-related loci (11, 12). In the genomic era, these analyses have been extended beyond a few well-characterized genes and a few-well studied species; for example, to reveal shared positive selection hotspots among viral immune pathways in both birds and mammals (13). At the population level, rapid evolution in response to disease can be detected by tracking changes in allele frequency before, during, and after the outbreak of disease (14, 15). However, such analyses are resource-intensive and therefore still challenging in wildlife systems (but see 16, 17, 18). Reduced representation techniques such as restriction-site associated DNA-sequencing (RADseq) (19) have made the acquisition of genome-wide, time-series genetic data more accessible in non-model systems (20). By integrating these resources and tests of selection at differing temporal scales, we can assess whether species that show rapid evolution in response to contemporary pathogens also show evidence of historical selection to similar pathogens.

A striking example of an EID acting as an extreme selective force in wildlife is devil facial tumour disease (DFTD), a transmissible cancer first described in 1996 afflicting wild Tasmanian devils (*Sarcophilus harrisii*) (21). Tasmanian devils are the largest extant carnivorous marsupial, with contemporary wild populations restricted to the Australian island of Tasmania. As a transmissible cancer, DFTD tumour cells are transmitted between hosts, behaving as a pathogen (22). Transmission typically occurs as devils bite each other during the mating season after devils have reached sexual maturity (23, 24). With few notable exceptions documenting regression (25), DFTD tumors escape recognition, become malignant, and can kill their hosts within six months (26). Starting from a single Schwann cell origin (27), DFTD has now swept across nearly the entire species range (Figure 1A). Devil populations have declined species-wide ~80% (28) with local declines in excess of 90%. Most populations will reach 90% decline after about 6 years, with just a few exceptions (29). This is particularly alarming because devils have notoriously low genome-wide diversity, attributed to climate- and anthropogenic-induced bottlenecks (30-32). Depleted genetic diversity at immune-related loci has likely further contributed to DFTD vulnerability (33).

79 Remarkably, a second independent transmissible cancer, DFT2, was described 20 years later in
80 2014 (34, 35). Comparative and functional analyses of DFTD and DFT2 showed similar drivers of
81 cancerous mutations and tissue type of origin (36). Low genetic diversity, chromosomal fragility
82 (37), a reportedly high incidence of non-transmissible neoplasms (38), and injury-prone biting
83 behaviour (39) may contribute to a predisposition to transmissible cancers in devils (40). Devils
84 have shown signs of resilience to DFTD. Females at five sites were found to exhibit precocial
85 breeding after the arrival of DFTD, potentially increasing their lifetime productivity despite high
86 mortality due to DFTD (41). DFTD has been spontaneously cleared (i.e., regressed) in some
87 individuals (25). Long-term, field-based pattern matching and individual-based simulations
88 predicted that cyclical co-existence or DFTD extirpation are more likely scenarios than devil
89 extinction (42). Incorporating evolutionary responses by devils into such predictive models
90 could also increase the likelihood of devil persistence. Taken together, these findings suggest
91 that transmissible cancers may be a recurring selective force in the Tasmanian devil lineage.
92
93 Because of the threat of DFTD and DFT2 to devil populations, there are ongoing conservation
94 efforts, including the establishment of a captive devil insurance meta-population. The insurance
95 population is managed to maintain genome-wide genetic diversity and serve as a source for re-
96 introductions in an effort to increase genetic diversity and size of wild populations (43). To
97 inform conservation efforts, it is important to understand what types of genetic variation in
98 natural populations may allow for evolutionary rescue from disease and maintain adaptive
99 potential for future threats (44). Given evidence for rapid evolution in response to DFTD,
100 monitoring of genetic variation at candidate adaptive loci could help evaluate the adaptive
101 potential of wild populations (44, 45). In heavily managed (e.g. captive) populations, loci
102 associated with an adaptive response to disease could be included in genotyping panels for
103 maintaining genetic diversity (46).
104
105 Ongoing long-term field and genomic studies have provided a wealth of resources to examine
106 responses to DFTD. Since the first disease detection in 1996, field sites have been established
107 across the island of Tasmania to consistently monitor the spread and effects of the disease and
108 wild devil populations (41). A high-quality reference genome for the devil yields deep
109 evolutionary history insight and fine-scale mapping for functional inference of individual
110 genotypes (27, 32). Population genomic studies have already shown that devils are rapidly
111 evolving in response to DFTD (2, 47, 48). From these data, a RAD-capture (Rapture; 49) panel
112 has been developed to target 16,000 putative candidates and demographically-informative loci,
113 facilitating the collection of data across hundreds of individuals in a single lane of sequencing.
114 This panel was used to identify genes associated with DFTD-related phenotypes, including
115 infection and survival in females (50). We also now have access to the shared and divergent
116 evolutionary histories owing to a robust, dated phylogenetic reconstruction (51), and high
117 quality, readily accessible, annotated reference genomes of the South American grey-tailed
118 opossum (*Monodelphis domestica*) (52) the tammar wallaby (*Notamacropus eugenii*) (53), and
119 the koala (*Phascolarctos cinereus*) (54).
120

121 Here we identify targets of selection and signatures of adaptation at both contemporary and
122 historical time scales in Tasmanian devils. We expand upon previous studies of contemporary
123 selection with a much larger sample size including several time points for sites across the entire
124 island, using a RAD-capture panel for targeted sequencing of almost 16,000 loci. Then, we test
125 for evidence of recurrent selection by examining shared contemporary and historical signatures
126 of selection, in terms of either specific genes or functional genetic pathways that could relate to
127 transmissible cancer. We propose three hypotheses: 1) If transmissible cancer is a novel
128 selective force acting on Tasmanian devils, genes under contemporary selection by DFTD will be
129 different from genes with a signature of historical positive selection. 2) If transmissible cancer is
130 a recurrent selective force in the devil lineage that targets the same set of genes repeatedly, we
131 may expect an overrepresentation of the same genes under both contemporary and historical
132 selection. Alternatively, 3) recurrent transmissible cancers may impose selection on different
133 genes within a common set of pathways, or even on genes in different functional groups. These
134 alternatives can inform conservation efforts to manage genetic diversity for resilience in natural
135 devil populations, and any genes or functional pathways that show both contemporary and
136 historical selection may be relevant to cancer resistance more broadly.
137
138
139 **Materials and Methods**
140 *Rapture Sequencing for Contemporary Selection*
141 We used the Rapture method (combining RAD and sequence capture; 49) to conduct targeted
142 genotyping of single-nucleotide polymorphisms (SNPs) across 2,562 individuals from multiple
143 Tasmanian devil populations, sampled both before and after DFTD appeared in each population
144 (Figure 1A, Supplemental Table S1). For SNP genotyping, we constructed Rapture libraries
145 following Ali et al (2016), using the restriction enzyme *PstI* and a capture array targeting 15,898
146 RAD loci selected for membership in one of three functional categories: 1) those showing
147 signatures of DFTD-related selection from previous work (Epstein et al. 2016), 2) loci close to
148 genes with known cancer or immune function, and 3) loci widely distributed across the genome
149 (See 48, 50 for more details on the devopment of this array.). See Supplemental Materials S1
150 for multiplexing, read processing, and genotyping details.
151
152 *Spatial and temporal analysis of contemporary selection*
153 We identified candidate SNPs by combining the results of three analyses: raw change in allele
154 frequency in each population after DFTD (hereafter Δaf), and two methods that estimate
155 strength of selection from allele frequencies at multiple time points in multiple populations,
156 the method of Mathieson & McVean (14), (hereafter *mm*), which allows the estimated selection
157 coefficient to vary over space; and *spatpg* (15), which allows the selection coefficient to vary
158 over time as well as space. To reduce the signal-to-noise ratio, we then combined three tests
159 for contemporary selection with a de-correlated composite of multiple signals (*DCMS*) (55). See
160 Supplemental Materials S1 for details of each analysis.
161
162 *Historical Selection*

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163 Today, devils are the only species of marsupials with any known history of transmissible cancer.
164 We combined existing genomic resources for the South American grey-tailed opossum and
165 tammar wallaby from the Ensembl database (56) and the recently published transcriptome
166 assembly of the koala (54) to identify signatures of positive selection in devils, relative to the
167 three other annotated marsupial genomes using the branch-site test of PAML (Phylogenetic
168 Analysis by Maximum Likelihood (57, 58). The branch-site test compares the likelihood scores
169 for two models which estimate dN/dS among site classes of the multi-sequence alignment and
170 branches. In the neutral model, all site classes and branches are constrained to dN/dS ≤ 1. In
171 the alternative model, dN/dS of site class 2 is allowed to exceed 1 for only the foreground
172 branch, while constraining the background branches to dN/dS ≤ 1. See Supplemental Materials
173 S1 for details regarding orthology identification and PAML implementation.
174
175 *Comparison of Contemporary and Historical Signatures*
176 To test whether the number of genes under contemporary selection differed from the genome-
177 wide background, we compared the number of overlapping candidate and non-candidate genes
178 with Fisher's one-tailed test. To test for differences in the strength of selection, we compared
179 the distributions of dN/dS magnitude > 1 and the proportion of sites per gene with dN/dS > 1
180 found in Site Class 2 of the branch-site test. We used two nonparametric tests of equality to
181 compare the distributions; our null hypothesis was that the two samples came from the same
182 population (59) (60). To identify key mechanisms of adaptation among candidate genes, we
183 used gene ontology (GO) term enrichment analysis using the SNP2GO package (61) and the
184 PANTHER web-interface (62). We capitalized on the wealth of ongoing research in devils and
185 DFTD by comparing our contemporary and historical candidates to those previously identified
186 using different datasets and analytical approaches (2, 47, 48, 50, 63). We then tested for
187 overrepresentation of contemporary and historical candidates in gene sets of the molecular
188 signatures database (MsigDB) (64). See Supplemental Materials S1 for details of these
189 comparisons.
190
191 **Results**
192 *Genomic Data Processing*
193 To test for contemporary selection, we sampled a total of 2,562 individuals across six localities
194 of Tasmania before and after DFTD prevalence (Table 1; Supplemental Table S1; Figure 1A),
195 with a RAD-capture array (50). After filtering, we retained 14,585 – 22,878 SNPs variants for
196 downstream analysis, depending on the sampled time point and population.
197
198 *Evidence for contemporary selection*
199 In the top 1% of DCMS scores, we identified 144 candidate SNPs for contemporary selection by
200 DFTD; of these, 79 had annotated genes (186 total) within 100 kb (Figure 2; Supplemental Table
201 S4). In our comparison of previously identified candidate genes in devils (47, 48, 50, 63), we
202 found many overlapping candidates (See *Discussion*), but only significant enrichment among
203 our contemporary candidates for those associated with disease-related phenotypes (50), with
204 the largest odds ratio for genes associated with phenotypes in females (14 genes, p-value=5.1e-
205 08, Odds ratio=7.1). Gene ontology enrichment analysis found middle ear morphogenesis

5

206 (GO:0042474) significantly enriched among contemporary candidate SNPs (FDR < 0.05). Five
207 candidate SNPs were within the 100 kb window of two genes associated with this term: EYA1
208 and PRKRA. Both EYA1 and PRKRA are involved in cell proliferation and migration and
209 implicated in tumour suppression and angiogenesis (65-67).
210
211 *Evidence for historical selection*
212 Using the branch-site test for positive selection in PAML, we found 1,982 genes with a signal of
213 historical positive selection (Figure 3, red); Supplementary Table S5). The majority of genes
214 were classified as having a molecular function of binding or catalytic activity; involved in
215 metabolic and cellular processes, or biological regulation; and a plurality as participating in the
216 Wnt signalling pathway, which is keystone regulator of stem cell proliferation, including in
217 cancer (68).
218
219 *Comparing historical and contemporary selection*
220 Twenty-one contemporary candidate genes were also candidates for historical selection
221 (Supplemental Table S6). The distributions of dN/dS estimates (Figure 3a) and proportion of
222 sites (Figure 3b) in genes under historical positive selection from the genome-wide background
223 and contemporary candidates similarly had an overall low magnitude of dN/dS and a low
224 proportion of sites under historical positive selection. The contemporary candidates did not
225 show evidence of more widespread historical selection compared to the genome-wide
226 background according to tests of equality (59, 60).
227
228 We identified 22 gene sets from the Molecular Signatures Database (64) independently
229 enriched within the contemporary candidate set and the historical candidate sets
230 (Supplemental Table S8). Note that no single gene was under both historical and contemporary
231 selection within these sets. The cancer gene neighbourhoods of correlated expression (CGN of
232 MSigDb) from the MORF compendium (64), shared among the highest rates of overlap (approx.
233 30-40%) for both contemporary and historical candidate sets (Supplemental Figure S4). After
234 correcting for multiple testing in each analysis, only the gene set for the gene expression
235 neighbourhood of RBBP8 was still significantly enriched. Importantly, the permutation test of
236 shared gene sets found *fewer* shared overlaps between historical and contemporary selection
237 than expected by chance (p = 0.012, Supplemental Figure S4).
238
239 **Discussion**
240 Here we used targeted sequencing and a molecular evolutionary analytical framework to show
241 widespread positive selection in the Tasmanian devil across the genome at both historical and
242 contemporary timescales. We identified 186 candidate genes for rapid allelic shifts following
243 the arrival of DFTD at the contemporary scale in wild devil populations and 1,982 candidate
244 genes for historical positive selection within devils relative to other marsupials. Statistical tests
245 comparing degree of overlap and distributions among these candidates did not support
246 recurrent selection on a common set of genes in response to transmissible cancer. Our results
247 suggest that the specific genes implicated in the contemporary evolutionary response to DFTD
248 are mostly novel targets compared to the genome-wide signature of historical selection. That is,

249 most of the genes under selection in devils in response to DFTD have not been under recurrent
250 selection over macroevolutionary timescales. However, we also found support for overlapping
251 contemporary and historical selection on some gene networks. Our work contributes to
252 mounting evidence identifying possible mechanisms by which devil populations are persisting
253 and rapidly evolving in the face of DFTD despite overall low genetic diversity and population
254 bottlenecks (2, 25, 50, 69, 70). Broadly, this type of approach can be applied to selection
255 analyses of novel threats in wildlife populations in the current era of anthropogenic global
256 change and guide monitoring and management actions focused on genetic adaptive potential.
257
258 *Contemporary Responses to DFTD*
259 Across six study sites that span the geographic range of Tasmanian devils, and genomic samples
260 of over 2500 devils collected over 15 years, we found widespread signatures of rapid evolution
261 in populations impacted by DFTD. Despite concerns that devils may not harbour sufficient
262 genetic diversity to sufficiently respond to DFTD, we identified 186 candidate genes
263 (Supplemental Table S4) with robust signatures of positive selection, reaffirming the adaptive
264 potential of wild devils described in a growing body of work (2, 47, 48, 70). Our targeted RAD-
265 capture sampling approach allowed us to sequence many more individual devils than previous
266 studies and examine specific loci and regions of interest. These samples spanned six genetic
267 populations shown to be locally adapted to differing habitats (48, 71). By incorporating
268 thorough time-series sampling at each site throughout the course of the disease and
269 bioinformatic methods that explicitly allow selection to vary over time and populations (*spatpg*
270 and *mm*), our results provide robust evidence of rapid evolution across the island in response to
271 DFTD-
272
273 In line with previous population genomic studies (2, 47, 48, 50, 63), our analysis of
274 contemporary evolution detected a putatively adaptive response related to the immune
275 system, cell adhesion, and cell-cycle regulation (Supplemental Table S4). Our GO enrichment
276 result for middle ear development (GO:0042474) among contemporary candidates may
277 highlight selection for interactions with the peripheral nervous system and cell proliferation.
278 Whereas Hubert and colleagues (47) suggested genes with nervous system annotations may be
279 indicative of selection for behavioural changes, others have pointed out the importance and
280 vulnerability of peripheral nerve repair by Schwann cells in devils, given the prevalence of biting
281 and Schwann cell origin of DFT (40). Regardless, genes and their products at the interface of the
282 nervous system and proliferation could prove to be important candidates. Significant
283 enrichment for genes associated with devil case-control, age, and survival (50) among our
284 contemporary candidates is a strong indicator that these contemporary candidates likely confer
285 relevant phenotypic change. We also confirmed five of the seven candidates identified
286 previously by Epstein and colleagues (2) with RADseq (approximately 90,000 SNPs) in just three
287 populations, specifically CRBN, ENSSHAG00000007088 (uncharacterized), and the previously
288 identified chromosome 3 region including THY1, USP2, C1QTNF5. In contrast, we identified only
289 two (TRNT1 and FSHB) of the 148 candidates from a re-analysis of that same dataset which
290 identified population-specific responses (47). Candidates associated with disease prevalence
291 identified with a genotype-environment-association approach (48) were also functionally

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292 associated with transcription and cellular regulation, but relatively few of the same specific
293 genes were identified (GRIP2, KYNU, ADAMST9, NDUFS3, PLEKHA3).

294

295 Among our contemporary candidates in the top 1% of DCMS scores across all tests, we did not
296 identify any of the genes or regions that had been associated with host variation responsible for
297 tumour regression (63). However, we found PAX3 ,which encodes a transcription factor
298 associated with angiogenesis (72), as a top candidate for DCMS of allele frequency change
299 (Δaf), possibly reflecting differences in test sensitivities. Among tumour genomic variants
300 associated with regression (73), only the gene encoding Janus kinase and microtubule
301 interacting protein 3 (JAKIMP3) was also found among our contemporary candidates. In
302 general, the paucity of candidates shared between our contemporary analysis and regression
303 studies suggests that regression is not the dominant form of phenotypic response to DFTD.

304

305 Our results affirm repeated, concordant responses across the island as described previously (2),
306 with greater similarity between analytical approaches than within populations. This result is
307 consistent with rapid evolution facilitated by selection for standing variation that was largely
308 shared across populations prior to disease arrival. This timescale would likely be too short for
309 new mutations to play a substantial role in DFTD response, and genetic variation is shared
310 across the species range, despite some geographic population structure (2, 71).

311

312 *Historical selection in devils*
313 With our genome-wide molecular evolution approach (57), we found similarly widespread
314 historical positive selection across the devil genome, in 1,977 genes, or about 31% of all 6,193
315 orthologs tested (Supplemental Table S5). The branch-site test is known to be less conservative
316 than related models, particularly when divergence among species is large (74), but the rates of
317 genome-wide selection we found in devils are similar to those described in other taxa; e.g. 17-
318 73% of avian orthologs (13). To our knowledge, this is the first genome-wide survey of positive
319 selection specifically among the publicly available annotated marsupials, spanning divergence
320 with origins in the Cretaceous (approx. 80 my) (51).

321

322 We did not find preferential positive selection for immunity-related genes, as has been shown
323 in primates (1), eutherian mammals more generally (75), and birds (13). Instead, we found the
324 highest proportion of pathways under historical selection to be functionally classified within the
325 Wnt pathway, a key signalling cascade regulating cell adhesion and implicated in carcinogenesis
326 (76). As genomic resources grow and improve in marsupials (10), interspecific analyses for
327 positive selection at finer scales may reveal more recent and specific responses in Tasmanian
328 devils. Analytical resolution could also be improved by finer-scale annotation and curated
329 databases of immune-system processes in marsupials to complement genome assembly efforts.
330 Interspecific analyses of more recently diverged species could also have greater power to
331 inform immune-system and cancer vulnerabilities in humans (77).

332

333 *Comparing Contemporary and Historical Timescales*

334   Remarkably few transmissible cancers have been discovered in nature (three in mammals, and
335   a small handful in bivalve molluscs) (78, 79), and yet two of those independent clonally-
336   transmitted cancers have been discovered in Tasmanian devils in less than 20 years. This, and
337   the observed rapid evolutionary response to disease would suggest that transmissible cancers
338   may be a recurrent event in devils. We tested for recurrent adaptation to transmissible cancers
339   in devils by examining whether the contemporary signature of selection in response to DFTD
340   (Figure 2) was reflected by positive selection at a deeper evolutionary time scale. We compared
341   the distributions of historical selection in contemporary candidates to those of the genome-
342   wide background (Figure 3) and by evaluating contemporary and historical gene set overlaps
343   (Supplemental Tables S7 and S8). Our results did not conclusively support or reject the
344   hypothesis of recurrent selection by repeated transmissible cancers. We found no significant
345   overlap between contemporary candidates with signatures of historical selection and there was
346   no significant difference in the proportion of sites or magnitude of historical selection between
347   contemporary and the genome-wide background selection (Figure 3). We broadened the gene-
348   by-gene comparison using the MSigDb gene sets to infer functional sets related to disease
349   responses and found that shared gene set enrichment occurred less than expected by chance
350   (Supplemental Figure S5). This suggests that these 22 gene sets could be specific targets
351   warranting further investigation, instead of a proportional representation of the broad
352   genome-wide background of historical selection. The 19 candidate genes (Supplemental Table
353   S6) and 22 shared gene sets (Supplemental Table S8, Supplemental Figure S4) raise interesting
354   targets for understanding functionally important variation in devils. Our results suggest a
355   largely novel response to DFTD, but still highlight potential mechanisms of species persistence.
356
357   The nineteen candidates for recurrent selection (Supplemental Table S6) are generally related
358   to three main themes: transcription regulation, the nervous system, and the centrosome. Four
359   of these candidates for recurrent selection were previously associated with disease-related
360   phenotypes by Margres and colleagues (50): SRP72, ARL9, ENSSHAG00000004590 (Novel,
361   Set1DB), ENSSHAG00000011607 (Novel, MAPK). We additionally found 82 historical candidates
362   previously identified in the top 1% of SNPs associated with disease-related phenotypes and
363   three genes in the top 0.1% associated with large-effect sizes for female case-control and
364   survival (50): PRKA10, ST8SIA2, and SLC12A8. PRKA10 binds to regulatory subunits of
365   proteinase K (80). These results lend support to the hypothesis of recurrent selection by
366   transmissible cancers, but the lack of significant overlap suggest that this could occur as a
367   random sampling from two large lists of candidates. These three genes were not identified in
368   our tests for contemporary selection. Both our contemporary selection analysis and the
369   genome-wide association study (GWAS) approach used by Margres and colleagues (50) are
370   statistically limited by small populations, sample size, and the time scale over which DFTD-
371   related selection has occurred. By considering the complement of these results together, the
372   overlapping historical, GWAS, and contemporary candidates may still be promising targets of
373   recurrent selection along similar functional axes.
374
375   Gene overlap analysis using the immunologic, curated, and cancer gene network sets of the
376   Molecular Signatures Database highlighted gene expression in cancer-related genes as a shared

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377 functional feature of both historical and contemporary selection in devils. We focus here on the
378 22 significant overlaps found with both contemporary and historical candidates (Supplemental
379 Table S8). The computationally mined co-expression neighbourhoods of cancer-related genes
380 from the MORF compendium (64) had the highest rates of overlap for both contemporary and
381 historical sets (Supplemental Figure S4). Among all gene sets, the strongest evidence for
382 recurrent selection in functional pathways was for the CGN of the retinoblastoma binding
383 protein 8 (MORF_RBBP8), which was the only significant set after correcting for multiple
384 testing. RBBP8 regulates cell proliferation and has been implicated as a tumour suppressor as a
385 modulator of BRCA1 (81). High rates of overlap among the MORF sets could be because co-
386 expressed genes are often subject to similar selective force at similar rates (75); or because co-
387 expression and signatures of selection are more likely to occur within close physical proximity
388 (82), although these genes are all on different super-contigs of the devil genome. Although
389 variation in host gene expression has been shown to confer rapid evolution of disease
390 resistance (83), differential expression among devils with differing disease status has not yet
391 been investigated.
392
393 *Transmissible Cancer as a Recurrent Selective Force?*
394 The low prevalence of genes with both contemporary and historical signatures of selection
395 suggest a novel response to DFTD compared to historic selection in the devil lineage. However,
396 our results do not conclusively eliminate or support the possibility that devils have previously
397 been impacted by transmissible cancers. The low genetic diversity observed in devils could be
398 the result of the widespread historical positive selection resulting from transmissible cancers or
399 other diseases (84), or historical bottlenecks due to climate change and habitat loss (30-32).The
400 genes (Supplemental Table S6) and functional gene sets (Supplemental Table 8; Supplemental
401 Figure S4) under selection at both timescales may reflect historical transmissible cancers, or
402 indicate ongoing selection by a similar selective force. A hypothesis for the lack of greater
403 overlap than we observed between historical and contemporary candidates is that there is
404 redundancy in the genetic mechanisms underlying resistance to transmissible cancers,
405 potentially as a result of repeated selection for resistance (85). It could be that historical
406 purifying selection already eliminated variation at genes associated with cancer resistance.
407
408 The widespread contemporary evolution we found in devils reflects the recent prediction (86)
409 that response to an emergent disease is most likely controlled by many genes conferring
410 quantitative resistance (87), for example by reducing the within-host growth rate of tumors. By
411 extension, DFTD is predicted to become less virulent in the short-term. If DFTD persists long-
412 term in the devil population with ongoing coevolution, it may lead to diversifying selection for
413 specific, qualitative gene-for-gene host resistance mechanisms (86). Phylodynamic study of
414 DFTD could help further test these hypotheses by testing for relationships between genomic
415 variation and transmission rates (88). Indeed, phylodynamic analysis of DFTD as it spread across
416 the island supports the hypothesis that devils may be mounting a response; transmission rates
417 have decayed such that DFTD appears to be shifting from emergence to endemism (Patton et
418 al. 2020, In Review). Although host-genomic variation was not jointly considered in this analysis,

419 the combined evidence of multiple studies demonstrating rapid evolutionary response of devils
420 to DFTD, including this one, support these interpretations.
421
422 *Conservation Implications*
423 Calls have been made to consider the historical context of adaptation when proposing
424 conservation management solutions based on genomic results (89). Our analysis of historical
425 selection largely supports the hypothesis that DFTD is a newly emerging and novel selective
426 force, distinctly shaping today's remaining wild devils. The targets of novel selection that we
427 identified (Figure 2, Supp Table S4) and their functional roles should be considered for
428 prioritization of monitoring and conservation in light of DFTD. At the same time, the wide
429 distribution of contemporary candidates across the genome also highlights the importance of
430 standing genetic variation to continue to respond to unique selective forces, including their
431 local environments (48). The low genetic diversity observed in devils may be the result of
432 historical population declines (30-32) and/or similarly diffuse selection operating historically in
433 the devil lineage. Genomic monitoring could be useful for maintaining both functional diversity
434 at candidate loci and genome-wide variation in breeding captive populations (46, 90, 91) and in
435 the wild. Multiple genomic tools are available for targeted monitoring of large sets of loci (e.g.
436 92, 93) and could be used to track adaptive evolution and potential in the form of genetic
437 diversity (44). However, before management decisions are made for specific genes further work
438 should first identify favoured alleles and functional fitness effects for the genes we found in
439 linkage with our genotyped SNP markers (Supplemental Table S4) .

440 At the time of writing, DFTD still has yet to reach all of the populations of devils in the far west
441 (Fig. 1a), and although wild populations have persisted, DFTD continues to circulate throughout
442 the island. We still do not know whether and how devils may be particularly susceptible to
443 transmissible cancers, and our work cannot shed light on possible mechanisms for origin of
444 these diseases. Instead our results inform mechanisms of tolerance/resistance to DFTD after it
445 has emerged. To maintain adaptive capacity over the long term in the face of similar recurrent
446 selective forces (e.g. transmissible cancers), our results warrant (1) the monitoring of genetic
447 variation in broad functional groups, for example in genes related to transcription and cell
448 proliferation; and (2) management strategies to maintain genetic diversity across those broad
449 groups. This study could provide a list of candidate loci for development of a genotyping panel
450 for either purpose, with flexibility to target many or fewer loci. At the same time, given urgent
451 and unpredictable present-day threats including not just emerging diseases but environmental
452 change and population fragmentation, it is important also for monitoring and population
453 management to account for genetic variation across the genome.
454
455 *Data and Script Accessibility*
456 Demultiplexed sequence data has been deposited at NCBI under Bio-Project PRJNA306495
457 (http://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA306495) and BioProject PRJNA634071
458 (http://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA634071). Code and tabular results are
459 available at **https://github.com/Astahlke/contemporary_historical_sel_devils**.
460
461 *Acknowledgements*

11

472   *Authors' contributions*

473   ARS, HIM, MEJ, AS, and PAH conceived and designed the study. RH and MEJ conducted

474   fieldwork and sampling. ARS, BE, SB, SAH, AV, BS conducted genomics labwork. ARS, BE, SB, AP,

475   SAH, AKF, and PAH conducted bioinformatic analyses. ARS wrote the manuscript with

476   contributions from all authors.

477

478 *Tables and Figures*

479 Table 1. Number of adults sampled before and after the year of first detection of DFTD at each

480 site. See Supplemental Table S1 for sample size for each year at each locality.

Location	Year of First Detection	Samples Before	Samples After	Total
Mt. William	1996	0	155	155
Freycinet	2001	300	382	682
Forestier	2004	131	552	683
Fentonbury	2005	99	169	268
West Pencil Pine	2006	52	348	400
Narawntapu	2007	224	150	374
Total		806	1756	2562

481

482   Figure 1. A) Map of the six contemporary sampling locations relative to disease prevalence over
483   time (red lines) with the year of first detection labled at each site. B) Pruned, unrooted
484   phylogenetic tree of the marsupials (51) used to estimate genome-wide historical selection on
485   the devil lineage. Devil cartoon by David Hamilton. From top to bottom: The tammar wallaby
486   (*Notamacropus eugenii*), koala (*Phascolarctos cinereus*), the Tasmanian devil (*Sarcophilus*
487   *harrisii*), and the South American grey-tailed opossum (*Monodelphis domestica*).
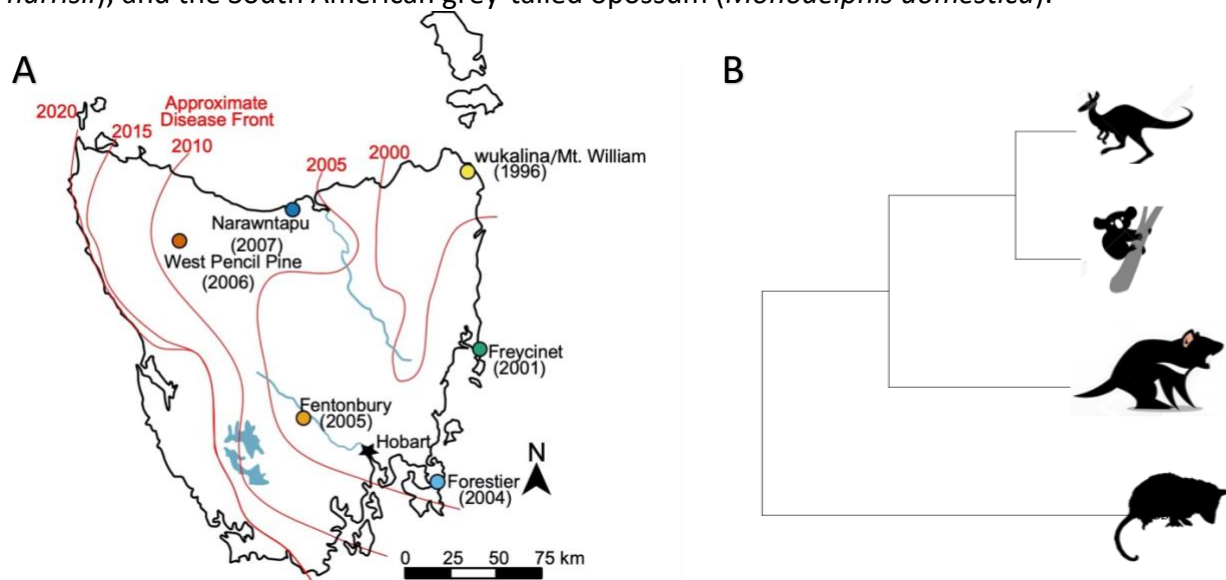

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491   Figure 2. Results of each elementary test of contemporary selection across sites and composite
492   score for candidate (in filled points) and noncandidate (in open squares) SNPs, ordered by
493   chromosome. From top to bottom: P-values for change in allele frequency (*Δaf*), Mathieson and
494   McVean (*mm*) (14), *spatpg* (15), and *DCMS* (55).
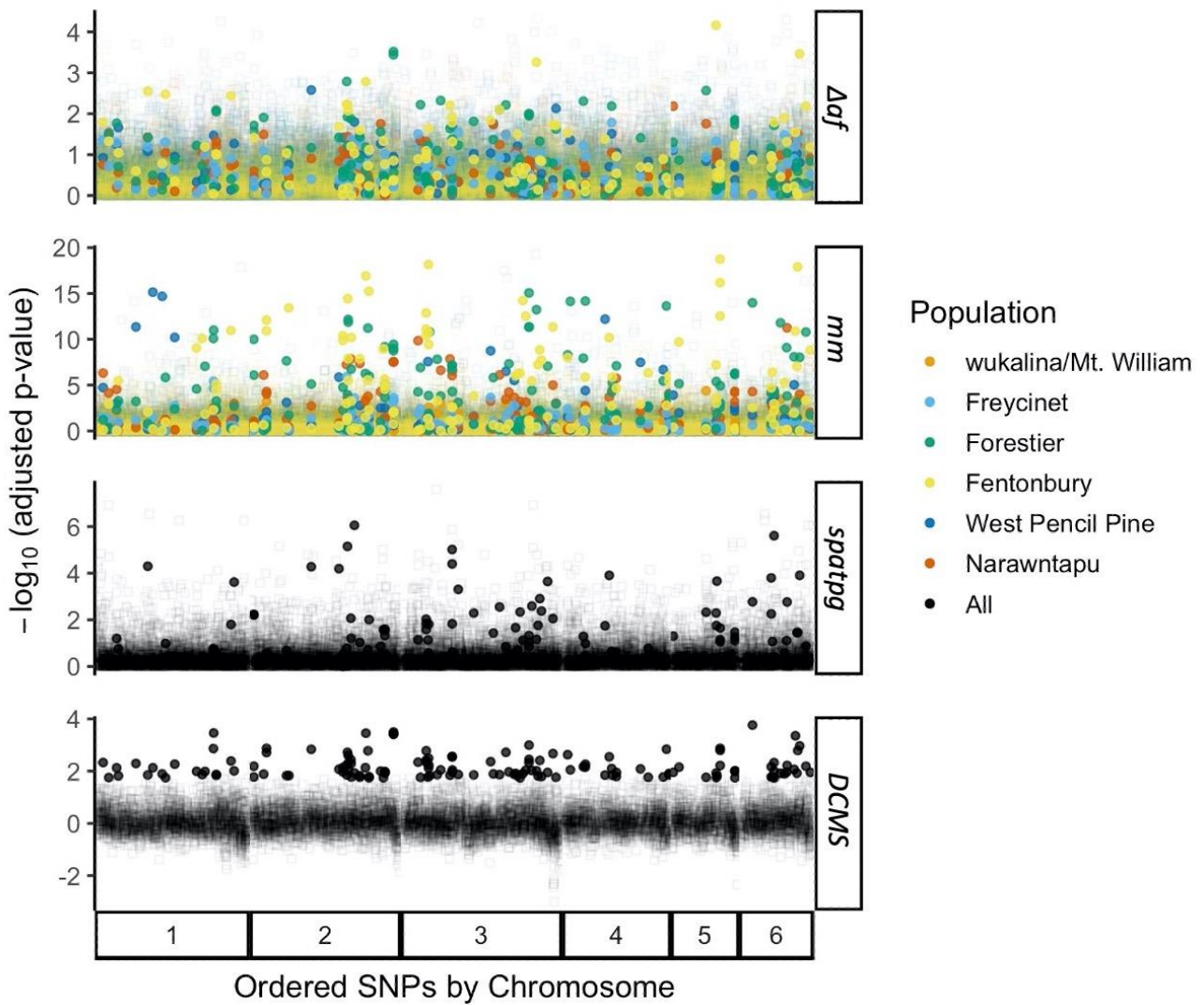

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497   Figure 3. Distributions of statistically significant historical positive selection (57) for
498   contemporary candidates (N=19) and the genome-wide background (N=1,982). Each point
499   represents the results for a single gene. A) Estimates of dN/dS and B) proportion of sites
500   classified as positive selection in the devil branch.
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508   References
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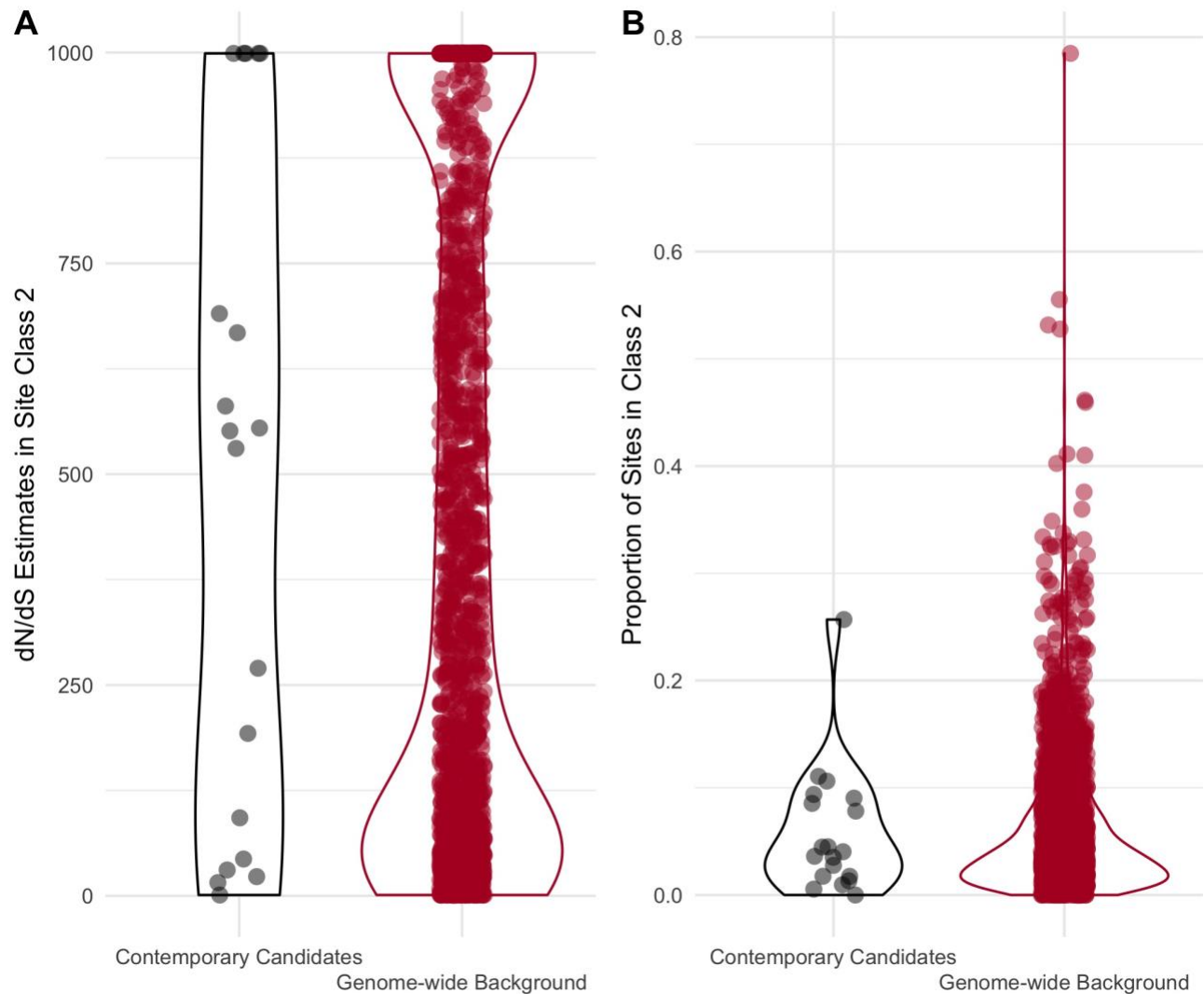
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