

# A comparison of genetic and genomic approaches to represent evolutionary potential in conservation planning

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#### 1 Highlights

2	•	Different molecular metrics should be used to meet specific conservation objectives
3	•	Single-species molecular data are inefficient at capturing multi-species evolutionary
4		potential
5	•	Genetic data may be a potential surrogate of genomic data within conservation
6		planning

7

#### 8 Abstract

9 Emerging global change stressors have underlined the importance of informing 10 conservation decisions with molecular diversity, particularly including intraspecific adaptive 11 or evolutionary potential across species and populations. Population-level evolutionary 12 potential is best captured by genomic approaches, yet these data types mostly remain 13 limited to model organisms. In contrast, traditional genetic data are broadly available. The 14 relevance of genomic metrics, and how they differ from genetic metrics in the context of 15 spatial conservation practices, remains unknown. This provides an opportunity to consider 16 the relative contribution and impact of genomic compared to genetic metrics in their 17 efficiency of selecting conservation areas of evolutionary importance. Here, we provide a 18 guideline to include metrics of genetic and genomic variation into spatial planning analyses 19 for multiple conservation objectives, and empirically explore how spatial prioritizations 20 change when including different types of molecular information across multiple species. 21 Specifically, we compare conservation solutions of scenarios including either an increase in 22 molecular information (i.e. either a single locus; mtDNA, or several thousand loci; SNPs), or 23 an increase in species included. We find that including less molecular information from 24 many species leads to similar outcomes to including more molecular information from

25	fewer species. Our work suggests that multi-species genetic data may be a cost- and time-
26	effective surrogate for genomic data in spatial planning.

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Keywords: evolutionary potential, spatial conservation planning, conservation genomics,
 genetic diversity, Marxan, surrogacy

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#### 31 Introduction

32 In a rapidly changing environment that is reshaping patterns of biodiversity across land- and 33 seascapes, it is more important than ever to focus conservation efforts on protecting 34 multiple facets of biodiversity, i.e. species, communities, and their evolutionary histories 35 (Carvalho et al., 2010). Landscape-specific evolutionary processes can help identify 36 conservation priority areas that achieve multiple objectives such as representing spatio-37 temporal patterns of genetic variation, population dynamics, and divergence (Funk et al., 38 2019, 2012). While patterns of evolutionary variation have been highlighted as an essential 39 biodiversity feature to ensure species' persistence through environmental change (Flanagan 40 et al., 2018; von der Heyden, 2017), there remains limited knowledge on the effectiveness 41 of different molecular metrics at capturing intraspecific evolutionary and adaptive potential 42 (Mittell et al., 2015).

43

#### 44 Integrating genetic and genomic variation into conservation planning

45 Over the past four decades, evolutionary processes have been captured by molecular
46 markers pertaining to a small portion of the genome, in particular mitochondrial DNA
47 sequences (mtDNA) and microsatellite loci (Moritz, 1994; Schlötterer, 2000). However, with
48 the advancement of next-generation sequencing (NGS), genome-wide single nucleotide

polymorphism (SNP) markers are steadily gaining popularity for both model and non-model
organisms (Ouborg et al., 2010). Therefore, theoretical calls for including evolutionary
processes in conservation decisions are shifting from incorporating genetic (e.g. AFLP:
Thomassen et al., 2011; mtDNA: Nielsen et al., 2017; and microsatellite: Beger et al., 2014;
Paz-Vinas et al., 2018) to genomic (e.g. SNP) variation (Allendorf et al., 2010; Shafer et al.,
2015).

55 With the advancement of genomic sequencing and the growing number of available 56 molecular markers, it is important to understand the advantages, as well as limitations, 57 unique to each marker type. Molecular markers can be classified as 'genetic', defined here 58 as the sequencing/genotyping of a specific region of the genome, or as 'genomic', defined 59 here as high-throughput sampling of the partial or entire genome (Table 1). Currently, 60 genetic markers, such as microsatellite loci, organelle-specific genes, and nuclear genes 61 (nDNA), make up most of the genetic data available to inform conservation decisions 62 (Lawrence et al., 2019; Schlötterer, 2004; Seeb et al., 2011). Yet, the use of genomic markers 63 is steadily increasing (Corlett, 2017), making more of such data available for conservation. 64 Genetic markers usually consist of few loci, with mitochondrial and chloroplast DNA (mtDNA 65 and cpDNA) sequences only containing DNA inherited via the maternal line. Most genetic 66 markers predominantly reflect neutral patterns (Table 1; Gebremedhin et al., 2009; Kohn et 67 al., 2006), but there is evidence that some regions of mtDNA (Bazin et al., 2006), as well as 68 microsatellite loci (Larsson et al., 2007) can be under selection, either directly or indirectly 69 via genetic hitchhiking, where markers are physically linked to regions under selection. 70 Comparatively, genomic markers can include thousands to millions of loci, 71 representing a much larger portion of the genome (Table 1; Allendorf et al., 2010; Garner et 72 al., 2016). Genome-wide SNPs are similar to nDNA and microsatellite loci in that they are bi-

73 parentally inherited, but SNPs better detect low levels of genetic structure at finer-scales 74 than the former (Puritz et al., 2012), and better represent genome-wide diversity (Fischer et 75 al., 2017). SNP datasets also provide an opportunity to identify markers putatively under 76 selection, termed candidate or outlier loci (from here onwards referred to as outlier loci; 77 Supplement 1), an important consideration given that these loci may provide adaptive 78 benefits to populations and species (Mahony et al., 2020; Narum et al., 2013). Even though 79 SNP datasets offer considerably more evolutionary information, biases can be introduced 80 during library preparation, sequencing, and bioinformatic analyses (Kofler et al., 2016; 81 Supplement 1). There are also limitations associated with restriction-site associated DNA 82 sequencing (RAD-Seq) to identify outlier loci (Lowry et al., 2017a), yet this approach is still 83 relevant and widely used (Lowry et al., 2017b; Supplement 1). Nevertheless, uncertainties 84 are also inherent in other biological data types that inform conservation decisions (Kujala et 85 al., 2013), and conservation scientists and managers must account for uncertainty 86 associated with genomic data.

87 Regardless of marker type, molecular data have the power to contribute a number 88 of metrics to the conservation planning toolbox. Three of the most commonly available, and 89 useful, metrics for conservation are those that describe patterns of genetic differentiation, 90 uniqueness, and diversity (Table 2; Beger et al., 2014). These include fixation indices, or F<sub>ST</sub>-91 based metrics, which report levels of intra-population heterozygosity in relation to other 92 populations, and, from a conservation perspective, form the basis for the concept of 93 Evolutionary Significant Units (ESUs; sensu Moritz, 1994; Table 2). For genomic data, these 94 metrics can be extended to outlier SNP  $F_{ST}$ -based metrics that can help identify different 95 selective pressures amongst populations (Table 2; Funk et al., 2012). Metrics of diversity are 96 also relevant for conservation, as populations with high levels of genetic diversity are

97 expected to exhibit longer persistence, as there is more standing variation for selection to 98 act on (Table 2; Hoffmann et al., 2015; Reed and Frankham, 2003). High genetic diversity 99 also correlates with increased ecosystem functioning and resilience (Ehlers et al., 2008; 100 Wernberg et al., 2018). Metrics of uniqueness, which for example can be measured by the 101 number of private alleles (i.e. alleles that are restricted to single populations), provide 102 further important criteria for conservation planning (Table 2; Nielsen et al., 2017; Paz-Vinas 103 et al., 2018; Slatkin, 1995). The percentage of private alleles/haplotypes in a population can 104 reflect past range expansions/contractions, as well as the amount of contemporary gene 105 flow with other populations (Maggs et al., 2008). Private alleles can inform two main 106 conservation objectives: i) to prioritize areas with high levels of private alleles, as they might 107 act as genetic reservoirs of variation, thus increasing the genetic insurance of the meta-108 population; or ii) to prioritize areas with low levels of private alleles, as these areas are likely 109 to be well-connected via gene flow, thus ensuring the persistence and functioning of the 110 meta-population. Further, genomic datasets can account for signals of local adaptation by 111 characterizing 'functional outlier loci', where the functionality of the locus is known via 112 genotype-phenotype analyses or expression profiling (Harrisson et al., 2014). However, 113 identifying 'true' outlier loci is challenging, potentially leading to the inclusion of false 114 positives (Hoban et al., 2016; Lotterhos et al., 2017; Lotterhos and Whitlock, 2015; 115 Supplement 1), and in many non-model species the functional relevance of outlier loci 116 remains unknown.

117

118 Investigating the surrogacy of genetic for genomic data within conservation planning
119 A key concern within conservation science is to determine how much information is
120 required to meet conservation targets defined *a priori*, as well as the trade-offs associated

121 with including broader versus finer scale biodiversity features (Wilson et al., 2007). 122 However, this question is rarely extended to molecular information (Goodwin et al., 2016), 123 despite the potential advantages of these metrics to identify areas of evolutionary 124 importance and resilience. While genomic approaches are well-suited to describe the 125 evolutionary potential of natural populations, such as populations distinguished by highly 126 differentiated loci that signal selection in response to local conditions (Funk et al., 2019), the 127 number of available genomic datasets for conservation is lagging behind those using 128 traditional markers (Ouborg et al., 2010; Seeb et al., 2011). In many parts of the world, 129 especially developing countries where conservation action is needed the most (Wilson et al., 130 2016), the only available multi-species molecular data will likely be genetic. Thus, it is 131 important to understand a) how spatial prioritizations change when including genetic or 132 genomic metrics and b) whether genetic metrics can adequately represent highly 133 differentiated areas (indicating unique evolutionary potential) identified by genomic 134 markers. In this study, we utilize genetic and genomic data generated for multiple marine 135 species to explore the applicability of genetic metrics as surrogates of genomic variation 136 within an empirical conservation planning framework.

137

#### 138 Methods

139 While genomic markers are considered the ideal approach to identify loci under selection,

140 genetic approaches may inadvertently also be able to capture patterns of selection for

141 conservation purposes (Bridge et al., 2016). To test how well different molecular

142 approaches capture selective signals in a conservation planning framework, we utilized

143 available datasets previously generated by Mertens et al. (2018) and Nielsen et al. (2018).

144 Here we took advantage of these unique datasets, which were sampled from the same six

145	sample sites for both genetic and genomic approaches, making them comparable in spatial
146	planning analyses (Supplement 2). Molecular metrics were derived from previous studies
147	including mtDNA data for five species (Granular limpet - Scutellastra granularis, Cape urchin
148	- Parechinus angulosus, Cushion star - Parvulastra exigua, Topshell winkle - Oxystele tigrina,
149	and Super klipfish - Clinus superciliosus; Mertens et al., 2018), and SNP data for two of these
150	species (S. granularis and P. angulosus; Supplement 3; Nielsen et al., 2018). These species
151	are characterized by different niches, life-history traits, reproductive strategies and larval
152	duration periods (Mertens et al. 2018), and as such represent the species and functional
153	diversity of rocky shore ecosystems of the study region. The mtDNA data consists of the
154	cytochrome oxidase 1 (CO1) gene for the invertebrates and the control region for C.
155	superciliosus, with total numbers of individuals collected ranging between 128 (C.
156	superciliosus) and 197 (S. granularis). Parechinus angulosus, C. superciliosus and P. exigua
157	show varying levels of population divergence, regardless of the mtDNA marker used,
158	whereas S. granularis and O. tigrina appear panmictic along the study region.
159	Due to cost restraints, SNP datasets could only be generated for two species, with <i>P</i> .
160	angulosus and S. granularis chosen as these display the highest and lowest levels of
161	population divergence using mtDNA data. Equal numbers of individuals were collected per
162	species (n = 40) for the same sampling points along the South African west coast and a
163	pooled RAD-seq approach (Supplement 1) was used to calculate metrics of population
164	diversity and divergence (Kahnt et al., 2018; Nielsen et al., 2018; Phair et al., 2019). The SNP
165	data, consisting of ~ 8 000 SNPs for <i>P. angulosus</i> and ~55 000 SNPs for <i>S. granularis</i> , showed
166	levels of population divergence, and high levels of private and outlier SNPs within the
167	northern populations for both species (Nielsen et al. 2018).

169 Empirical spatial prioritization analyses

We chose to include metrics of both diversity and uniqueness (Table 2) and therefore
included nucleotide diversity (π) and percentage of private alleles, respectively, for both
mtDNA and SNP markers. In addition, the genomic dataset allowed us to include one metric
of putative selection (measured as the percent of outlier SNPs).

174 To compare the prioritizations of different molecular metrics, we utilized the 175 decision-support tool Marxan (Ball et al., 2009), which uses a simulated annealing algorithm 176 to identify sets of sites that meet given conservation targets at minimum costs. We included 177 the rocky shore and mixed shore habitat types as our domain, which were divided into 3 x 3 178 km hexagonal planning units. As conservation planning efforts often have to consider an 179 array of socio-economic pressures in balance with meeting biological targets, we included 180 an 'opportunity cost' information in all scenarios. The Marxan algorithm uses cost to 181 prioritize planning units that capture the greatest amount of each biological feature, whilst 182 keeping the total cost of the prioritized set of sites at a minimum (Margules et al., 1988). We 183 applied opportunity cost data derived from Majiedt et al. (2013), which is based on 27 184 marine pressure factors combining extractive practices and marine resource uses, and 185 ranging from commercial fishing, shipping, diamond and titanium mining, to coastal 186 development. This dataset was specifically assembled for the South African west coast and 187 is thus representative of the economic activities of the region. 188 As a baseline scenario, we included five rocky shore substrate types (boulder, 189 exposed, very exposed, sheltered and mixed coast; Sink et al., 2012) as conservation 190 features, to which all molecular metrics were subsequently added (Table 3). Following best 191 practice for generating 'genetic surfaces' (Murphy et al., 2008; Vandergast et al., 2011), we

192 interpolated the point values over the planning region using the Inverse Distance Weighting

193 (IDW) algorithm in ArcGIS v.10.3.1 (Desktop, 2011). We reclassified the IDW raster layers 194 with the 'reclassify' tool in ArcGIS, normalizing the continuous point values of each metric 195 into three equal-interval bins or classes: low, medium, high. For each species, each metric 196 was a single feature with three different levels pertaining to the low, medium and high 197 classes derived from the range of values specific to that metric for that species. We chose to 198 specifically explore the conservation objective of protecting the evolutionary potential 199 (Table 2), by selecting a range of conservation targets (i.e. 20-80%) for solely the high-200 ranking classes of each molecular feature.

201 We ran five spatial prioritization scenarios to test the effect of increasing molecular 202 information (Table 3): coastal habitat types (base), mtDNA diversity and uniqueness metrics 203 for two species (mt2), or five species (mt5), two SNP metrics (diversity and uniqueness) for 204 two species (*snp2*), and three SNP metrics (diversity, uniqueness, and selection) for two 205 species (*snp3*). We did not run a prioritization based solely on putative outlier SNPs as the 206 potential function of such SNPs remains unknown (Table 2; Harrisson et al., 2014). For each 207 scenario, we ran Marxan with default parameters, a boundary length modifier of zero, and 208 100 repeats to account for variability within the conservation solutions. To compare the 209 conservation solutions, we conducted hierarchical clustering using Euclidean dissimilarities 210 from the selection frequencies (i.e. the number of times the unit was selected out of 100 211 runs) per scenario in RStudio v.1.1.423 (Team, 2015).

To assess how well each conservation scenario captured putative selection patterns of *P. angulosus* and *S. granularis*, we calculated a population-specific Selection Index in a similar manner as the Population Adaptive Index described by Bonin et al. (2007b). We created an allele frequency distribution for each outlier SNP, and if in a specific population, the outlier was in the 95<sup>th</sup> percentile of the overall allele frequency distribution, then it was

217 counted towards the Selection Index of that specific population. The Selection Index was 218 interpolated across the entire planning region employing the IDW technique. We calculated 219 the Selection Index captured per scenario by multiplying the Marxan selection frequency by 220 the Selection Index of each planning unit, which was summed across all planning units per 221 scenario. 222 To test the effect of increasing species, we ran another set of scenarios (Table 3): 223 mtDNA data from one (*mt1r*), two (*mt2r*), three (*mt3r*), and four species (*mt4r*), ran five 224 separate times each, including randomly selected species. We compared the total Selection 225 Index captured by these scenarios as above. 226 227 Results 228 Spatial prioritization analyses 229 The scenarios comparing different amounts of molecular information showed a variety of 230 spatial priorities, with the two most similar being mt5 and snp3, followed by mt2 and snp2, 231 and the baseline being the most distinct (Fig. 1). Most of the conservation targets displayed 232 the same patterns between scenarios, with the exception of the 20% target, which showed 233 *mt5* as the most divergent scenario, and *mt2* and *snp3* being the most similar (Fig. 1). 234 235 Performance of conservation objectives in capturing local selective signals 236 When comparing the Selection Index between scenarios varying in the amount of molecular 237 information, the two worst performing scenarios were *base* and *snp2*, with both only 238 capturing a small portion of the *P. angulosus* Selection Index (Fig. 2). In contrast, the two 239 best performing scenarios were *mt5* and *snp3*, which consistently had the highest Selection 240 Index for both species across the conservation targets (Fig. 2).

The scenarios comparing molecular data from different numbers of species show a positive relationship between the number of species included and the Selection Index for *S. granularis*, but the Selection Index captured plateaus around three species for *P. angulosus* for each target coverage (Fig. 3)

245

#### 246 **Discussion**

247 This study offers the first experimental comparison of multi-species genetic and genomic 248 datasets within spatial conservation prioritizations. We present initial evidence of genetic 249 markers acting as adequate surrogates of genomic data in capturing evolutionary potential 250 in spatial plans (Fig 1, Fig. 2). We find that including measures from either putatively neutral 251 or outlier genomic regions will change conservation solutions (Fig. 1), highlighting the 252 potential of NGS techniques, specifically the identification of outlier loci, to enhance reserve 253 designs (Ouborg et al., 2010). Our results also show that habitat types, single-species 254 genetic data, and multi-species neutral genomic data inadequately capture evolutionary 255 potential (Fig. 2, Fig. 3). Broadly, the findings suggest a trade-off between the number of 256 species and the amount of molecular information included (Fig. 1, Fig. 2). This has 257 implications for spatial conservation planning, as genetic data may in many cases be more 258 economical to generate compared to NGS (Langmead and Nellore, 2018), and is readily 259 available, especially for a wider array of species (Lawrence et al., 2019; Seeb et al., 2011). 260 Our results also illustrate how spatial priorities are highly dependent on the number 261 of species included regardless of conservation target (Fig. 3), and thus multi-species 262 approaches are essential for conservation prioritizations with genetic data (Fig. 2, Fig. 3; 263 Paz-Vinas et al. 2018). This is most likely driven by differences in ecological traits and 264 evolutionary histories of species, where co-distributed and even closely related species can

265 display highly divergent patterns of spatial genetic variation (Borsa et al., 2016;

Papadopoulou and Knowles, 2016). As umbrella- and surrogate-species show inconsistent
efficiency in representing species distributions and assemblages, as well as genetic patterns
(Carvalho et al., 2010; Ponce-Reyes et al., 2014), basing conservation plans on multi-species
datasets should increase the likelihood of protecting complex evolutionary histories within a
region (Nielsen et al. 2017; Paz-Vinas et al. 2018).

271

272 Potential surrogates of genomic selective signals in spatial conservation planning

273 From a spatial planning perspective focused on capturing evolutionary potential, we find 274 that including less molecular information (i.e. a single mtDNA locus) for several species can 275 effectively represent putative adaptive variation identified from vastly more genomic 276 information (i.e. thousands of SNP loci) from fewer species (Fig. 1, Fig. 2). The results also 277 show that the overall Selection Index increased with the number of species included (Fig. 3), 278 suggesting that including genetic variation for multiple species may inadvertently capture 279 local selective pressures. However, this pattern was mainly seen in S. granularis (Fig. 3), 280 which highlights how the signals of selective pressures are likely to be species-specific. As 281 spatial plans may only include genomic signals from one or a few species that are likely 282 species-specific, it is essential that surrogates of selection are included for additional species 283 to achieve multi-species complementarity within this biodiversity feature.

Our findings corroborate those of Hermoso et al. (2016), who found that species distribution patterns of 46 freshwater fish were effective surrogates for the genetic patterns of four individual fish species. Furthermore, Wright et al. (2015) found that species richness patterns of over 2 500 species mirrored the patterns of genetic diversity of 11 rocky shore species, suggesting that species- and molecular-level patterns may be a product of similar

processes (Vellend, 2005). Conversely, Paz-Vinas et al. (2018) showed that conservation
solutions based on the co-occurrence of high numbers of species did not capture the
intraspecific genetic diversity of a set of six fish species. Species richness patterns have also
been shown to insufficiently represent phylogenetic diversity (Pio et al., 2011), supporting
the need to incorporate fine-scale molecular data to adequately conserve evolutionary
patterns.

295 In addition to using species-level data as surrogates of genomic variation, abiotic 296 factors may also be potential proxies of local selection in spatial conservation plans. For 297 example, when species distribution data is incomplete, partitioning reserves along 298 biophysical gradients will incidentally capture a representative amount of areas pertaining 299 to intraspecific evolutionary potential (Bridge et al., 2016; Carvalho et al., 2010). Further, 300 Hanson et al. (2017) found that environmental features are adequate surrogates of adaptive 301 variation in AFLPs across ten alpine plant species. Yet the effectiveness of selecting 302 environmentally diverse areas to incidentally capture genomic adaptive potential is still 303 largely unexplored, especially within the marine environment.

304

305 A way forward comparing molecular approaches in conservation planning

306 Capturing multi-species genomic signals with genetic metrics provides exciting opportunities

307 to utilize available genetic datasets within conservation planning efforts. Our study provides

308 a baseline for comparing genetic and genomic approaches in capturing evolutionary

309 potential, however further work is required to truly expand the results obtained here to

- 310 other natural systems. As our study domain is over a relatively small region, it is crucial to
- 311 test whether similar results are found over larger scales and across different environmental
- 312 systems. While this study would benefit from additional sample sites, we were restricted to

313 using genetic and genomic datasets from the same locations, as different sample sites 314 would influence conservation outputs and bias comparisons between data types. Our work 315 also included genetic data from mtDNA loci, leaving the ability of other genetic metrics to 316 capture genomic signatures unexplored. For example, microsatellite-derived metrics will 317 likely be more effective surrogates of genomic datasets as they are bi-parentally inherited 318 and show higher intraspecific variability, although this also remains to be formally tested. 319 Furthermore, our SNPs were obtained from a pooled, reduced representation 320 sequencing approach, which is increasingly being used to generate allelic frequencies for 321 populations (Kurland et al., 2019). This methodology has several merits, namely a cost-322 effective increase in the number of individuals that can be sequenced per population, which 323 can increase the accuracy of allele frequencies, but also a few limitations, such as low 324 frequency alleles and portions of the genome outside of restriction cut sites may be 325 undetected (see Supplement 1 for further details). However, it was recently found that 326 abundant/fine-scale sampling has greater influence on genomic patterns than more in-327 depth genomic sequencing (D'Aloia et al., 2020), and thus we chose to include more sample 328 sites and individuals per sample site, rather than more detailed genomic information per 329 individual. To further strengthen our findings, additional efforts comparing trade-offs 330 between traditional genetic markers and whole-genome sequencing data in conservation 331 planning scenarios is recommended.

Our analyses were focused on comparing genetic and genomic metrics of diversity rather than differentiation, and as such we did not include scenarios based on genetic clusters, although it is essential that these approaches are compared in their efficiency in capturing putatively neutral and adaptive population clusters. Several studies have found similar differentiation patterns between the two molecular marker types (e.g. Benestan et

al., 2015; Dowle et al., 2015; Fernández et al., 2016; Ford et al., 2015), while, alternatively,
several others have identified fine-scale structuring with genomic data where genetic data
did not (e.g. Blanco-Bercial and Bucklin, 2016; Castellani et al., 2012; Dierickx et al., 2015;
Maroso et al., 2016). Therefore, a comprehensive analysis should be conducted to
understand if genomic-derived clusters significantly alter conservation scenarios, in order to
better assess the trade-offs between the two marker types.

343 Assessing different marker types, population differentiation metrics, and broader 344 study regions will further resolve the trade-offs between molecular information and species 345 included into conservation plans. In addition, while this study compares molecular metrics 346 of differentiation, uniqueness, and diversity, we recognize that these are not the only 347 molecular features of conservation importance. Many others, including estimates of 348 effective population size (Frankham et al., 2014), demographic change (Garza and 349 Williamson, 2001), inbreeding (Marshall et al., 1999) and hybridization (Buonaccorsi et al., 350 2005), as well as migration estimates (von der Heyden et al., 2008) may all be relevant 351 measures to support conservation objectives in spatial planning. 352 Overall, this study highlights how genomic signals of evolutionary potential in two 353 species can be adequately represented by putatively neutral genetic variation across five

354 species, even at low conservation targets. We offer preliminary evidence that genetic data

355 may be a cost- and time-effective surrogate for genomic data when seeking to conserve

356 putative adaptive variation, but ultimately more work is needed to confirm these results

357 within larger conservation settings.

358

359 Appendix A. Supplementary data

- 360 A comparison of different genomic sequencing and outlier detection methodologies
- 361 (Appendix S1), as well as sample sites (Appendix S2) and sampling information (Appendix

362 S3) on datasets used within the spatial analyses are available online. The authors are solely

- 363 responsible for the content and functionality of these materials. Queries (other than
- 364 absence of the material) should be directed to the corresponding author.
- 365

#### 366 Declaration of Competing Interest

367 There are no perceived actual or potential conflicts of interest including any financial,

- 368 personal or other relationships with other people or organizations that could
- 369 inappropriately influence, or be perceived to influence, our work. All funding agencies are
- acknowledged.
- 371

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- 671 **Table 1.** Comparisons of genetic and genomic approaches, including markers available, as
- 672 well as marker-specific pros and cons.

Data	Marker	Markers	Pros	Cons
type	characteristics			
Genetic	Maternally or	Amplified	Standardised analyses,	Less power to detect
data	bi-parentally	Fragment	less researcher-based	genetic differentiation
	inherited	Length	bias, easy to interpret	
		Polymorphisms		Usually detects
	Dominant or	(AFLPs),	Lower costs as fewer	historical measures of
	co-dominant	chloroplast DNA	markers sequenced,	diversity (unless
		(cpDNA),	requires greater number	microsatellites)
	One or a few	mitochondrial	of individuals sampled in	
	loci	DNA (mtDNA),	each population	Generally not capable
		nuclear DNA		of detecting adaptive
		(nDNA) <i>,</i>		variation (unless
		microsatellites		indirectly – e.g.
				genetic hitchhiking in
				microsatellites)
Genomic	<b>Bi-parentally</b>	SNPs, outlier	Potentially provides	Data analysis often
data	inherited	SNPs	greater resolution of	requires training in
			population structure,	command-line or
	Co-dominant		demographic processes	outsourcing of
			etc., due to the	bioinformatic
	Potentially		availability of thousands	analyses
	thousands of		of markers for both	
	loci		neutral and adaptive loci	Lack of standardized
				analyses
				Outlier detection

methods prone to false positives

**Table 2.** Definitions of genetic and genomic features, their conservation relevance and how they contribute towards spatial planning by capturing either spatial-temporal processes or evolutionary potential.

Molecular features	Conservation relevance	Potential conservation objectives
Measure: differentiation		
- Genetic & Genomic: Local <i>F</i> <sub>ST</sub>	Low distinctiveness indicates that a site is connected to others by high levels of gene flow, meaning it can increase resilience by supplementing individuals to the meta-population	Avoid low differentiation regions (prioritize evolutionary potential)
Measures how much the genetic diversity		
of a population differs from the mean of		Protect low differentiation
all other populations combined	High distinctiveness indicates low levels of gene flow, meaning it might have lower resilience to stochastic change due to it not	regions (prioritize connectivity)
- Genetic & Genomic: Pairwise <i>F<sub>ST</sub></i>	being connected to the meta-population. It could also indicate	
Measures how much the genetic diversity differs between two populations	local adaptation or recent bottleneck events. In this case, a site can potentially play an important role in increasing the resilience of the meta-population by harboring unique alleles that can be advantageous in future environmental changes	Give highly differentiated regions conservation priority (prioritize evolutionary potential)
		Prioritize connected regions as pairs instead of as individual sites (prioritize connectivity)
Measure: diversitv		
- Genetic: Haplotype diversity	Low diversity may indicate small effective population sizes, and a lower likelihood of adaptation from standing variation. These sites are at greater risk to inbreeding depression and stochastic change	Protect high diversity regions (prioritize evolutionary potential)

Probability that two randomly selected individuals differ in their haplotypes

Unlike nucleotide diversity, which is site specific, haplotype diversity is calculated from all populations, and therefore incorporates gene flow

- Genetic & Genomic: Nucleotide diversity

Average number of nucleotide differences per genomic site, between randomly chosen SNPs from within a population

#### Measure: uniqueness

- Genetic: Private haplotypes/alleles

Haplotypes/alleles unique to a single geographic area

- Genomic: Private SNPs

Neutral loci that are unique to particular areas. They are different to outlier loci (see below), as they are not under selection High diversity is beneficial for long-term persistence and adaptation as there is more 'raw material' for selection to act on. These regions are assumed to be more resilient to environmental change

Low levels of private alleles indicate that the region is connected to others by high levels of gene flow, meaning it can increase resilience by supplementing individuals to the meta-population

A high percentage of private alleles could indicate low levels of gene flow, meaning lower resilience to stochastic change. High levels of private alleles could also denote local adaptation or recent bottleneck events. In this case, the region can potentially play an important role in increasing the resilience of the metapopulation by harboring unique alleles that can be advantageous to future environmental changes Protect regions with few private alleles (prioritize connectivity)

Protect unique regions with many private alleles (prioritize evolutionary potential)

### <u>Measure: selection / evolutionary</u> potential

- Genomic: Non-functional outlier loci richness

Loci that are statistically significantly different from other regions of the genome. The function roles of the loci are often unknown

## <u>Measure: selection / evolutionary</u> <u>potential</u>

- Genomic: Non-functional outlier loci structure

Genetic clusters that are inferred from differentiation of outlier loci. The clusters can be displayed geographically to illustrate where genetic breaks occur

## Measure: selection / evolutionary potential

- Genomic: Functional outlier loci

Loci that are shown to under selection through experiments or through strong correlations with environmental A high percentage of outliers indicates that selection is occurring on individuals in that area. It may be assumed that the selection leads to increased fitness and that this selection will continue to benefit the individual fitness under future environmental conditions Protect populations with high levels of outlier loci (prioritize evolutionary potential)

Different genetic clusters are likely to be under different selective regimes; therefore, each cluster is assumed to have its own unique evolutionary trajectory. Each cluster can potentially act as genetic insurance for the meta-population

Aim to protect a percentage of each cluster in an adaptive landscape (prioritize evolutionary potential)

If environmental feature(s) driving selection and/or functional role of the outliers are known, these features would indicate which regions are of conservation importance

This scenario could also be of importance for human-mediated adaptive assistance. It is also important to note that this scenario is still quite rare and mainly confined to model organisms Protect areas in which environmental features are driving known adaptation

Protect areas where known adaptive genes occur

variables. The genes corresponding to the loci, as well as their functional roles, are often identified

Assist un-adapted sites with breeding programs

**Table 3**. The various prioritizations run in Marxan, with their included biodiversity features and species (CS = *Clinus superciliosus*, PA = *Parechinus angulosus*, PE = *Parvulastra exigua*, OT = *Oxystele tigrina*, SG = *Scutellastra granularis*)

Scenario I.D.	Biodiversity features compared	Species included
	Scenarios increasing in molecular data	
base	Five different habitat types; no genetic/genomic system features	NA
mt2	Diversity and uniqueness metrics from mtDNA markers for the two species with genomic data	PA, SG
mt5	Diversity and uniqueness metrics from mtDNA markers for five species	All
snp2	Diversity and uniqueness metrics from putatively neutral SNPs for the two species with genomic data	PA, SG
snp3	Diversity, uniqueness, and selection metrics for putatively neutral and highly differentiated SNPs for the two species with genomic data	PA, SG
	Scenarios increasing in species	
mt1r	Neutral metrics (diversity and uniqueness) from mtDNA markers for one randomly chosen species	All
mt2r	Neutral metrics (diversity and uniqueness) from mtDNA markers for two randomly chosen species	PE, CS PA, SG OT, PE OT, SG PE, SG
mt3r	Neutral metrics (diversity and uniqueness) from mtDNA markers for three randomly chosen species	PE, OT, SG CS, OT, PA CS, PA, PE CS, SG, PA PE, SG, PA
mt4r	Neutral metrics (diversity and uniqueness) from mtDNA markers for four randomly chosen species	PE, SG, CS, PA SG, OT, CS, PA CS, PE, SG, OT SG, OT, PE, PA PE, PA, OT, CS



**Figure 1.** Hierarchical clustering dendrograms illustrating the similarities between scenarios for conservation targets ranging from 20-80% of each biodiversity feature.



**Figure 2.** The Selection Index (SI) captured per species for genetic and genomic scenarios (see Table 3 for scenario explanations), shown for targets ranging from 20-80% (0.2-0.8) of each biodiversity feature.



**Figure 3.** The Selection Index (SI) captured per species for genetic scenarios with varying number of species (see Table 3 for scenario explanations), shown for targets ranging from 20-80% (0.2-0.8) of each biodiversity feature.

#### Supplementary data

**Appendix S1-** The definitions, as well as advantages and disadvantages, of the methods used obtain genomic inferences in the present study, compared to other possible molecular approaches. This is not an exhaustive list of molecular methodologies for conservation, but is rather restricted to those relating to next generation sequencing (NGS) methods for DNA-based sequencing, pertaining to sequencing and identification of outlier loci, and some considerations with regards to their use for conservation. The methods used to generate the genomic data within the present study are indicated in bold. It should also be noted that even using the NGS techniques with lowest costs/highest uncertainties, the amount of genomic information, and sequencing costs, are still substantially greater than traditional genetic techniques (see Table 1 for genetic and genomic comparisons).

1: Allocation of samples for sequencing					
Method	Definition	Advantages	Disadvantages	References	
Individual	Individuals have distinct barcodes	Individual haplotypes/genotypes are	High sequencing costs, likely leading to	Anderson et al.	
sequencing	and are sequenced with other	available	trade-offs between individuals and	(2014)	
	barcoded individuals		sample sites sequenced, as well as the		
		Can conduct assignment tests, and	depth of coverage	Dorant et al. (2019)	
		calculate clonality, relatedness,			
		inbreeding coefficients, and linkage		Ferretti et al. (2013)	
		disequilibrium			

Pooled sequencing	Individuals are pooled into a single	Low sequencing costs, making it feasible	Individual genotypes and inferences	Gautier et al. (2013)
(Pool-seq)	sample (usually pertaining to a	to sample many locations and individuals	from them are unavailable	
	sample site/population). Each	per location, as well as making NGS		Hivert et al. (2018)
	pooled sample receives a barcode	available for many groups in developing	Need accurate equimolar pooling	
	and is sequenced with other	regions, especially when combined with a	across individuals to best attain allelic	Inbar et al. (2020)
	barcoded samples	reduced-representation sequencing type	variance. Errors from unequal	
		(i.e. Probe-/RAD-seq detailed below)	representation between individuals in	Kofler et al. (2016)
			a sample can be reduced by large pool	
		Accurate allele frequencies for outlier	sizes and removal of PCR duplicates	Kurland et al. (2019)
		detection methods, as the number of		
		sequenced individuals is usually higher	Contamination and sequencing biases	Lynch et al. (2014)
		per population than individual	can occur on the population rather	
		sequencing	than individual level	Rellstab et al. (2013)
			Low frequency alleles will be lost in	Schlötterer et al.
			bioinformatic stages aimed at filtering	(2014)
			sequencing artefacts	
				Tilk et al. (2019)
		2: Sequencing type		
Whole genome	Portions of the genome (termed	Extensive and complete genomic	Exceedingly high costs, especially when	Andrews et al.
sequencing (WGS)/	'reads') are sequenced and either:	information	using individual sequencing at high	(2016)
Whole genome	1) assembled to create a genomic		coverage across the genome. To	
resequencing (WGR)	sequence for the first time (WGS),	One single continuous genomic	counteract the high costs, many	Andrews and Luikart
	or 2) mapped onto an existing	sequence, allowing for no gaps in the	studies use Pool-seq WGS or low-	(2014)
	reference genome to compare	data	coverage WGS (IcWGS)	
	genomic variability between			Carpenter et al.
	individuals/populations (WGR)	Increased power for statistical analyses	Many research questions, and	(2013)
			inferences for conservation (such as	
			population structure and diversity) do	Catchen et al. (2017)
			not require the entire genomic	
			sequence, and thus the high	da Fonseca et al.
			sequencing costs can be avoided by	(2016)

			using reduced-representation sequencing instead	Díaz-Arce and
Probe-based/exome	The complexity of the genome is	Lower costs than WGS/WGR	The development of panels for capture	Rodríguez-Ezpeleta
capture sequencing	reduced by pre-selecting genomic		leads to increased costs and time spent	(2019)
	regions (such protein coding	Provides high depth and uniformity of	on preparation for sequencing	
	regions, termed 'exons'), and using	coverage within the designated sequence		Fuentes-Pardo and
	a DNA probe to target the regions	regions	Designated sequence regions need to	Ruzzante (2017)
	of interest for sequencing. These		be known <i>a priori,</i> making it unsuitable	
	regions are mapped onto an	The precise, targeted nature of probe-	for many non-model organisms which	Goodwin et al.
	existing reference genome	based sequencing can be beneficial when	lack any prior genomic information	(2016)
		DNA is degraded	(but see Puritz and Lotterhos, 2018)	
				Graham et al. (2020)
		Can be used to assess epigenetic	May not be suitable to for exploratory	
		modifications (which affect how genes	questions regarding genome-wide	Lowry et al. (2017a,
		are expressed in the genome)	evolutionary processes	2017b)
Restriction site-	The complexity of the genome is	Low costs allow for sequencing of	Possibility of mutations at restriction	
associated	reduced by using restriction	multiple individuals/populations, and at	sites leading to ineffective cutting of	Mckinney et al.
sequencing	enzymes to cut out regions	higher coverage. It may also be the most	fragments (i.e. allele dropout)	(2017)
(RAD-seq)*	throughout the genome (both	feasible method for smaller research		
	coding and non-coding). The areas	groups, especially within developing	Sequence amplification during library	Meek and Larson
	adjacent to the cut sites are then	countries, to perform genome-wide	preparation can lead to unequal	(2019)
	sequenced. The sequenced	studies	duplication of one allele over another	
	fragments can be assembled de		within an individual. These 'PCR	Narum et al. (2013)
	novo, or mapped onto an existing	No genomic information is needed a	duplicates' can however be identified	
	reference genome	<i>priori,</i> making it suitable for non-model	and removed during bioinformatic	Nielsen et al. (2011)
		organisms, and with <i>de novo</i> genome	processing	
		assembly		Reitzel et al. (2013)
			The length of the areas adjacent to the	
		Flexibility of restriction enzymes make it	cut sites (i.e. the sequenced fragments	Rowe et al. (2011)
		easily scalable, sequencing few loci at	linked to these sites) varies both	
		high coverage or more loci at low	between individuals and species,	leer and Mullikin
		coverage	meaning that the portion of the	(2010)
			genome sequenced can be more or	

			less incomplete depending on the level of linkage disequilibrium	
		<i>3: Outlier detection</i>	-	
Genetic– environment	The variance in allele frequencies is statistically compared with the	Can detect signals of selection which do not necessarily lead to high	Genomic sample sites need to cover the entire environmental gradient	Beaumont (2005)
associations (GEAs)	variance with environmental	differentiation between populations	Pequires high quality environmental	Bierne et al. (2013)
	selective pressures. Thus, loci which have allele frequencies	Multivariate GEAs (specifically constrained ordinations) may be more	data, and results may differ depending on the environmental data included	Booker et al. (2020)
	strongly associated with an environmental variable are identified as outliers	powerful in detecting weak, multilocus selection signatures	A high rate of false negatives may occur if the direction of past	Dalongeville et al. (2018)
			population expansion mirrors the environmental gradient	Foll and Gaggiotti (2008)
			Results have been shown to differ between outlier detection models	Forester et al. (2017)
			(Dalongeville et al., 2018; Forester et al., 2017)	Grummer et al. (2019)

Differentiation/Fsr-	<i>F</i> <sub><i>st</i></sub> is a commonly used measure of	Does not require high quality	Cannot test specific hypotheses as to	
based	population differentiation. $F_{ST}$ –	environmental data or large number of	which environmental selection	Helyar et al. (2011)
	based outlier tests are built on the	genomic sample sites	pressures are acting on outlier loci	
	concept of neutral SNPs being			Lotterhos and
	affected in a similar manner by	Outliers can be identified as either under	May not perform well when there are	Whitlock (2015)
	processes such as gene flow and	balancing or divergent selection	exceedingly high levels of selection	
	genetic drift, while outlier SNPs will		biasing the baseline <i>F</i> <sub>ST</sub> variation	Luikart et al. (2003)
	be affected in a distinct manner by			
	the process of selection. Outliers		Neutral variation is increased when	Narum and Hess
	are thus selected from loci which		demographic processes occur within a	(2011)
	have significantly high (indicating		linear fashion, which may lead to high	
	divergent selection) or low		rates of false positives	Rellstab et al. (2015)
	(indicating balancing selection) $F_{ST}$			
	values		Results have been shown to differ	
			between outlier detection models	
			(Lotterhos and Whitlock 2015; Narum	
			and Hess 2011)	

\* There is a plethora of RAD-seq methods, which are discussed in detail within Andrews et al. (2016). The RAD-seq method used to obtain the data within the present study was a paired-end ezRAD approach (sequenced on the Illumina Mi-seq platform), which was selected as it does not rely on a PCR step to amplify sequences during library preparation, its use with Pool-seq has been compared and validated against individual sequencing, and has been shown to produce high quality data for non-model marine invertebrates (Toonen et al., 2013).

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**Appendix S2.** The six sample sites pertaining to the genetic and genomic material.



**Appendix S3**. The genetic and genomic datasets, and the associated molecular marker and species, which were included into conservation planning scenarios. The gene region and base pair (bp) length is shown for mtDNA markers, and the number of putatively neutral and adaptive single nucleotide polymorphisms (SNPs) are shown for Pool/RAD-seq SNP markers.

Species	Reference
Cape urchin ( <i>Parechinus angulosus</i> )	Mertens et al. (2018)
Granular limpet ( <i>Scutellastra</i> granularis)	Mertens et al. (2018)
Tiger topshell winkel ( <i>Oxystele</i> <i>tigrina</i> )	Mertens et al. (2018)
Cushion star ( <i>Parvulastra exigua</i> )	Mertens et al. (2018)
Super klipfish ( <i>Clinus superciliosus</i> )	Mertens et al. (2018)
Granular limpet ( <i>Scutellastra</i> granularis)	Nielsen et al. (2018)
Cape urchin (Parechinus angulosus)	Nielsen et al. (2018)
	Species Cape urchin (Parechinus angulosus) Granular limpet (Scutellastra granularis) Tiger topshell winkel (Oxystele tigrina) Cushion star (Parvulastra exigua) Super klipfish (Clinus superciliosus) Granular limpet (Scutellastra granularis) Cape urchin (Parechinus angulosus)