Highly replicated evolution of parapatric ecotypes

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Abstract

Parallel evolution of ecotypes occurs when selection independently drives the evolution of similar traits across similar environments. The multiple origin of ecotypes is often inferred on the basis of a phylogeny which clusters populations according to geographic location and not by the environment they occupy. In contrast, when ecotypes arise once, expand their range and colonise similar environments, their populations cluster by ecology and not geography. However, discriminating between these scenarios is difficult because gene flow upon secondary contact can create the appearance of multiple origins despite a true single origin history. Here, we convincingly demonstrate multiple origins within the Dune and Headland ecotypes of an Australian wildflower, *Senecio lautus*. We observed phylogenetic clustering by geography and strong genetic structure between populations. There was surprisingly little gene flow between parapatric ecotypes, which is not high enough to obscure a single origin history. Overall, our work highlights the importance of demonstrating that populations have arisen repeatedly and independently within studies of parallel evolution.

Introduction

Governed by natural selection, parallel evolution occurs when populations evolve similar traits after repeatedly and independently colonising similar habitats (Schluter & Nagel, 1995). The patchy distribution of phenotypically similar populations means they frequently occur next to other contrasting forms (e.g., plant species inhabiting serpentine and non-serpentine soils in Scandinavia (Berglund *et al.*, 2003), and marine snails adapted to crab predators or wave action along the rocky coasts of Spain (Johannesson *et al.*, 2010). Parallel evolution by natural selection creates consistent patterns of phenotypic similarity and divergence that can extend to morphological (Elmer *et al.*, 2010; Ravinet *et al.*, 2013; Perreault-Payette *et al.*, 2017), behavioural (York & Fernald, 2017), and reproductive (Smith & Rausher, 2011) traits. The nature of parallel trait evolution largely depends on the demographic history of the system under investigation, where the interplay of geography, gene flow, and natural selection with the genetic architecture of traits determines its repeatability (Orr, 2005; Stern & Orgogozo, 2009; Rosenblum *et al.*, 2014; Lenormand *et al.*, 2016; Stoltzfus & McCandlish, 2017; Blount *et al.*, 2018; Yeaman *et al.*, 2018). However, it is surprisingly rare for studies of parallel evolution to convincingly demonstrate that populations have arisen in an independent and repeated fashion (hereafter multiple origin). Ruling out alternative demographic scenarios, such as a single origin of forms followed by gene flow upon secondary contact, is seldomly performed (but see Quesada *et al.*, 2007; Bierne *et al.*, 2013; Butlin *et al.*, 2014; Pérez-Pereira *et al.*, 2017, and see Ostevik *et al.*, 2012 for a critical review of the evidence in plants). In light of this, researchers may incorrectly assume a parallel colonisation history, leading to inaccurate inferences about the prevalence of parallel evolution in nature.

Typically, researchers of parallel evolution by natural selection ask whether phylogenetic clustering of populations coincides with the geography and not with the ecology of populations (Allender *et al.*, 2003; Quesada *et al.*, 2007; Johannesson *et al.*, 2010; Butlin *et al.*, 2014; Trucchi *et al.*, 2017). This is because genetic clustering of geographically close populations implies dispersal might be geographically restricted (i.e., isolation by distance; Wright, 1943), and colonisation of contrasting and neighbouring habitats might have occurred independently many times. However, alternative historical scenarios could also lead to clustering of populations by geography, and must be ruled out before examining the evolution of traits in light of parallel evolution (Endler, 1977; Barton & Hewitt, 1985; Coyne

52 & Orr, 2004; Bierne *et al.*, 2013). To understand this problem, first consider a scenario where
53 an ancestral population gives rise to two locally adapted populations that occupy distinct yet
54 geographically proximate habitats (hereafter ecotypes, Figure 1A). These two populations
55 migrate and colonise new localities, where the same contrasting habitats are geographically
56 close each time. This scenario of a single split followed by range expansion of two ecotypes
57 does not have a parallel colonisation and adaptation history because each ecotype only arose
58 once (rather than multiple independent times after independent colonisation of contrasting
59 habitats). Because gene flow is either not possible after the original ecotypic split, or does not
60 homogenise adjacent populations after range expansion, populations sharing the same
61 ecology form reciprocally monophyletic clades in a phylogeny (Figure 1A).

62 Nevertheless, if there is sufficient gene flow between geographically close populations from
63 two ecotypes that originated only once, the original phylogenetic signal of reciprocal
64 monophyly can be eroded (Endler, 1977; Barton & Hewitt, 1985; Coyne & Orr, 2004; Bierne
65 *et al.*, 2013). In other words, as the original signal of a single origin disappears, populations
66 become most related to their neighbouring population and not to the other populations of the
67 same ecotype. Therefore, gene flow can result in grouping of populations by geography
68 rather than ecology if many loci are homogenised (Figure 1B). This phylogenetic signal is
69 identical to that of true parallel evolution (a multiple origin scenario), where populations
70 from two ecotypes arise multiple independent times (Figure 1C). Gene flow dynamics can
71 thus fundamentally alter our interpretation of parallel evolution, to the extent that we can
72 mistakenly infer parallel evolution in systems where secondary contact after range expansion
73 of a single origin fused the history of locally adapted populations (Endler, 1977; Barton &
74 Hewitt, 1985; Coyne & Orr, 2004; Bierne *et al.*, 2013).

75 However, not all levels of gene flow have the same equivocal effect in the genetic record of
76 colonisation history (Bierne *et al.*, 2013). This makes it difficult to distinguish single from
77 multiple origins of ecotypes. Systems of parallel evolution frequently detect gene flow
78 between populations, especially when contrasting ecotypes are in close geographic proximity
79 (i.e. parapatry). However, only few studies comprehensively model the demographic history
80 of populations (Quesada *et al.*, 2007; Bierne *et al.*, 2013; Butlin *et al.*, 2014; Meier *et al.*,
81 2017; Pérez-Pereira *et al.*, 2017; Trucchi *et al.*, 2017), and even fewer have used simulations
82 to address whether the levels of gene flow can obscure the observed phylogeny (Bierne *et al.*,
83 2013; Pérez-Pereira *et al.*, 2017). The system that has perhaps most clearly demonstrated the

84 parallel origins of forms in the presence of gene flow is the marine snail *Littorina saxatilis*.
85 Multiple lines of evidence suggest the wave and crab ecotypes have evolved multiple
86 independent times along rocky coastlines (Quesada *et al.*, 2007; Johannesson *et al.*, 2010;
87 Bierne *et al.*, 2013; Butlin *et al.*, 2014; Pérez-Pereira *et al.*, 2017). Also, an obvious extreme
88 case of multiple origins arises when parallel evolution occurs between geographically distant
89 populations where lack of gene flow cannot obscure the history of colonisation (e.g.,
90 threespine stickleback populations that colonise separate continents (Magalhaes *et al.*, 2019) .
91 However, in other systems where gene flow is moderate between ecotypes (Rougemont *et al.*,
92 2015; Le Moan *et al.*, 2016; Rougeux *et al.*, 2017, 2019; Herman *et al.*, 2018), it remains
93 unclear to what extent gene flow contributed to the signal of parallel evolution.

94 Identifying the genetic basis of parallel trait evolution often provides unambiguous evidence
95 for parallel evolution of ecotypes. For instance, in sticklebacks, the repeated evolution of
96 pelvic loss in separate populations relied on different mutations in the same gene, suggesting
97 this adaptive trait has arisen multiple independent times (Chan *et al.*, 2010). Conversely, in
98 systems where the exact same mutation is repeatedly involved in adaptation (Colosimo *et al.*,
99 2005), it is more difficult to identify whether the trait was repeatedly selected for (i.e. via
100 standing genetic variation), rather than arising once followed by the repeated colonisation of
101 similar environments (Lee & Coop, 2019). Knowing the causal genes of adaptation is ideal as
102 the demographic history of individual adaptive loci can be modelled, avoiding the
103 complications of distinguishing between single and multiple origins using neutral
104 polymorphisms (as described above). However, directly isolating the specific genes involved
105 in adaptation is infeasible in most non-model organisms, particularly because genetic
106 experiments are not feasible or the genetic architecture of adaptation is highly polygenic
107 (Tiffin & Ross-Ibarra, 2014; Yeaman, 2015).

108 The above considerations suggest we need to characterise the origin and colonisation history
109 of forms as well as the repeated evolution of traits in systems where populations have adapted
110 to similar environments. Such an approach will clarify the possible role of natural selection in
111 shaping diversity in systems with broad geographic and ecological ranges, thus paving the
112 way for understanding the molecular basis of adaptation and its implications for our
113 understanding of predictability and repeatability in evolution. In this work, we characterise
114 the origin and colonisation history of *Senecio lautus*, an Australian wildflower that appears to
115 have evolved multiple times in parapatry into two contrasting coastal forms called Dune and

116 Headland ecotypes (Roda *et al.*, 2013; Melo *et al.*, 2014). The two forms differ in their
117 growth habit: the Dune ecotype is erect and colonises sand dunes, and the Headland ecotype
118 is prostrate, forming matts on the ground of rocky headlands (Ali, 1964; Radford *et al.*, 2004;
119 Thompson, 2005). These locally adapted populations (Richards & Ortiz-Barrientos, 2016;
120 Walter *et al.*, 2016) are separated by strong extrinsic reproductive isolation (Melo *et al.*,
121 2014; Richards *et al.*, 2016) and exhibit similar morphology of each ecotype across
122 populations (James *et al.*, 2020). With this work we hope to clearly illustrate how the
123 demographic history of populations affects the evidence for the independent and repeated
124 origins of parapatric populations.

125 Previous work using pools of DNA sequences from multiple coastal, inland, alpine, and
126 woodland *S. lautus* ecotypes found that strong isolation by distance separated all populations
127 along the coast and that geography, not ecology, explained the phylogenetic clustering of its
128 coastal populations (Roda *et al.*, 2013). Although these results suggest that the Dune and
129 Headland ecotypes have evolved in parallel, it remains unclear if gene flow could be
130 responsible for this pattern of ecotypic and geographic differentiation, thus potentially
131 affecting our inferences on the number of independent colonisations and origins of Dune and
132 Headland populations. Here, we directly estimate patterns of gene flow within and between
133 Dune and Headland ecotypes, as well as other demographic parameters important for
134 characterising the colonisation history of this system. We use estimates of demographic
135 parameters in forward population genetic simulations to explore the conditions that would
136 favour a phylogenetic transition from clustering by their ecology to clustering by their
137 geography, thus helping us gain further confidence on our conclusions about parallel
138 parapatric divergence in this system. Our results illustrate the way we understand parallel
139 evolution and pave the way for analyses of parallel trait evolution driven by natural selection
140 in plants, where cases of parallelism remain understudied.

141 # Methods

142 ## Sample collection and DNA extraction

143 Leaf samples for DNA extraction were collected from 23 Dune and Headland populations of
144 *Senecio lautus* along the coast of Australia, which included eight parapatric Dune-Headland
145 population pairs, three allopatric Headland populations, and three allopatric Dune populations
146 (n_{mean} = 58, n_{total} = 1338; Figure 2A, Table S1). We sampled mature (flowering) plants evenly

147 across the geographic range of each population, ensuring that sampled plants were at least
148 one metre apart. DNA was extracted using a modified CTAB protocol (Clarke, 2009) and
149 cleaned with Epoch Life Sciences spin columns. We quantified sample concentration with the
150 Invitrogen Quant-iT PicoGreen dsDNA Assay Kit, and used the BioTek Take3 Micro-
151 Volume Plate to ensure DNA samples were pure. Samples were standardised to 10ng/uL.

152 **GBS library construction**

153 We created reduced representation libraries by using a two-enzyme Genotyping-by-
154 Sequencing (GBS) approach (modified from Poland *et al.*, 2012). We created seven libraries,
155 each containing 192 barcoded individuals. For each individual, genomic DNA was digested
156 with the restriction enzymes Pst1-HF (New England Biosciences; NEB) and Msp1 (NEB).
157 Forward and reverse barcodes were ligated to fragments from each sample, and subsequently
158 cleaned with homemade Serapure beads (Faircloth & Glenn, 2011; Rohland & Reich, 2012).
159 For each sample we amplified the fragments and added Illumina sequencing primers via
160 PCRs. Each sample was quantified with the Invitrogen Quant-iT PicoGreen dsDNA Assay
161 Kit. We created seven equimolar pools (192 individuals per pool), ensuring each population
162 was evenly distributed across the pools. Each pool was size-selected on the BluePippin (2%
163 DF Marker V1, 300-500bp; Sage Science), and cleaned with the Monarch PCR & DNA
164 cleanup kit (NEB). Pooled libraries were sent to Beijing Genomics Institute for sequencing
165 on seven lanes of the HiSeq4000, with 100bp paired-end sequencing.

166 **Bioinformatics**

167 The Beijing Genomics Institute removed forward barcodes and quality filtered the raw reads
168 to remove reads containing Illumina adaptors, low quality reads (> 50% of bases < Q10), and
169 reads with > 10% Ns. We trimmed reverse barcodes with *TagCleaner* standalone v0.12
170 (Schmieder *et al.*, 2010). We retained an average of 2,849,159 clean reads (SD = 827,036)
171 across the 1,319 individuals (after the removal of 19 individuals with high missing data, see
172 below; Table S2). Reads were mapped to the *S. lautus* reference PacBio genome v1.0
173 (Wilkinson, 2019) with *BWA-MEM* v0.7.15 (Li & Durbin, 2009; Li, 2013). On average, 86%
174 of reads (SD = 15) mapped to the reference genome, and 81% (SD = 15) mapped properly
175 with their paired read (Table S2). *PicardTools* v2.7.0 (Broad Institute, 2019) was used to
176 clean aligned reads and to add read groups (PCR duplicates were not marked for removal).
177 We jointly called all variant and invariant sites for each population with *FreeBayes* v1.1.0

178 (Garrison & Marth, 2012). Because SNPs were separately called for each of the 23
179 populations, we first normalised the 23 VCF files before merging them together. This was
180 achieved by first using BCFtools v1.4.1 (Li *et al.*, 2009) to split multiallelic sites into biallelic
181 records. Each file was then normalised by re-joining biallelic sites into multiallelic records.
182 We then left-aligned and normalised indels, and used *vt* (Tan *et al.*, 2015) to decompose
183 biallelic block substitutions into separate SNPs for each population. We then merged the 23
184 per-population VCF files into one large file for subsequent SNP filtering.

185 We largely followed the *dDocent* pipeline for SNP filtering (Puritz *et al.*, 2014a; b), including
186 iterative filtering to maximise the number of sampled SNPs (O'Leary *et al.*, 2018). Using
187 *VCFtools* v0.1.15 (Danecek *et al.*, 2011), we first retained sites if they were present in > 50%
188 of individuals, had a minimum quality score of 30, and a minimum minor allele count of 1.
189 We then filtered for a minimum depth of 3 for a genotype call. Individuals were removed if
190 they contained > 40% missing data. We then filtered for a maximum mean depth of 100, and
191 a minimum mean depth of 10. We filtered for missing data per population, removing sites if
192 they contained > 50% of missing data within each population. We refiltered for an overall
193 missing data of 20%. Indels were removed with *vcflib* (Garrison, 2016). We then filtered for
194 population-specific Hardy Weinberg Equilibrium using the *filter_hwe_by_pop.pl* script
195 within *dDocent*. See below for the minor allele frequency thresholds for each analysis.

196 **Do populations cluster by geography or ecotype?**

197 To explore the broad patterns of genetic clustering of populations, we performed two separate
198 analyses: phylogeny construction and *fastSTRUCTURE* (Raj *et al.*, 2014). We used *PLINK*
199 v1.9 (Purcell *et al.*, 2007) to filter for a minor allele frequency of 0.05 and also to thin SNPs
200 by retaining one unlinked SNP per rad locus. This dataset contained 3,844 unlinked SNPs
201 across the 1,319 individuals. We generated a maximum likelihood phylogeny within *IQ-*
202 *TREE* v1.6.0 (Nguyen *et al.*, 2015) using the polymorphisms-aware phylogenetic model
203 (Schrempf *et al.*, 2016). We first used ModelFinder (Kalyaanamoorthy *et al.*, 2017) to
204 determine the best-fit substitution model for the data (TVMe+FQ+P+N9+G4), and increased
205 the virtual population size (N) to the maximum value of 19 (as recommended by Schrempf *et*
206 *al.*, 2016). Default parameters were used for tree construction, with the western Australia
207 D09 population assigned as the outgroup. To assess convergence, we undertook 10 separate
208 runs of IQ-TREE and examined tree topology (which remained unchanged with 10
209 independent runs). We also ensured that the log-likelihood values were stable at the end of

210 each run. Branch support was performed using 10,000 replicates of UFboot (Hoang *et al.*,
211 2018), and 10,000 replicates of SH-aLRT (Guindon *et al.*, 2010).

212 We further explored broad patterns of population structure using the variational Bayesian
213 framework, *fastSTRUCTURE* v1.0 (Raj *et al.*, 2014). Here, we implement *fastSTRUCTURE*
214 as extra evidence for whether populations genetically cluster by geography or ecotype. We do
215 not infer specific historical admixture scenarios from *fastSTRUCTURE*, as different
216 demographic scenarios can give rise to indistinguishable structure plots (Lawson *et al.*,
217 2018). The *fastSTRUCTURE* algorithm assigns individuals into genetic clusters (K) by
218 minimising departures from Hardy-Weinberg equilibrium and inferring individual ancestry
219 proportions to each genetic cluster. We ran the simple prior (K = 1-30) with 100 independent
220 runs per K-value. In order to determine the most likely number of genetic clusters (the
221 optimal K), we used the *chooseK.py* script from *fastSTRUCTURE* to examine (1) the K-value
222 that best explained the structure in the data (the smallest number of model components that
223 accounted for almost all of the ancestry in the sample), and (2) the K-value that maximised
224 the marginal likelihood of the data. Results were summarised and plotted in the R package
225 *pophelper* v2.2.7 (Francis, 2017).

226 **Is there gene flow across the system?**

227 To explore patterns of gene flow in a phylogenetic context, we used *TreeMix* v1.13 (Pickrell
228 & Pritchard, 2012). *TreeMix* constructs a bifurcating maximum likelihood tree, identifies
229 populations that are poor fits to the model, and sequentially adds migration events that
230 improve the fit of the data. We filtered our data for MAF 0.01, retaining 24,933 SNPs across
231 the 1,319 individuals. We constructed an initial 25 maximum likelihood trees with no
232 migration, 1000 bootstrap replicates in blocks of 50 SNPs with D09 as the assigned outgroup,
233 and selected the tree with the highest log-likelihood as the input tree for all subsequent
234 analyses. We then tested between 1-25 migration events in blocks of 50 SNPs. Trees and
235 migration events were robust to varying the size of the linkage blocks as well as the MAF
236 threshold of the dataset (data not shown). To select the number of migration events, we
237 examined the log-likelihoods and cumulative variance explained by each model, as well as
238 performed jackknife estimates to obtain the standard error and significance of the weight of
239 each migration event. However, the interpretation of these P-values should be treated with
240 caution due to possible errors in the tree structure as well as the inference of incorrect
241 migration events (Pickrell & Pritchard, 2012).

242 To more formally test for admixture, we used the *threepop* function in *TreeMix* to calculate
243 *f3*-statistics (Reich *et al.*, 2009). The *f3*-statistic determines whether a particular population
244 (*A*) is the result of admixture between two other populations (*B* and *C*). It measures the
245 difference in allele frequencies between populations *A* and *B*, and populations *A* and *C*, so *f3*
246 can be interpreted as the amount of shared genetic drift between two populations from a
247 common ancestor. In the absence of admixture, *f3* (*A*; *B*, *C*) will be positive, whereas a
248 significantly negative value of *f3* provides evidence for *A* being admixed from *B* and *C*. We
249 calculated *f3* for all triads of populations with jackknifing in blocks of 50 SNPs to obtain Z-
250 scores for calculating statistical significance (Z-score < -3.8 = P < 0.0001).

251 The erect phenotype is common across Australian species of the genus *Senecio* (Thompson,
252 2005), except the prostrate *S. lautus* Headland ecotype and a few Alpine populations,
253 suggesting these prostrate forms are derived. We tested for isolation by distance (IBD;
254 Wright, 1943) in the ancestral and derived ecotypes to evaluate similarities in their dispersal
255 dynamics (Slatkin, 1993). We tested for IBD using migration rates (2Nm) inferred in
256 *fastsimcoal2* (see below) as well as Slatkin's $\widehat{M}$, $(1 / F_{ST} - 1)/4$, as a proxy for gene flow
257 (Slatkin, 1993). For Slatkin's $\widehat{M}$, we used the dataset excluding the western Australia
258 populations (D09 and D35), with a MAF of 0.05, and calculated pairwise F_{ST} in VCFtools.
259 We calculated pairwise geographic distances using the following formula, which uses the
260 spherical law of cosines to consider the curvature of the earth:
261 *6378137*acos(sin(lat1)*sin(lat2)+cos(lat1)*cos(lat2)*cos(long1-long2))*, where 6378137 is
262 earth's radius in meters, and *lat* and *long* are the latitude and longitude (in radians) of the two
263 populations compared. For the *fastsimcoal2* migration rates, we tested for IBD between the
264 Dune and Headland of each population pair using a linear model in R (R Core Team, 2017),
265 using an average of the bidirectional gene flow rates for each pair (log-log scale). For
266 Slatkin's $\widehat{M}$, we also tested for IBD between the Dune and Headland of each population pair
267 (log-log scale) using a linear model in R, and tested for IBD within the Dunes, and within the
268 Headlands (log-log scale) using Mantel tests with 9,999 permutations in R (*mantel* in the
269 *vegan* package (Blanchet *et al.*, 2018).

270 **Is there gene flow between parapatric populations?**

271 We examined levels of admixture between parapatric populations with *STRUCTURE* v2.3.4
272 (Pickrell & Pritchard, 2012). *STRUCTURE* is a Bayesian MCMC approach that assigns
273 populations into genetic clusters (K) based on individual genotypes by assuming Hardy-

274 Weinberg Equilibrium within a population. It assigns each individual an admixture
275 coefficient to depict the proportion of the genome that originated from a particular K cluster.
276 To increase the numbers of SNPs we took a subset of the data by excluding the two
277 populations from the west coast of Australia (D09 and D35). Excluding these most divergent
278 populations decreased the amount of missing data and thus increased the number of common
279 SNPs in the south-eastern populations. We used the same filtering procedure as above,
280 filtered for MAF 0.05 and thinned SNPs in *PLINK* to retain one SNP per rad locus. Each
281 population pair was extracted and subsequently filtered for MAF 0.05. We retained between
282 837 and 2,606 unlinked SNPs per pair (mean = 1,905 SNPs; SD = 575). *STRUCTURE*
283 analysis was run using the admixture model and the correlated allele frequency model
284 (Falush *et al.*, 2003) with 10 independent runs for K = 1-6 (50,000 burn-in and 200,000
285 MCMC). We ensured convergence of all summary statistics. As we were specifically
286 interested in detecting admixed individuals between the two ecotypes, we plot results for K =
287 2. To explore any additional genetic structure within a pair, we also estimated the optimal K-
288 value with the Evanno method (Evanno *et al.*, 2005), by examining the maximum value for
289 ΔK (the second order rate of change in the log probability of data between successive K-
290 values). The R package *pophelper* was used to calculate the ΔK, summarise results and plot
291 the data.

292 We directly estimated levels of gene flow between population pairs from the site frequency
293 spectrum (SFS) using the composite-likelihood method implemented in *fastsimcoal2* v2.6.0.3
294 (Excoffier *et al.*, 2013). The joint SFS of two populations is sensitive to demographic
295 processes. For instance, gene flow will result in more low-frequency shared polymorphisms
296 than expected under a non-migration scenario (Hahn, 2018). We tested eight demographic
297 models (Figure 4A), and inferred migration rates, as well as other demographic parameters
298 including current population sizes, ancestral population size, divergence time, time of
299 secondary contact, and gene flow cessation time, for eight Dune-Headland population pairs.
300 We additionally asked whether gene flow was occurring in a linear fashion down the coast
301 within each ecotype, by testing eight Dune-Dune and eleven Headland-Headland pairs (Table
302 S2). To determine the baseline level of gene flow inferred by *fastsimcoal2* between isolated
303 populations, namely the null gene flow expectation, we estimated migration rates for three
304 very divergent allopatric populations (>1,500 km apart, between the eastern and south-eastern
305 clades; D03-D32, D03-H12, and H02-H12), and took the highest detected migration rate
306 from these allopatric comparisons as the baseline rate.

307 As above, the western Australia populations (D09 and D35) were excluded from this dataset
308 to increase the number of sampled SNPs. For each pair, we filtered for a minor allele count of
309 one (MAC1), retaining between 6,679 and 19,951 variable sites per pair (mean = 12,155
310 SNPs, SD = 3,316). By using a MAC1 and a relatively high number of samples per
311 population (mean = 57, SD = 15), we retain rare alleles that are informative about migration
312 events between the populations (Slatkin, 1985b). Since we cannot distinguish ancestral from
313 derived alleles, we used the minor allele SFS (folded SFS). We used an *ad hoc* approach to
314 estimate the number of monomorphic sites (see Supplementary Methods). Gene flow
315 estimates were robust to varying the number of monomorphic sites (data not shown). We
316 used custom R functions (modified from Liu *et al.*, 2018) to generate the joint folded SFS per
317 population pair without downsampling.

318 We performed 50 independent *fastsimcoal2* runs per model per population pair. Each run
319 consisted of 100,000 coalescent simulations and 40 expectation-maximisation cycles for
320 parameter optimisation. We used a mutation rate of 1.0×10^{-8} based on Asteraceae EST
321 sequence comparisons and fossil calibrations (Strasburg & Rieseberg, 2008). We ranked the
322 models based on the Kullback–Leibler information value which was estimated from the AIC
323 scores of the best run per model. Here, the normalisation of the difference between the AIC
324 scores of a particular model and the best model in the set provides a measure of the degree of
325 support for a particular model, namely model likelihood (w_i) (Thomé & Carstens, 2016).
326 Since the use of linked-SNPs might lead to pseudo-replication issues when comparing
327 models based on *fastsimcoal2* likelihood values (Bagley *et al.*, 2017) and the SFS discards
328 linkage information, we verified SNPs were largely unlinked by calculating linkage-
329 disequilibrium in PLINK (data not shown).

330 As *fastsimcoal2* uses simulations to approximate the likelihood values, there is variance in
331 the likelihood estimates. To test whether the best model significantly differs from alternative
332 models with negligible gene flow (2Nm = 0.01) but the same values at other parameters, we
333 compared their likelihood distributions based on 100 expected SFS from 100,000 coalescent
334 simulations per model (Bagley *et al.*, 2017). If likelihood distributions overlap, there is no
335 significant differences between the fit of both models (Meier *et al.*, 2017). To obtain
336 confidence intervals for all demographic parameters, we performed parametric bootstrapping.
337 Given the parameter values of the best run of the best model, we simulated 100 SFS and re-
338 estimated the parameter values from them. Each run consisted of 100,000 coalescent

339 simulations and 30 expectation-maximisation cycles. The parameter values of the best run of
340 the best model were specified as initial values of each bootstrapping run. We computed the
341 95% confidence intervals of all parameters with the *groupwiseMean* function of *rcompanion*
342 R package (Mangiafico, 2015).

343 **Is gene flow high enough to obscure a single origin scenario?**

344 To ask under what conditions gene flow can erode a signal of phylogenetic monophyly of
345 each ecotype, we ran forward simulations of neutral polymorphism in *SLiM2* (Haller &
346 Messer, 2017), see Supplementary Methods. *SLiM2* simulates diploid genomes using a
347 Wright-Fisher model, and tracks derived mutations within simulated genomes. We mimicked
348 a model of a single-origin scenario, where an ancestral population splits into two populations
349 (Figure 5A). We can think of this split as an initial single origin of the Dune and Headland
350 ecotypes. To represent two parapatric population pairs, each of these two 'ecotypes' further
351 splits again and one population of one ecotype exchanges genes with the other ecotype
352 (representing one parapatric pair at *location 1*, and this also occurs at *location 2* to represent
353 the other parapatric pair). We varied the following parameters: population size, migration
354 rate, time from the present to the second split (*T1*), and time from the second split to the split
355 of the ancestral population (*T2*). In addition, an outgroup population was retained after the
356 first split, in order to construct a rooted tree (see below). Each model used a heuristic burn-in
357 period of 10 x N (population size) generations to reach mutation-drift balance in the ancestral
358 population. After each simulation, 30 individuals per population were sampled and output in
359 a VCF file.

360 We calculated a distance matrix between individual genotypes and constructed a rooted
361 neighbour-joining tree within the *ape* R package (Paradis *et al.*, 2004; Popescu *et al.*, 2012).
362 We calculated the genealogical sorting index (GSI; Cummings *et al.*, 2008), see
363 Supplementary Methods. GSI is a measure of how monophyletic an arbitrary set of tips are
364 on a tree. If all the tips form a monophyletic group, GSI will be 1, whereas if the tips are
365 dispersed throughout the phylogeny, then GSI will be closer to 0. We calculated GSI for four
366 sets of tips on each tree: for all 'Dunes', for all 'Headlands', for both populations from
367 *location 1*, and for both populations from *location 2*. We took the average of the first two,
368 and the average of the second two, then the log of the ratio of these two GSI values. When
369 positive, the GSI ratio indicates a false signal of parallel origins, as the populations that are

370 parapatric appear as each other's closest relatives. Conversely, when the GSI ratio is negative,
371 it indicates that the 'true' signal of the single origin is stronger.

372 We also asked where our observed data fall in the parameter space, and whether the inferred
373 migration rates between the Dune and Headland of each replicate pair is high enough to
374 obscure the signal of a single origin. To first estimate which panel of the parameter space the
375 *S. lautus* system is located, we estimated the internal branch (T1 of Figure 5A) by averaging
376 the direct estimates of divergence times within ecotypes (calculated from *fastsimcoal2*, see
377 above for details). We assumed a similar divergence time for the internal branch (T2 of
378 Figure 5A). We then asked whether our observed D-H migration rates fall within the region
379 where the phylogeny is not distorted.

380 To further explore the effect of gene flow in phylogenetic distortion, we compared the
381 relative node order of the observed phylogeny (where the topology is estimated in the
382 absence of gene flow) to the *fastsimcoal2* models (where gene flow is taken into account).
383 We did this for four population pairs (D04-H05 and D05-H06; D14-H15 and D32-H12).
384 Specifically, if the observed phylogeny represents a true parallel origin scenario, then
385 isolation-with-migration models which jointly estimate gene flow and divergence time should
386 infer deeper divergence times for populations of the same ecotype, compared to comparisons
387 between putative sister populations of divergent ecotypes. We used divergence times
388 estimated in *fastsimcoal2* to compare divergence within and between ecotypes, and asked
389 whether these estimated divergence times were in accordance with the topology of a
390 phylogeny.

391 **Results**

392 **Populations cluster by geography and not by ecology**

393 Phylogenetic inference reveals that neither ecotype forms a monophyletic clade, providing
394 evidence against a single origin scenario (Figure 2B). Parapatric Dune-Headland populations
395 are also often sister-taxa, giving evidence for the multiple origin of ecotypes. To visualise the
396 major genetic structure within *fastSTRUCTURE*, we plotted the lowest K-values that capture
397 the major structure in the data (Pritchard *et al.*, 2000; Lawson *et al.*, 2018; although the
398 "best" K-value across all populations was higher – see below). The clustering of populations
399 into two genetic groups (K=2) revealed a striking correspondence to geography (Figure 2C),

400 where the eastern populations (dark blue) are separated from those populations further south
401 and to the west (light blue). This strong genetic structuring into two main clades suggests
402 there are at least two independent origins within the system. When three genetic groups
403 (K=3) are considered, the eastern populations are further separated into two clusters, again
404 largely corresponding to geography and reflecting the phylogenetic structure of the data; K=4
405 distinguishes the west Australia populations from those on the south-eastern coast. This
406 genetic clustering of populations according to their geographic distribution provides further
407 evidence against a single origin scenario, and is consistent with previous work in this system
408 (Roda *et al.*, 2013; Melo *et al.*, 2019).

409 **Minimal gene flow across the system**

410 In the absence of migration, the *TreeMix* phylogeny explained 95.9% of the data, with the 24
411 additional migration events augmenting this value to 98.9 % (Figure S1). Figure 3A shows
412 the first migration event ($P < 2.2x10^{-308}$) with a migration weight (*w*) of 0.40. Although the
413 24 other migration events were also significant ($P_{average} = 2.92x10^{-3}$, SD = 0.0062), their
414 individual weightings were small (see Figure S2 for 1-10 migration events), most of them
415 were not between parapatric pairs, and the addition of these migration events did not
416 substantially alter the topology from its estimation in the absence of gene flow. Although
417 these results could suggest a potential complex colonisation history including long distance
418 yet rare migration events, these P-values should be treated with caution. This is because
419 model comparisons in *TreeMix* suffers from multiple testing, a large number of parameters,
420 and the estimated graph can be inaccurate (Pickrell & Pritchard, 2012). We therefore tested
421 the robustness of this inference using *f3-statistics*. All *f3-statistics* were positive (Figure S3),
422 giving no evidence of admixture between any populations. Strong isolation by distance
423 within each ecotype further supports this contention using $\widehat{M}$ as a proxy for migration rates
424 (IBD within Dunes: Mantel test, r = -0.83, P = 0.0001; within Headlands r = -0.73, P =
425 <0.0001; Figure 3B). A strong IBD trend exists between ecotypes for the eight pairs studied
426 here ($\widehat{M}$: $F_{1,6} = 0.55$, P = 0.05661, multiple $R^2 = 0.48$, Figure 3C). Although the same trend
427 was seen in the migration rate estimates from *fastsimcoal2* it was not statistically significant,
428 perhaps due to the low sample size (*fastsimcoal2*: $F_{1,6} = 0.53$, P = 0.4953, multiple $R^2 = 0.08$,
429 Figure 3B). Overall, this pattern of IBD implies that there is geographically restricted
430 dispersal within the system and populations are evolving largely independently from one
431 another.

432 The absence of admixture across the system is also supported by *fastSTRUCTURE* across all
433 populations. The inferred value of K is close to the number of sampled populations (Figure
434 S4B) and each population is genetically distinct, suggesting that *S. lautus* has a simple
435 demographic history with limited admixture (Lawson *et al.*, 2018). Specifically, the K-value
436 that best explained the structure in the data was 22, the rate of change in the likelihood of
437 each K-value (Figure S4C) was negligible for K = 24-28, and the K-value that maximised the
438 marginal likelihood of the data was 28, together suggesting that the optimal K-value is
439 around 23 (Figure S4A,B). The *fastSTRUCTURE* results for K=23 show that each population
440 forms a distinct genetic cluster (Figure 3D), suggesting very little, if any, admixture between
441 them, further implying that each sampled population has been separated from other
442 populations long enough to be genetically distinct (see pairwise F_{ST} values in Table S3) and
443 with insufficient levels of gene flow to homogenise their genomes (Lawson *et al.*, 2018).
444 Further, when we examine all K-values from 1-23, there is a distinct hierarchical structure
445 that mirrors the phylogeny suggesting that such structure is an accurate representation of the
446 history of the populations. The Tasmania population pair (D14-H15) should be treated with
447 caution due to the smaller sample size (n_{mean} = 11.5) compared to other populations (n_{mean} =
448 62). For groups with fewer samples, genetic clustering programs such as *fastSTRUCTURE*
449 are likely to assign them as mixtures of multiple populations rather than their own distinct
450 population (Lawson *et al.*, 2018). This is evident for K = 22, where the Tasmania populations
451 appear admixed (Figure S4A).

452 **Minimal gene flow between parapatric ecotypes and distant populations**

453 We observed very few admixed individuals between the parapatric Dune-Headland
454 populations at each locality within the *STRUCTURE* analysis for K = 2 (Figure 4B). On
455 average, 9.36% of individuals were admixed per population, although their admixture
456 proportions were on average less than 1% (mean = 0.008, SD = 0.018). This suggests that
457 gene flow between parapatric populations might have stopped back in the past, and lineage
458 sorting of many alleles has already taken place. For all pairs, the best K-value based on the
459 Evanno method (Evanno *et al.*, 2005) was K = 2 (Figure S5). Demographic modelling in
460 *fastsimcoal2* revealed the most likely divergence model for all population pairs within and
461 between ecotypes was bidirectional gene flow after secondary contact (w_i > 0.99; Figure S6).
462 However, for parapatric Dune-Headland population comparisons, direct measurements of
463 migration rates revealed that most migration rates were very low (2Nm < 1.00), with the

464 exception of D04-H05 and D32-H12 (Figure 3B upper section, 4B; Table S4, S5). For Dune-
465 Dune population comparisons we also detected very low migration rates ($2Nm_{mean}$ = 0.23, SD
466 = 0.09), with all pairs containing 2Nm < 1.00. For Headland-Headland comparisons we again
467 detected very low migration rates ($2Nm_{mean}$ = 0.57, SD = 1.01), with all pairs containing
468 2Nm < 1.00, with the exception of H12-H12A (Figure 3B upper section, 4B; Table S4, S5).
469 Across all comparisons, all Dune-Dune pairs and most Headland-Headland pairs exhibited
470 gene flow levels lower than the maximum migration rate of allopatric populations separated
471 by more than 1,500km (i.e. the null expectation; 2Nm = 0.39; Figure 3B). Three Dune-
472 Headland pairs (D00-H00, D03-H02 and D12-H14) were also within this null range.
473 Alternative models with negligible gene flow did not fit the data better with the exception of
474 the D03-H02 pair (Figure S7), as previously detected in Melo *et al.*, (2019). Overall, the
475 observed magnitude of gene flow and previous lines of evidence, make us conclude that most
476 parapatric Dune-Headland populations in *S. lautus* are effectively allopatric.

477 **Levels of gene flow do not obscure a single origin scenario**

478 Simulation of a single origin scenario with gene flow (Figure 5A) in *SLiM* revealed how gene
479 flow can erode the true signal of monophyly of each ecotype (true signal = Figure 5B blue
480 regions, negative GSI ratios) leading to a distorted phylogeny where populations cluster by
481 geography (distorted signal = Figure 5B red regions, positive GSI ratios). We detected this
482 phylogenetic shift when *T1* was long and *T2* was short (Figure 5B top right panel) even for
483 small amounts of gene flow. In contrast, we did not detect it when *T1* was short and *T2* was
484 long (Figure 5B bottom left panel). For intermediate lengths of *T1* and *T2*, increasing
485 migration rates lead to an increase in GSI ratios. In general, population size did not
486 dramatically altered GSI ratios, except when both *T1* and *T2* were short (Figure 5B, upper left
487 quadrats). Overall, these patterns suggest that as the speed of diversification increases (from
488 long to short internal branches, *T2*), even small amounts of gene flow are likely to erode the
489 true signal of a single origin where populations cluster by ecology and not by geography.
490 This is because short internal branches are already likely to distort the phylogenetic signal
491 due to high levels of ancestral polymorphism in each parapatric pair.

492 Our observed data are located in the bottom right panel of Figure 5B: the estimated
493 divergence time within ecotypes (branch T1 of Figure 5A) ranged between 43,928 and
494 128,159 generations (mean = 84,559, SD = 22,916), which is closest to T1 = 100,000, also
495 assuming a similar time between splitting events between parapatric pairs (branch T2 of

496 Figure 5A). None of our observed bidirectional migration rates (mean m = $1.5x10^{-05}$, range =
497 $1.7x10^{-06}$ to $5.2x10^{-05}$) fall within the region of the parameter space that would create full
498 phylogenetic distortion (i.e. darkest red region of Figure 5B, bottom right panel). Most
499 observed migration rates are low, bidirectional and less than $1.0x10^{-05}$ (Figure 5C, light grey),
500 which are in regions of the parameter space that are unlikely to distort a single origin
501 scenario. Only two population pairs (D04-H05 and D32-H12) had levels of gene flow that
502 could have partially distorted a single origin scenario (Figure C, dark grey).

503 Divergence time estimations in *fastsimcoal2* (which considers gene flow) were in accordance
504 with the observed phylogeny: we observed deeper divergence times for populations of the
505 same ecotype compared to sister-taxa of different ecotypes. More specifically, for D04-H05
506 and D05-H06, the average divergence time between populations of the same ecotype (i.e.
507 D04-D05 and H05-H06) was 79,801 (SD = 2,698), whereas the average divergence time
508 between populations at each locality (i.e. D04-H05 and D05-H06) was 49,317 (SD = 26,319).
509 This is also true for D14-H15 and D32-H12, where the average divergence time between
510 populations of the same ecotype (i.e. D14-D32 and H15-H12) was 68,723 (SD = 17,526), and
511 the average divergence time between populations at each locality (i.e. D14-H15 and D32-
512 H12) was 43,318 (SD = 6,522). Overall, this gives further evidence that the phylogenetic
513 topology (estimated in the absence of gene flow) has not resulted from gene flow distortion.

514 Discussion

515 We have used an array of complementary approaches to disentangle the demographic history
516 of the coastal *Senecio lautus* ecotypes. In this system, many lines of evidence support a
517 multiple origin scenario for the parapatric Dune and Headland populations. The demographic
518 history of this system reveals striking population structure and a strong effect of geography
519 and restricted dispersal, to the extent that all populations are evolving largely independently
520 from each other. Together with previous results from transplant experiments, our results
521 convincingly show that parapatric Dune and Headland populations have evolved multiple
522 times repeatedly and independently, and that selection and drift, rather than gene flow, play a
523 predominant role in the distribution of genetic diversity in this system. Below we discuss
524 these results in light of parallel parapatric divergence in this highly replicated system of
525 evolution.

526 **Strong genetic structure between *Senecio lautus* coastal populations**

527 The dispersal of gametes and seeds within a landscape depends upon the physical distance
528 they can move and the availability of suitable habitats (Hansson, 1991). Within highly patchy
529 environments, most gametes and seeds are restricted to disperse locally. Within coastal
530 populations, where suitable habitats are largely limited, the dispersal kernel of a species
531 highly restricts gene flow (Nathan *et al.*, 2012). In *S. lautus*, dispersal is governed by
532 pollinators (including native bees, moths and butterflies) which can transport pollen grains up
533 to 2 km in a day (White, 2008), and by wind that drives dispersal of seeds with pappi
534 (Andersen, 1993). Although there is potential for long distance movement within the system
535 both within and between ecotypes (Roda *et al.*, 2013), strong local adaptation prevents
536 migrants and hybrids from effectively establishing beyond their local site (Richards & Ortiz-
537 Barrientos, 2016; Walter *et al.*, 2016). This, coupled with a landscape of patchy environments
538 along the coast, suggests that population structure in *S. lautus* is expected to be pronounced.
539 Our findings are in accord with this expectation, and highlight various levels of population
540 structure and history in the coastal system.

541 Genetic structure within *S. lautus* clusters populations according to their geographic
542 distribution along the Australian coast, and not by the environment they occupy (Figure 2B,
543 2C). Within *fastSTRUCTURE*, the largest genetic groups within *S. lautus* encompass two
544 clades (Figure 2C) which are called the eastern and south-eastern clades. Each clade can be
545 further subdivided into two subclades (Figure 2C). These four clades are largely independent
546 of each other, do not have evidence of long-distance gene flow between them (Figure 3A),
547 and appear to contain multiple repeated instances of parapatric divergence. This genetic
548 structure, where populations group by geography and not ecology is mirrored in the
549 phylogeny (Figure 2B), and is consistent with our previous work using targeted sequencing
550 of neutral genes (Melo *et al.*, 2019) and RADseq using pools of individuals (Roda *et al.*,
551 2013).

552 A further level of structure can be visualised at the locality (i.e. parapatric Dune-Headland
553 populations), where each population is unique in this system: F_{ST} values are above 0.2 in each
554 population comparison (Table S3), and *fastSTRUCTURE* supports K-values equal to the
555 same number of populations sampled in this study (Figure 3D). Also, all parapatric pairs are
556 fully differentiated with little admixture (Figure 4B), even those such as D04-H05 at Coffs
557 Harbour (NSW) that have adjacent habitats, i.e. where the potential for gene flow between

558 ecotypes is high. Previous ecological experiments in this population pair have demonstrated
559 strong extrinsic reproductive isolation against migrants and hybrids (Richards *et al.*, 2016;
560 Richards & Ortiz-Barrientos, 2016), and cline analyses revealed that the barrier to gene flow
561 is complete (North, 2015). Finally, no single estimate of the *f3-statistic* for any population
562 triad was negative (Figure S3), further supporting that there are negligible levels of gene flow
563 between populations across the entire system.

564 There is a strong signal of isolation by distance (Wright, 1943) within each ecotype as well as the
565 Dune-Headland parapatric pairs, where there is an increase in genetic differentiation between
566 populations with increasing geographic distance. This pattern arises when populations are
567 geographically restricted and are at an equilibrium of dispersal and drift. Isolation by distance also
568 suggests that long distance dispersal within the system is not pervasive, and populations have
569 colonised their habitats far enough in the past to approach an equilibrium under the current patterns
570 of dispersal (Slatkin, 1993). Overall, a combination of strong selection and limited dispersal can
571 explain why parapatric populations persist despite the opportunity for homogenising gene flow
572 between them. Future ecological studies that directly estimate seed and pollen dispersal kernels will
573 help clarify the relative contributions of movement and local adaptation to divergence in parapatry.

574 **Parallel evolution of parapatric *S. lautus* ecotypes with minimal levels of gene flow**

575 A common doubt arising in purported cases of parallel evolution is whether gene flow is
576 responsible for the grouping of populations by geography and not by ecology (Quesada *et al.*,
577 2007; Johannesson *et al.*, 2010; Bierne *et al.*, 2013; Butlin *et al.*, 2014; Martin *et al.*, 2015;
578 Rougemont *et al.*, 2015; Le Moan *et al.*, 2016; Meier *et al.*, 2017; Pérez-Pereira *et al.*, 2017;
579 Trucchi *et al.*, 2017; Rougeux *et al.*, 2019). A single origin scenario combined with high
580 levels of gene flow can alter the phylogenetic relationships of populations, falsely suggesting
581 multiple independent origins (Endler, 1977; Barton & Hewitt, 1985; Coyne & Orr, 2004;
582 Bierne *et al.*, 2013). This is because genetic structure at neutral markers can be decoupled
583 from colonisation history via introgression and incomplete lineage sorting. This needs careful
584 scrutiny in our system: there are multiple parapatric divergences that have the potential for
585 high gene flow due to their close geographic proximity. Surprisingly, we observed minimal
586 levels of gene flow between parapatric *S. lautus* Dune-Headland pairs, similar levels of gene
587 flow between parapatric and allopatric population pairs (Figure 3B), as well as linearly down
588 the coast for populations within each ecotype (Figure 3B, 3C; Table S4, S5). However, two
589 parapatric population pairs (D04-H05 and D32-H12) had an estimated number of migrants

590 per generation above one (Figure 3B; Table S4, S5). This consistent with population genetic
591 theory stating that, in the absence of divergent selection, these levels of gene flow would
592 homogenise them (Slatkin, 1985a), potentially obscuring a single origin scenario. Therefore,
593 we further addressed this problem by using forward simulations of the neutral divergence
594 process to ask if the demographic parameters estimated in this study are likely to obscure the
595 history of colonisation and divergence in *S. lautus*. This simulation approach is conservative
596 because previous transplant experiments in the system (Melo *et al.*, 2014; Richards *et al.*,
597 2016; Richards & Ortiz-Barrientos, 2016; Walter *et al.*, 2016, 2018b; a) as well as clinal
598 analyses (North, 2015), have shown that divergent natural selection is strong and creates
599 extrinsic reproductive isolation between Dune and Headland populations.

600 In our simulations, we investigated the interaction of gene flow, incomplete lineage sorting,
601 and drift on phylogenetic distortion. Although it is clear that there are regions in the
602 parameter space that completely erode the signal of a single origin of ecotypes and falsely
603 suggest their parallel origins (Figure 5B, red regions), there is also large fraction of the
604 parameter space that does not (Figure 5B, blue regions). Although our simulations revealed
605 that even small amounts of gene flow can distort the phylogeny, our observed levels of gene
606 flow between parapatric Dune-Headland *S. lautus* populations are not high enough to distort
607 the phylogenetic relationships amongst populations from different ecotypes. Even the two
608 parapatric pairs (D04-H05 and D32-H12) that experience the most bidirectional gene flow do
609 not fall within the region of parameter space where complete distortion of the phylogeny
610 occurs (i.e. darkest red region of Figure 5B, bottom right panel). In addition, these population
611 pairs are geographically and genetically distant from pairs at other localities, suggesting that
612 their divergence likely occurred in parapatry. Further evidence that gene flow has not
613 obscured a single origin scenario in *S. lautus* comes from comparing joint estimates of gene
614 flow and divergence times (as implemented in isolation with migration models) between
615 population pairs of the same ecotype and putative sister populations of divergent ecotypes.
616 We observed that population pairs of the same ecotype and not those from different ecotypes
617 show deeper divergence times. In addition, constructing the phylogeny taking into account
618 gene flow did not alter the topology from its estimation in the absence of gene flow (Figure
619 3A), and parapatric pairs were not better explained by the presence of gene flow. Together,
620 these results imply that phylogenetic distortion is highly unlikely in *S. lautus* and that such
621 relationships reflect the true history of populations and ecotypes.

622 Overall, our results indicate that coastal Dune-Headland *S. lautus* populations are highly
623 replicated, having originated multiple independent times in parapatry with limited levels of
624 gene flow. Within the system we have high confidence for at least six (and potentially eight)
625 independent parapatric Dune-Headland divergences. We treat the divergences at two
626 localities (D04-H05 and D32-H12) with some caution: this is because their estimated number
627 of migrants per generation is above one (which can lead to population homogenisation;
628 Slatkin, 1985a), and they also fall within the simulated parameter space where there is some
629 potential for phylogenetic distortion. Nevertheless, these pairs are from the two separate
630 clades, and are genetically isolated from other such pairs, so even moderate levels of gene
631 flow within each of these distant pairs will not substantially distort the phylogeny. In
632 addition, the estimates of divergence times for these populations (taking into account gene
633 flow) do not suggest homogenisation after secondary contact has occurred. Therefore, all
634 eight parapatric divergences sampled within this study appear to have originated
635 independently, thus evolving in parallel. Furthermore, in comparison to other systems (e.g.,
636 Le Moan *et al.*, 2016; Meier *et al.*, 2017; Trucchi *et al.*, 2017; Rougeux *et al.*, 2019), our
637 observed migration rates between ecotypes of each pair are generally lower (*S. lautus* mean m
638 $= 1.5 \times 10^{-5}$, range $= 5.3 \times 10^{-5}$ to 1.7×10^{-6}), yet are most similar to the *Littorina saxatilis*
639 system (mean $m \sim 1.0 \times 10^{-6}$; Butlin *et al.*, 2014), which is perhaps the clearest example of
640 parallel evolution in nature.

641 **The limits of inference from parallel evolution**

642 Parallel evolution allows the study of deterministic evolution in multiple ways. It not only
643 helps us understand whether populations adapting to similar conditions evolve similar
644 phenotypes, but whether this repeated adaptation is driven by the same or different genetic
645 mechanisms in replicate populations (Lenormand *et al.*, 2016). Furthermore, in systems
646 where reproductive isolation has evolved, we can begin to understand the relative
647 contributions of prezygotic and postzygotic barriers to divergence (Nosil *et al.*, 2002; Rogers
648 & Bernatchez, 2006; Stankowski, 2013). However, much remains unknown about the tempo
649 and mode of adaptation and speciation and particularly whether the two processes share a
650 common genetic basis. As such, studies of parallel evolution can help uncover further "rules
651 of speciation" (Coyne & Orr, 1989), particularly with regard to the role of natural selection in
652 creating diversity at different levels of organisation.

653 In coastal *S. lautus* we can start answering these questions. On one hand, our study helps us
654 better interpret the multiple transplant experiments carried out in this system, and suggests
655 that we can compare them as replicates of the adaptation and speciation process. For instance,
656 it is common to four different coastal localities to find selection against migrants and hybrids
657 in the field (Walter *et al.*, 2016), but very weak intrinsic reproductive isolation in F1 hybrids
658 (Melo *et al.*, 2014; Richards *et al.*, 2016; Walter *et al.*, 2016), suggesting that parapatric
659 ecological divergence is a major driver of diversification under natural conditions in the
660 system. Nonetheless, two different studies have found strong intrinsic reproductive isolation
661 in F2 hybrids (Richards *et al.*, 2016; Walter *et al.*, 2016), suggesting that genetic
662 incompatibilities are indeed accumulating and segregating within populations. Polymorphic
663 genetic incompatibilities have been discovered in many systems now (Scopece *et al.*, 2010;
664 Cutter, 2012; Larson *et al.*, 2018), and perhaps monkeyflowers best illustrate how they are
665 contributing to plant speciation (Lowry & Willis, 2010; Oneal *et al.*, 2014; Sweigart &
666 Flagel, 2015; Zuellig & Sweigart, 2018). Within *S. lautus*, intrinsic reproductive isolation in
667 F1 hybrids is almost complete between very divergent lineages (between the eastern and
668 south-eastern clades), but also according to ecology: although Dune populations are
669 interfertile despite half a million years of divergence, crosses between ecotypes, and crosses
670 between divergent Headlands are almost fully infertile (Melo *et al.*, 2019). Given that these
671 clades have independently evolved Dune and Headland forms, we can infer that adaptation to
672 similar conditions is only concordant for certain habitats but not for others. This variable
673 level of predictability might relate to the form of selection acting in each environment, or to
674 the ruggedness of fitness landscapes across geography (Lenormand *et al.*, 2009, 2016;
675 Salazar-Ciudad & Marín-Riera, 2013; de Visser & Krug, 2014; Blount *et al.*, 2018).

676 A major step forward to make sense of patterns of evolution across multiple replicates of
677 parapatric divergence would be to isolate the actual genes responsible for adaptation and
678 speciation, and to model their individual demographic history (Lee & Coop, 2017, 2019).
679 This could help us better understand if adaptation arises from new mutations or from standing
680 genetic variation, as well as reveal the nature of parallelism in a given system. For instance,
681 we could describe parallel evolution in terms of repeated fixation of the same alleles (e.g.,
682 Colosimo *et al.*, 2005), or fixation of functionally equivalent alleles, as it might be plausible
683 during polygenic adaptation (Berg & Coop, 2014; Tiffin & Ross-Ibarra, 2014; Yeaman,
684 2015), or when phenotypes arise from loss-of-function mutations (e.g., Chan *et al.*, 2010).
685 Furthermore, to understand how patterns of evolution at the genotypic level manifest at the

686 phenotype, studies of parallel evolution should directly link adaptive loci to phenotypic traits
687 and further demonstrate that the trait(s) itself has been under repeated selection in
688 independent populations (Storz & Wheat, 2010; Pardo-Diaz *et al.*, 2015; Hoban *et al.*, 2016).
689 Nonetheless, our study demonstrates that studying neutral loci can uncover patterns of
690 colonisation and migration that are consistent with parallel evolution, or even reveal alternate
691 divergence scenarios (e.g., Roesti *et al.*, 2015). Given the strong correlation between coastal
692 environment and growth habit in *S. lautus* (James *et al.*, 2020) and the results presented here,
693 studying the genetics of adaptation across this highly replicated system will reveal the mode
694 and tempo of adaptation and speciation. Our work also implies that previous discoveries in
695 this system implicating divergence in hormone signalling, flowering, and stress-related
696 pathways (Wilkinson *et al.*, 2019) are worth studying under the umbrella of parallel evolution
697 thus helping us better frame divergence at different levels of organisation and development.

698 Finally, in our work we have unusually high power to detect gene flow, as the number of
699 individuals sequenced in each population is large ($N_{mean} = 57$, $2N_{mean} > 100$ chromosomes per
700 population). This sampling regime allows me to sample of many rare variants and therefore
701 better distinguish ancestral polymorphism from migration. Studies undertaking demographic
702 modelling often sample 10-25 individuals per population (e.g., Roesti *et al.*, 2015; Kautt *et*
703 *al.*, 2016; Trucchi *et al.*, 2017) and occasionally even less than 10 (e.g., Meier *et al.*, 2017),
704 thus cannot easily distinguish shared variants due to gene flow from ancestral polymorphism,
705 which can make results biased to detecting moderate to high levels of gene flow (Slatkin,
706 1985b; Hey & Nielsen, 2007; Strasburg & Rieseberg, 2010; Cruickshank & Hahn, 2014). As
707 our simulations reveal that even small amounts of gene flow can obscure a phylogenetic
708 topology, studies that fail to detect gene flow with few numbers of individuals should treat
709 results with caution.

710 Overall, here we provide strong evidence for multiple origins of parapatric Dune and
711 Headland populations within *S. lautus*. Across this highly replicated system we observed
712 phylogenetic clustering by geography, with strong genetic structure between populations,
713 isolation by distance, and surprisingly minimal gene flow between parapatric populations at
714 each locality as well as the system as a whole. Simulations confirmed that gene flow levels
715 are not high enough to obscure a single origin scenario. This makes *S. lautus* a highly
716 replicated system of parapatric divergence and one of the clearest examples of the parallel
717 evolution of ecotypes discovered yet, adding to the increasing number of potential cases of

parallel evolution in plants (Foster *et al.*, 2007; Trucchi *et al.*, 2017; Cai *et al.*, 2019; Konečná *et al.*, 2019). Our work emphasises that researchers in the field of parallel evolution should strive to rule out a single origin scenario to demonstrate that populations within a system have arisen repeatedly and independently.

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Author contributions

MEJ and DO conceived the project. MEJ and JE undertook sample collection. MEJ extracted DNA, prepared libraries, performed bioinformatics, and undertook the *IQ-Tree*, *fastSTRUCTURE*, *STRUCTURE* and *TreeMix* analyses. HA conducted the *fastsimcoal2* analyses. JSG performed the *SLiM* simulations. MEJ and DO wrote the paper with input from all authors. DO is the mentor and supervisor for the research program.

Conflicts of interest

We do not have any conflicts of interest.

Data archival

Data will be uploaded to Dryad upon acceptance of the manuscript.

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Figures

Figure 1. The colonisation history and phylogenetic topology for alternate origin scenarios

Schematic diagram representing the colonisation history and phylogenetic topology of two ecotypes (dark green and light green) from an ancestral population (grey) for three origin scenarios. Solid arrows depict the sequence of colonisation. Double headed dotted arrows represent gene flow (*m*) between the ecotypes within each locality. L1, L2 and L3 represent three geographically distant localities, where a population of each ecotype resides. **(A)** Within a single origin scenario, the two ecotypes arise once from the ancestor, followed by range expansion. In the absence of gene flow, ecotypes form monophyletic clades within the phylogeny. **(B)** The single origin with gene flow scenario involves gene flow upon secondary contact between the ecotypes within each locality. Here, the observed phylogenetic topology shows populations clustering according to their geographic distribution. **(C)** Within a multiple origin scenario, the ancestral (dark green) ecotype arises once from the ancestor followed by range expansion, with the derived (light green) populations independently arising from each orange population. Populations phylogenetically cluster according to their geographic distribution, which can be indistinguishable from a single origin with gene flow scenario **(B)**.

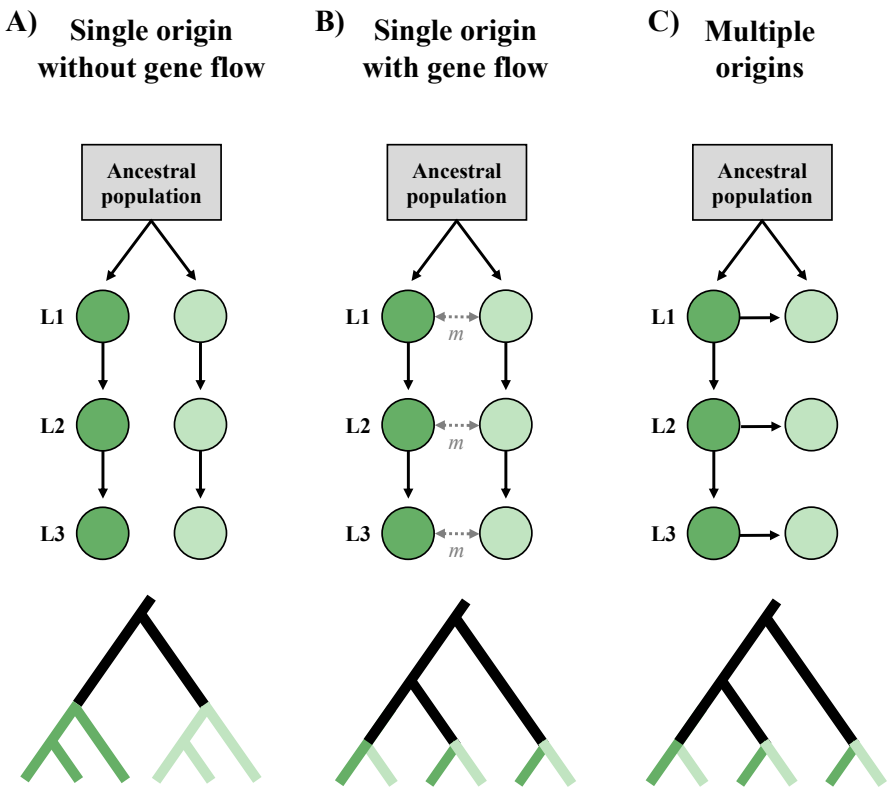


Figure 2. Sampling locations and genetic clustering of *Senecio lautus* populations

(A) Sampling locations of the 23 Dune (orange) and Headland (green) *Senecio lautus* populations along the coast of Australia. **(B)** Maximum likelihood phylogeny of Dune and Headland populations implemented in IQ-TREE. Numbers on each node represent the SH-alRT support (%), followed by the ultrafast bootstrap support (%). **(C)** Bayesian assignment of individuals to genetic clusters within *fastSTRUCTURE* for K=2-4. Each of the 1,319 individuals is depicted as a bar, with colours representing ancestry proportions to each cluster. Populations are ordered according to their geographic distribution along the coast.

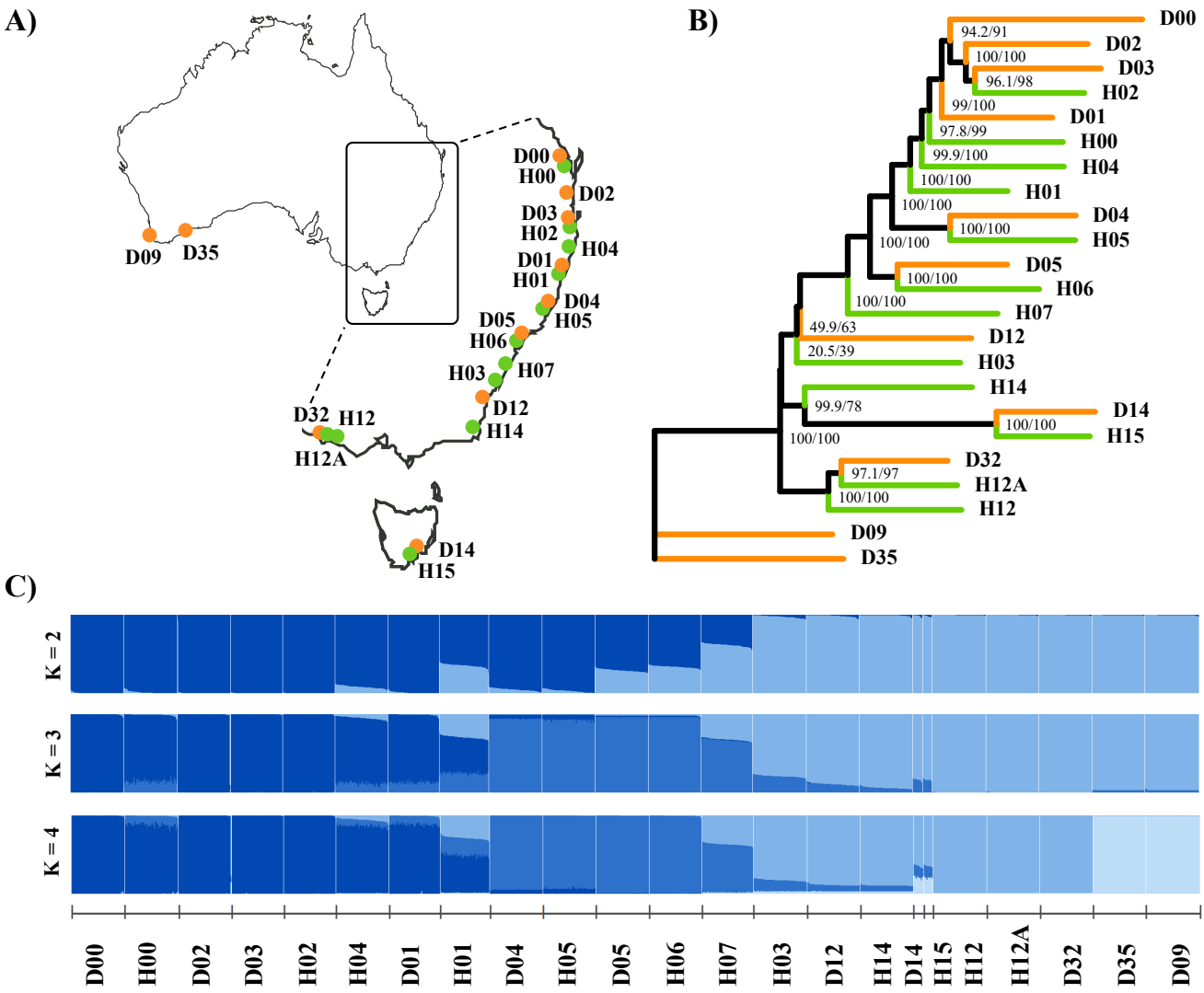


Figure 3. Patterns of long-distance gene flow, IBD, and genetic clustering

(A) Maximum likelihood tree with one migration event inferred in *TreeMix*, the x-axis representing genetic drift. The arrow represents the migration event (w = 0.40, P < 2.2x10^{-308}). **(B)** Patterns of isolation by distance across Dune and Headland populations for Dune-Headland pairs (black), Dune-Dune (orange) and Headland-Headland (green). Average migration rate is the mean bidirectional migration for each pair. Grey shading represents the null model for migration rates, inferred from the maximum migration value from three allopatric comparisons. Grey horizontal dashed line represents migration (2Nm) of one. Pairs falling above this line are labelled. Black dashed line represents the linear model for the DH comparisons. **(C)** Relationship between geographic distance and divergence time for parapatric Dune-Headland pairs (black), Dune-Dune (orange) and Headland-Headland (green). Black, orange and green dashed line represent the linear model for the DH, DD and HH comparisons respectively. **(D)** Bayesian assignment of individuals to genetic clusters within *fastSTRUCTURE* for K=23. Each of the 1,319 individuals is depicted as a bar, with colours representing ancestry proportions to each cluster. Populations are ordered according to their geographic distribution along the coast.

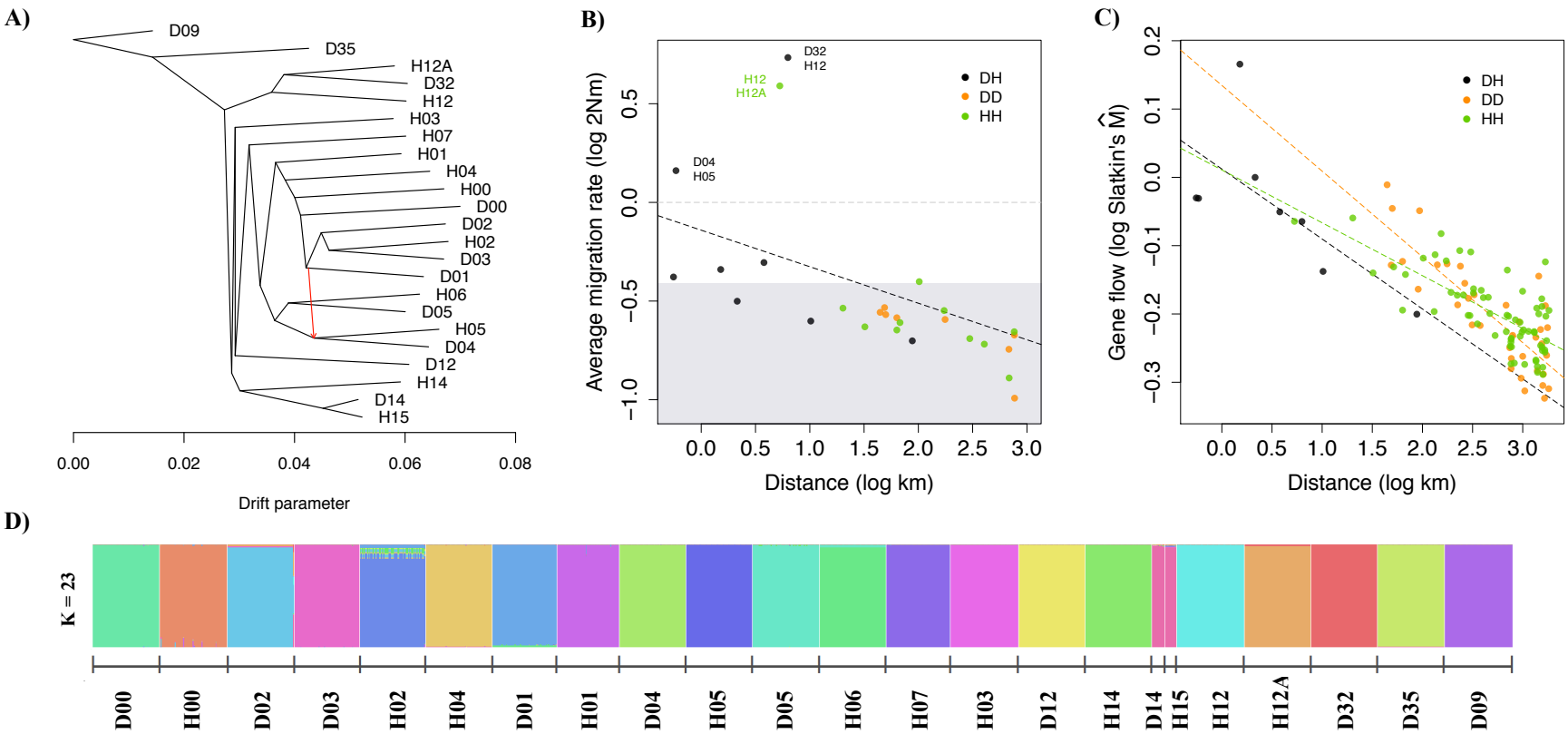


Figure 4. Patterns of gene flow and admixture between parapatric Dune-Headland populations

(A) Schematic diagram representing the eight demographic models and estimated parameters in *fastsimcoal2*: no migration, bidirectional migration, Dune to Headland migration, Headland to Dune migration, bidirectional migration after secondary contact, Dune to Headland migration after secondary contact, Headland to Dune migration after secondary contact, bidirectional migration after population splitting with cessation of gene flow. **(B)** Bayesian assignment of individuals to genetic clusters within *STRUCTURE* for K=2 for the Dune (orange) and Headland (green) ecotypes at each locality. Each individual is depicted as a bar, with colours representing ancestry proportions to each cluster. Below are the migration rates (*m*) from the Dune to Headland, and Headland to Dune within each locality estimated within *fastsimcoal2*. Asterisks denote pairs with 2Nm > 1.

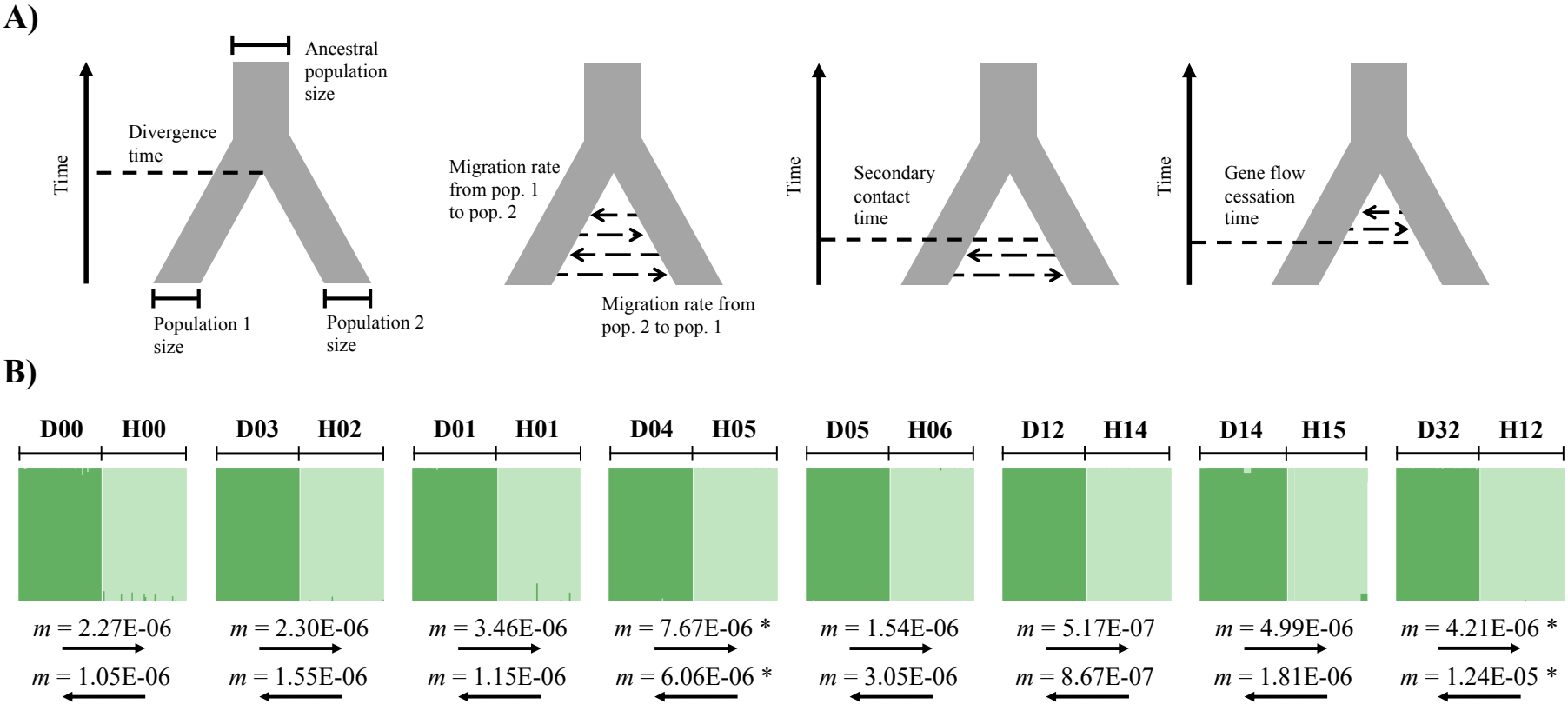
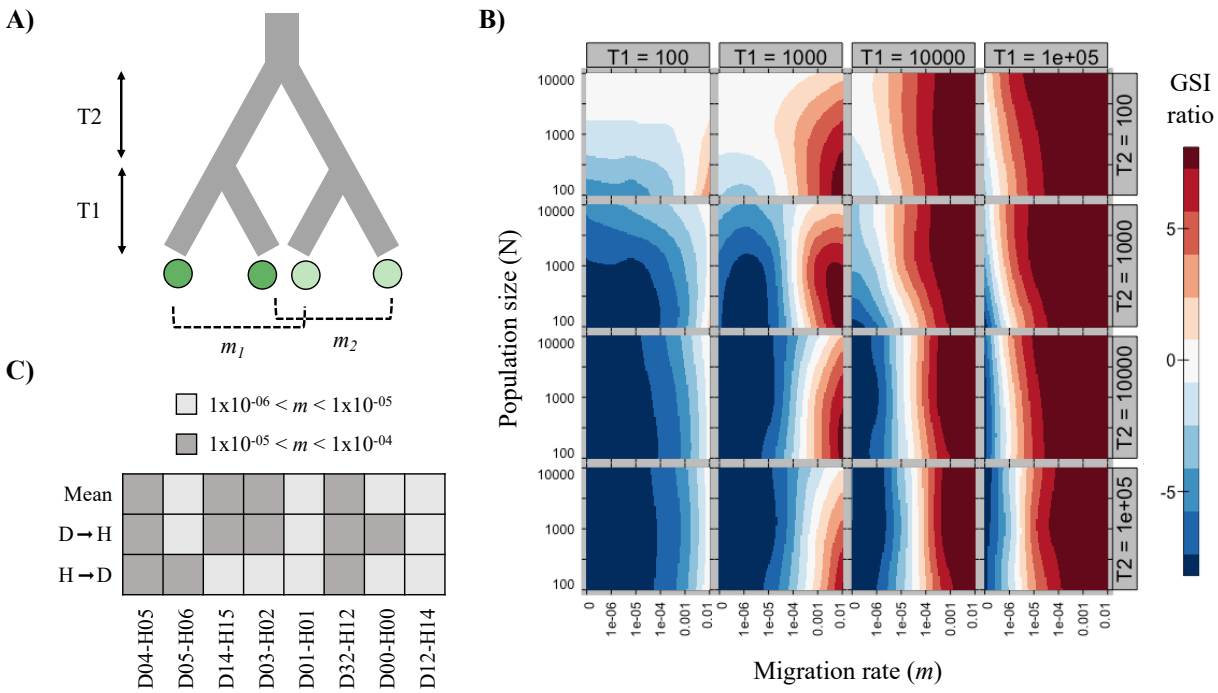


Figure 5. Forward population genetic simulations in *SLiM*

(A) Schematic diagram representing the single origin scenario simulated in *SLiM*. *T1* represents the time from the present to the second split, *T2* represents the time from the second split to the split of the ancestral population. Gene flow (denoted by dotted lines) occurs between populations within each locality. Light green and dark green circles represent populations from different ecotypes. **(B)** Logarithm of the ratio of mean Genealogical Sorting Index (GSI) calculated for parapatric populations, over mean GSI for sister populations of the same ecotype from a reconstructed phylogeny from simulation output in *SLiM2*. Increasingly positive GSI values (red) denote increasing levels of polyphyly due to distortion of the single origin scenario, whereas increasingly negative GSI values (blue) denote increasing levels of the true signal of monophyly due to lack of gene flow between ecotypes. **(C)** Summary of observed migration rates in *fastsimcoal2* for the eight replicate parapatric pairs for mean bidirectional migration (mean), Dune to Headland migration (D→H), and Headland to Dune migration (H→D). Light grey boxes indicate migration rate (m) values between $1x10^{-06}$ and $1x10^{-05}$, corresponding to regions of the parameter space in **(B)**, bottom right panel, where phylogeny distortion is unlikely. Dark grey boxes indicate migration rates between $1x10^{-05}$ and $1x10^{-04}$, corresponding to regions of the parameter space where there is some concern for phylogenetic distortion. Population pairs are ordered from left to right in accordance with increasing geographic distance between ecotypes.



Supplementary tables and figures

Table S1. Sampling locations

Sampling locations of the 23 *Senecio lautus* Dune and Headland populations. Coordinates represent the mid-point of each population. N corresponds to the final number of individuals after removing those with low coverage. Parapatric pairs in bold are sister-taxa within the phylogeny. H12A is a population found within an ecotone between the Dune (D32) and Headland (H12) at this locality.

Clade	Population code	Location	Ecotype	Pair	Coordinates	N
Eastern	D00	QLD: Stradbroke Island	Dune	D00-H00	S27° 31.153' E153° 30.189'	62
Eastern	H00	QLD: Stradbroke Island	Headland	D00-H00	S27° 26.140' E153° 32.749'	63
Eastern	D02	QLD: Southport	Dune	-	S27° 56.846' E153° 25.736'	62
Eastern	D03	NSW: Cabarita	Dune	**D03-H02**	S28° 19.794' E153° 34.264'	61
Eastern	H02	NSW: Cabarita	Headland	**D03-H02**	S28° 21.013' E153° 34.676'	61
Eastern	H04	NSW: Byron Bay	Headland	-	S28° 38.060' E153° 38.268'	62
Eastern	D01	QLD: Lennox Head	Dune	D01-H01	S28° 46.858' E153° 35.655'	60
Eastern	H01	QLD: Lennox Head	Headland	D01-H01	S28° 48.813' E153° 36.313'	58
Eastern	D04	NSW: Coffs Harbour	Dune	**D04-H05**	S30° 18.946' E153° 08.142'	62
Eastern	H05	NSW: Coffs Harbour	Headland	**D04-H05**	S30° 18.741' E153° 08.676'	62
Eastern	D05	NSW: South West Rocks	Dune	**D05-H06**	S30° 53.027' E153° 04.037'	62
Eastern	H06	NSW: South West Rocks	Headland	**D05-H06**	S30° 52.710' E153° 04.549'	62
South-eastern	H07	NSW: Port Macquarie	Headland	-	S31° 28.526' E152° 56.219	60
South-eastern	H03	NSW: Kiama	Headland	-	S34° 40.301' E150° 51.704'	63
South-eastern	D12	NSW: Bermagui	Dune	D12-H14	S36° 28.346' E150° 03.581'	62
South-eastern	H14	NSW: Green Cape	Headland	D12-H14	S37° 15.748' E150° 02.991'	62
South-eastern	D32	VIC: Cape Bridgewater	Dune	**D32-H12**	S38° 19.631' E141° 23.772'	62
South-eastern	H12	VIC: Cape Bridgewater	Headland	**D32-H12**	S38° 22.728' E141° 22.018'	63
South-eastern	H12A	VIC: Cape Bridgewater	Intermediate	-	S38° 20.282' E141° 23.896'	62
South-eastern	D14	TAS: Port Arthur	Dune	**D14-H15**	S43° 10.550' E147° 51.267'	12
South-eastern	H15	TAS: Port Arthur	Headland	**D14-H15**	S43° 11.240' E147° 50.672'	11
Western	D35	WA: Isthmus Hill	Dune	-	S35° 05.885' E117° 59.182'	62
Western	D09	WA: Leeuwin-Naturaliste National Park	Dune	-	S33° 46.239' E114° 59.541'	63

Table S2. Sequencing and alignment summary for *Senecio lautus* individuals

Summary statistics for the 23 populations used within the study. Excluded from the table are the 19 individuals removed due to high missing data.

Population code	Mean # clean reads (range)	Mean % mapped reads (range)	% mapped reads properly paired (range)
D00	2,138,896 (971,466 - 3,506,240)	94 (62 - 98)	92 (61 - 96)
H00	3,075,580 (1,528,536 - 6,198,407)	81 (16 - 97)	79 (16 - 95)
D02	2,714,361 (895,858 - 5,258,091)	80 (18 - 96)	76 (17 - 94)
D03	3,160,935 (2,015,566 - 8,748,545)	84 (21 - 97)	78 (20 - 95)
H02	2,772,081 (1,408,465 - 4,192,718)	85 (34 - 96)	83 (33 - 94)
H04	3,176,210 (1,695,120 - 5,950,574)	90 (72 - 97)	79 (60 - 95)
D01	3,061,253 (1,318,262 - 4,548,766)	96 (83 - 98)	90 (72 - 96)
H01	2,770,561 (1,105,881 - 6,164,034)	93 (42 - 98)	91 (36 - 96)
D04	2,922,712 (2,146,253 - 3,718,635)	91 (62 - 98)	83 (61 - 96)
H05	2,866,233 (1,754,603 - 4,696,562)	92 (71 - 97)	85 (67 - 95)
D05	2,854,456 (1,554,814 - 4,156,601)	93 (48 - 97)	87 (44 - 94)
H06	2,112,573 (1,253,010 - 3,538,428)	84 (37 - 97)	82 (36 - 95)
H07	3,116,096 (1,646,581 - 10,437,355)	82 (27 - 98)	73 (21 - 96)
H03	2,795,169 (1,593,958 - 5,514,042)	77 (15 - 97)	76 (14 - 95)
D12	2,700,235 (1,448,045 - 5,032,607)	90 (45 - 98)	83 (39 - 94)
H14	3,033,007 (1,661,205 - 8,349,758)	71 (11 - 96)	67 (11 - 95)
D32	2,854,449 (1,517,908 - 5,609,011)	79 (19 - 97)	76 (17 - 95)
H12	2,892,473 (1,220,369 - 4,774,451)	83 (34 - 97)	80 (33 - 94)
H12A	2,614,734 (1,509,934 - 8,120,979)	85 (27 - 98)	82 (27 - 95)
D14	2,894,283 (1,704,586 - 4,893,613)	94 (75 - 98)	85 (58 - 95)
H15	3,229,783 (1,823,447 - 4,958,055)	90 (33 - 97)	84 (29 - 94)
D35	2,987,725 (1,754,767 - 6,004,276)	90 (62 - 98)	78 (44 - 95)
D09	3,008,471 (1,794,627 - 4,826,686)	67 (21 - 96)	63 (20 - 92)

Table S3. Pairwise F_{ST} for *S. lautus* populations

Pairwise FST values between all 21 populations of the south and south-eastern clades.

	D00	D01	D02	D03	D04	D05	D12	D14	D32	H00	H01	H02	H03	H04	H05	H06	H07	H12	H12A	H14	H15
D00	-																				
D01	0.25	-																			
D02	0.25	0.22	-																		
D03	0.27	0.22	0.20	-																	
D04	0.29	0.25	0.26	0.28	-																
D05	0.29	0.25	0.27	0.27	0.25	-															
D12	0.34	0.29	0.31	0.33	0.31	0.28	-														
D14	0.34	0.28	0.29	0.31	0.29	0.26	0.32	-													
D32	0.34	0.30	0.33	0.34	0.32	0.30	0.30	0.32	-												
H00	0.26	0.23	0.24	0.25	0.26	0.26	0.29	0.28	0.31	-											
H01	0.26	0.22	0.25	0.25	0.25	0.24	0.26	0.25	0.28	0.23	-										
H02	0.26	0.21	0.21	0.20	0.27	0.27	0.31	0.30	0.33	0.25	0.25	-									
H03	0.33	0.29	0.31	0.32	0.30	0.27	0.28	0.30	0.30	0.29	0.25	0.31	-								
H04	0.28	0.23	0.25	0.26	0.27	0.26	0.30	0.29	0.31	0.24	0.22	0.26	0.29	-							
H05	0.29	0.25	0.27	0.28	0.21	0.26	0.31	0.30	0.32	0.27	0.25	0.27	0.30	0.27	-						
H06	0.30	0.27	0.28	0.29	0.27	0.21	0.30	0.28	0.31	0.27	0.24	0.28	0.28	0.27	0.28	-					
H07	0.31	0.27	0.28	0.29	0.28	0.24	0.28	0.28	0.30	0.27	0.24	0.29	0.27	0.27	0.28	0.26	-				
H12	0.35	0.31	0.33	0.34	0.33	0.30	0.31	0.32	0.22	0.31	0.28	0.33	0.30	0.31	0.33	0.32	0.30	-			
H12A	0.34	0.30	0.33	0.33	0.32	0.30	0.29	0.31	0.20	0.31	0.27	0.32	0.29	0.31	0.32	0.32	0.30	0.22	-		
H14	0.34	0.30	0.31	0.33	0.31	0.28	0.28	0.30	0.30	0.30	0.27	0.32	0.28	0.29	0.32	0.30	0.28	0.31	0.30	-	
H15	0.34	0.28	0.29	0.31	0.30	0.26	0.32	0.15	0.32	0.28	0.25	0.30	0.30	0.29	0.31	0.28	0.28	0.32	0.30	0.30	-

Table S4. Estimation of gene flow rates and population parameters in *fastsimcoal2*

Populations: the two populations used for each comparison (population 1 is on the left, and population 2 on the right). Asize: ancestral effective population size. Pop1size: effective population size of population 1. Pop2size: effective population size of population 2. DivTime: divergence time. SecTime: time since gene flow upon secondary contact. 2NmP1->P2: gene flow (2Nm) from population 1 to population 2. 2NmP2->P1: gene flow (2Nm) from population 2 to population 1. Values in bold represent 2Nm > 1.

Comparison	Populations	Asize	Pop1size	Pop2size	DivTime	SecTime	2NmP1->P2	2NmP2->P1
Dune-Headland	D00-H00	100497	47926	134364	71945	18690	0.2176	0.2830
	D03-H02	88035	34637	152616	44190	15031	0.1590	0.4722
	D01-H01	72385	90270	159101	71918	13268	0.6241	0.3671
	D04-H05	87603	90859	123949	30707	6329	**1.3942**	**1.5024**
	D05-H06	97653	131873	70970	67927	16810	0.4049	0.4325
	D12-H14	56510	211701	103102	110018	11783	0.2188	0.1787
	D14-H15	102574	39573	143420	47929	39730	0.3952	0.5187
	D32-H12	56568	661726	212041	38706	11290	**5.5694**	**5.2694**
Dune-Dune	D00-D02	97055	63843	113624	52711	6436	0.3242	0.2617
	D01-D03	99168	142624	51613	58652	23772	0.3280	0.2119
	D01-D04	92638	121936	74257	66970	11319	0.2901	0.2200
	D02-D03	93440	116800	48492	54857	23163	0.3514	0.2029
	D04-D05	78638	86635	110138	77895	18111	0.3178	0.2027
	D05-D12	35322	103044	223346	128159	21689	0.1562	0.2041
	D12-D14	22223	259172	38450	118024	35758	0.0991	0.1046
	D14-D32	47348	27002	641721	56330	12595	0.3179	0.1074
Headland-Headland	H00-H02	97070	107516	94259	81290	9622	0.3920	0.4012
	H01-H04	78784	171269	86431	81443	19633	0.2561	0.3261
	H01-H05	61893	173061	89468	95145	17016	0.2546	0.3116
	H02-H04	78009	107213	91698	87055	20222	0.2913	0.1768
	H03-H07	57850	147099	125603	109197	12874	0.1904	0.1921
	H03-H14	63207	157400	119559	108683	9099	0.2068	0.2012
	H05-H06	84257	109077	88382	81710	10907	0.2559	0.1953
	H06-H07	67117	89121	141737	88627	14422	0.2532	0.2387
	H12-H12A	52196	286574	322443	43928	14082	**3.7584**	**4.0315**
	H12-H15	46457	362929	51902	81116	9800	0.1921	0.2501
	H14-H15	46091	168257	72243	101092	18596	0.1396	0.1181
Allopatric	D03-D32	35657	53174	566115	76621	5201	0.3873	0.3238
	D03-H12	37227	67316	333665	99511	8840	0.1984	0.2642
	H02-H12	33876	78181	313687	111278	9257	0.1884	0.2707

Table S5. Bootstrap values for gene flow estimates inferred in *fastsimcoal2*

Populations: the two populations used for each comparison (population 1 is on the left, and population 2 on the right. 2NmP1->P2min and max: lower and upper 95% confidence intervals for gene flow from population 1 to population 2, respectively. 2NmP2->P1min and max: lower and upper 95% confidence intervals for gene flow from population 2 to population 1, respectively. Populations in bold represent 2Nm > 1.

Comparison	Populations	2NmP1->P2min	2NmP1->P2max	2NmP2->P1min	2NmP2->P1max
Dune-Headland	D00-H00	0.2181	0.2281	0.2769	0.2865
	D03-H02	0.1511	0.1611	0.4576	0.4758
	D01-H01	0.5991	0.6174	0.3554	0.3655
	D04-H05	**1.3120**	**1.3648**	**1.4347**	**1.4903**
	D05-H06	0.3924	0.4054	0.4172	0.4321
	D12-H14	0.2174	0.2236	0.1775	0.1840
	D14-H15	0.3776	0.4010	0.5001	0.5215
	D32-H12	**4.9046**	**5.0810**	**5.2088**	**5.3999**
Dune-Dune	D00-D02	0.3186	0.3344	0.2576	0.2686
	D01-D03	0.3183	0.3305	0.2040	0.2137
	D01-D04	0.2818	0.2911	0.2148	0.2226
	D02-D03	0.3417	0.3563	0.2000	0.2096
	D04-D05	0.3106	0.3228	0.2008	0.2078
	D05-D12	0.1533	0.1584	0.2020	0.2077
	D12-D14	0.0976	0.1011	0.1037	0.1068
	D14-D32	0.3109	0.3274	0.1050	0.1106
Headland-Headland	H00-H02	0.3828	0.3952	0.3901	0.4038
	H01-H04	0.2504	0.2574	0.3183	0.3290
	H01-H05	0.2485	0.2558	0.3033	0.3139
	H02-H04	0.2892	0.2978	0.1741	0.1802
	H03-H07	0.1882	0.1945	0.1917	0.1975
	H03-H14	0.2029	0.2089	0.1976	0.2038
	H05-H06	0.2526	0.2610	0.1926	0.1992
	H06-H07	0.2460	0.2537	0.2310	0.2382
	H12-H12A	**3.6292**	**3.7754**	**3.9901**	**4.1278**
	H12-H15	0.1878	0.1954	0.2484	0.2574
	H14-H15	0.1357	0.1415	0.1163	0.1202
Allopatric	D03-D32	0.3699	0.3893	0.3174	0.3273
	D03-H12	0.1932	0.2013	0.2604	0.2680
	H02-H12	0.1832	0.1900	0.2645	0.2731

Figure S1. Summary of TreeMix runs

(A) Maximum likelihood tree with no migration. **(B)** Residuals for the no migration tree. **(C)** Log-likelihoods for each model for 1-25 migration events. **(D)** Proportion variance explain for each model for 1-25 migration events.

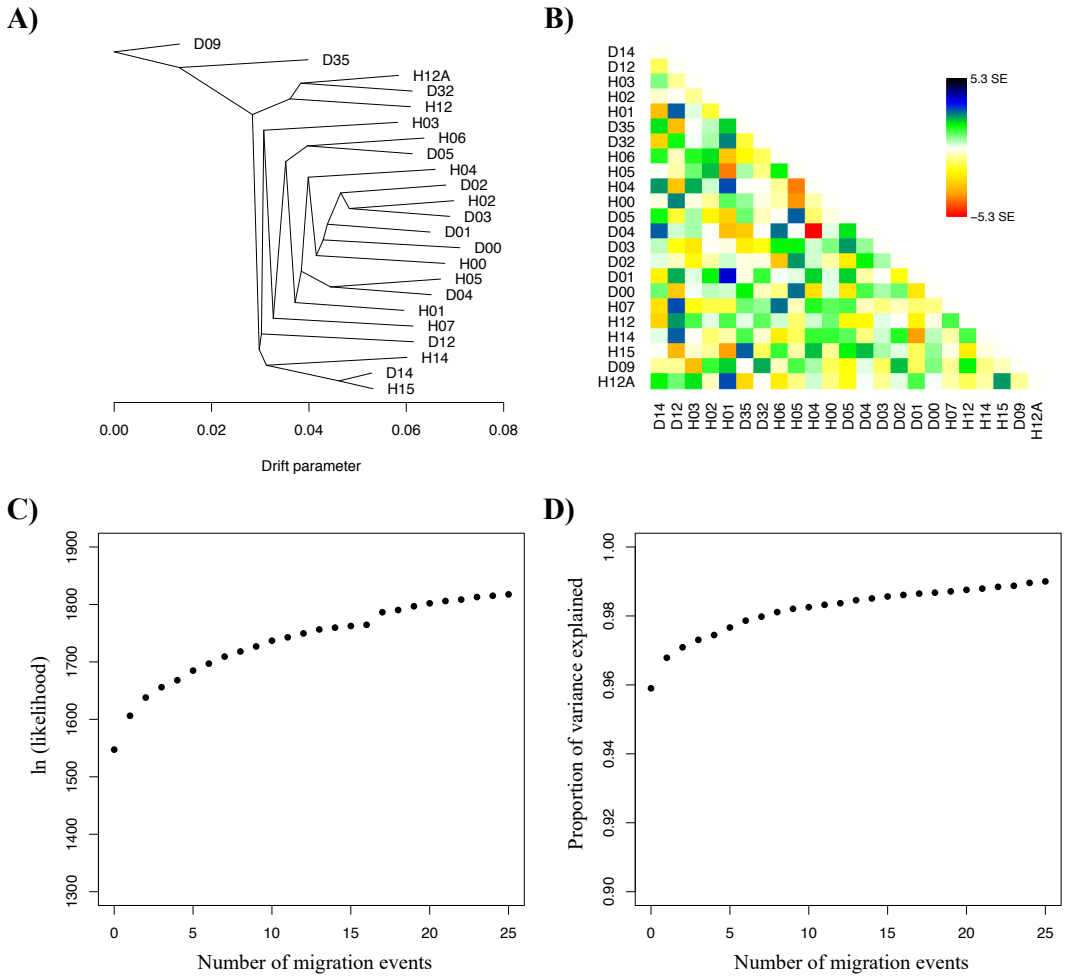

A)
D09
D35
H12A
D32
H12
H03
H06
D05
H04
D02
H02
D03
D01
D00
H00
H05
D04
H01
H07
D12
H14
D14
H15
0.00
0.02
0.04
0.06
0.08
Drift parameter
B)
D14
D12
H03
H02
H01
D35
D32
H06
H05
H04
H00
D05
D04
D03
D02
D01
D00
H07
H12
H14
H15
D09
H12A
5.3 SE
−5.3 SE
C)
ln (likelihood)
1300
1400
1500
1600
1700
1800
1900
0
5
10
15
20
25
Number of migration events
D)
Proportion of variance explained
0.90
0.92
0.94
0.96
0.98
1.00
0
5
10
15
20
25
Number of migration events

Figure S2. TreeMix migration events 1-10

Maximum likelihood tree with 10 migration events. Coloured arrows denote the intensity and direction of migration events.

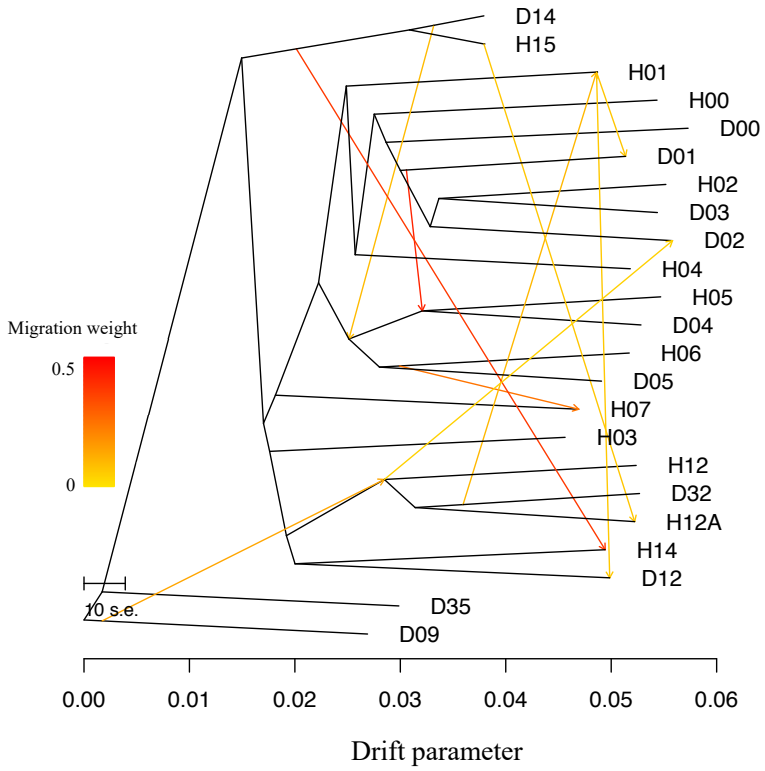


Figure S3. Frequency distribution of *f3*-statistics

Frequency distribution of *f3*-statistics calculated in *TreeMix* across all populations.

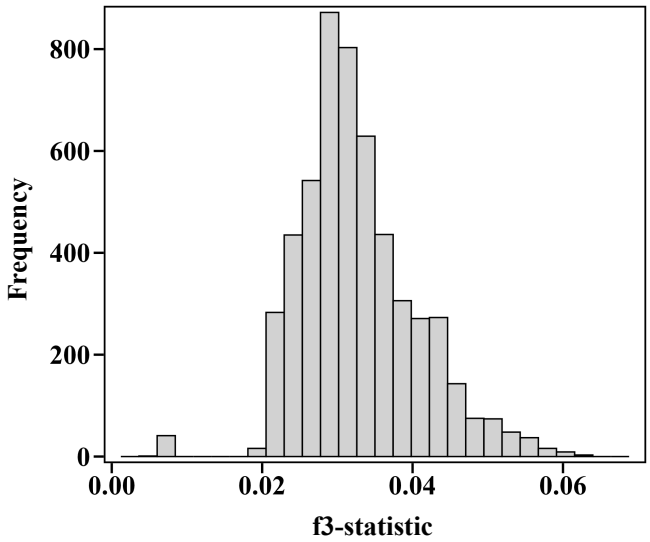


Figure S4. *fastSTRUCTURE* K=22, K=28 and marginal likelihoods

(A) Bayesian assignment of individuals to genetic clusters within *fastSTRUCTURE* for K=22 and K = 28. Each of the 1,319 individuals is depicted as a bar, with colours representing ancestry proportions to each cluster. Populations are ordered according to their geographic distribution along the coast. **(B)** Marginal likelihood values for successive K-values within *fastSTRUCTURE*. Red dashed lines denote the K-value that best explained the structure in the data (K = 22), as well as the K-value that maximised the marginal likelihood of the data (K = 28). **(C)** Change in marginal likelihoods from *fastSTRUCTURE* for successive K-values. Red dashed line denotes K = 23, higher K-values with negligible change in likelihood values.

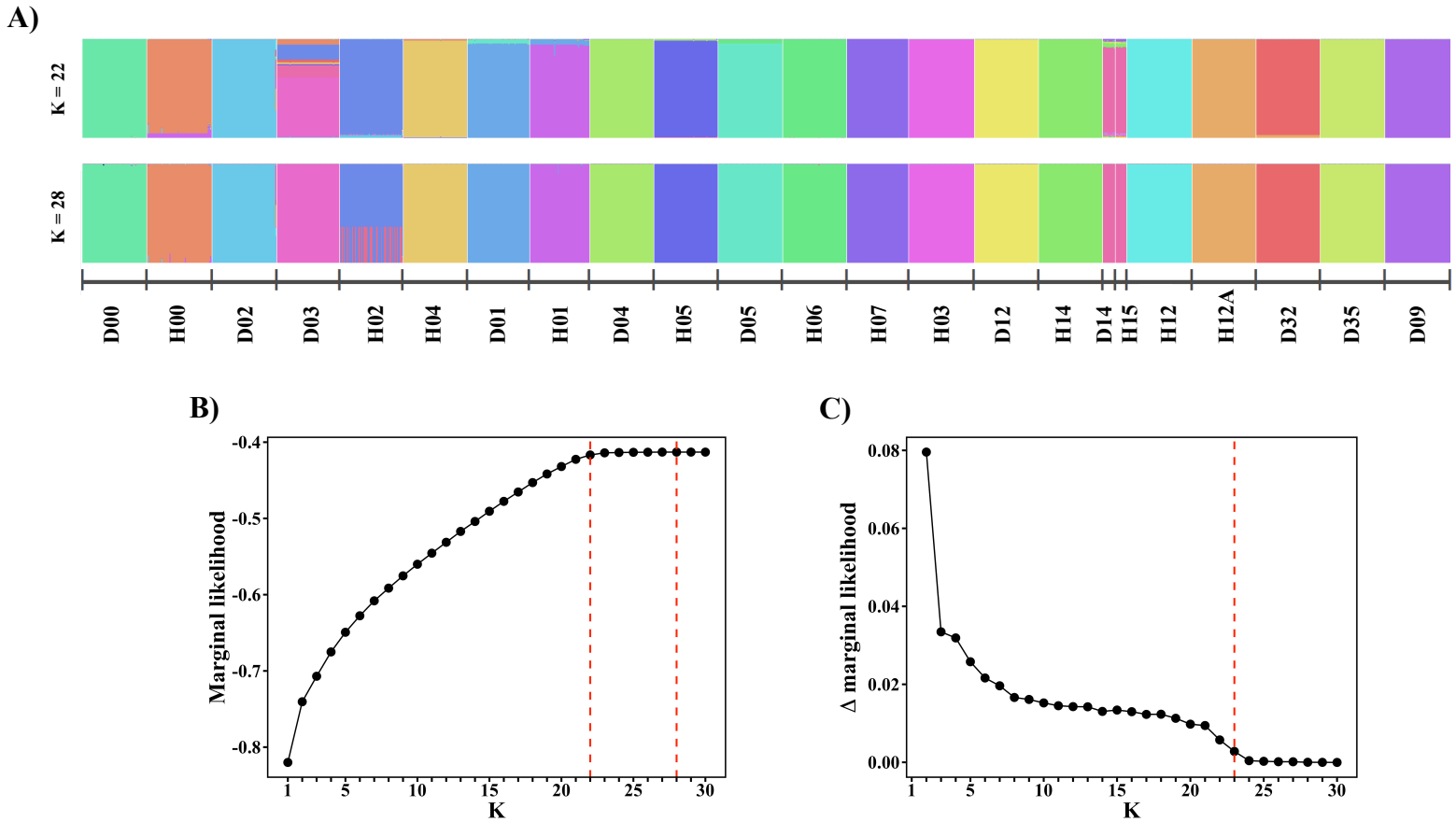


Figure S5. *STRUCTURE* best K-values for Dune-Headland pairs

STRUCTURE best K-values for the eight Dune-Headland replicate pairs, based on the maximum value for ΔK (the second order rate of change in the log probability of data between successive K-values).

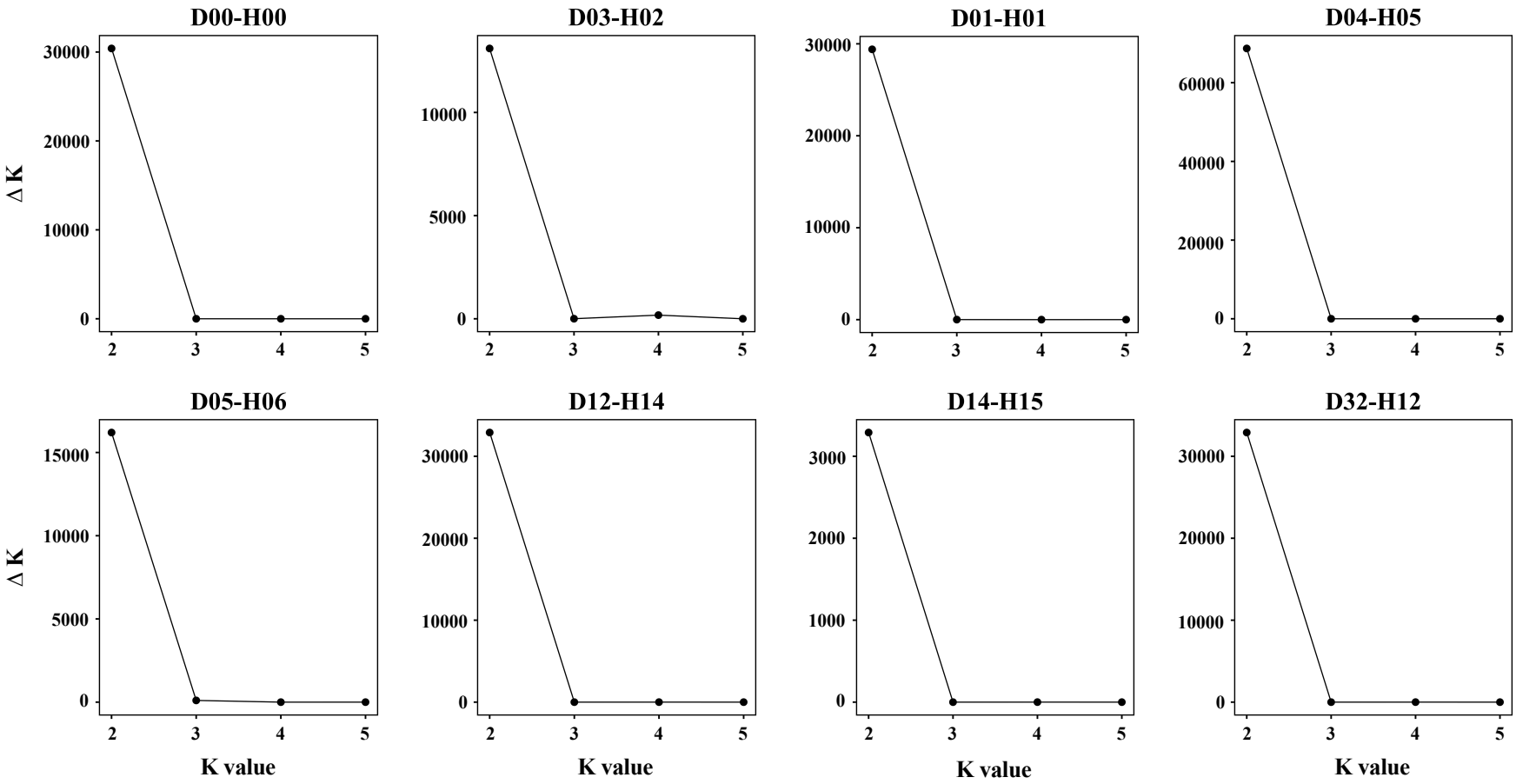


Figure S6. Log-likelihood values for the eight demographic models tested in *fastsimcoal2* per pair

NM: no migration. BM: bidirectional migration. M21: migration from population 2 to 1. M12: migration from population 1 to 2. BSC: bidirectional migration after secondary contact. SC21: migration from population 2 to 1 after secondary contact. SC12: migration from population 1 to 2 after secondary contact. EBM: bidirectional migration after population splitting with cessation of gene flow.

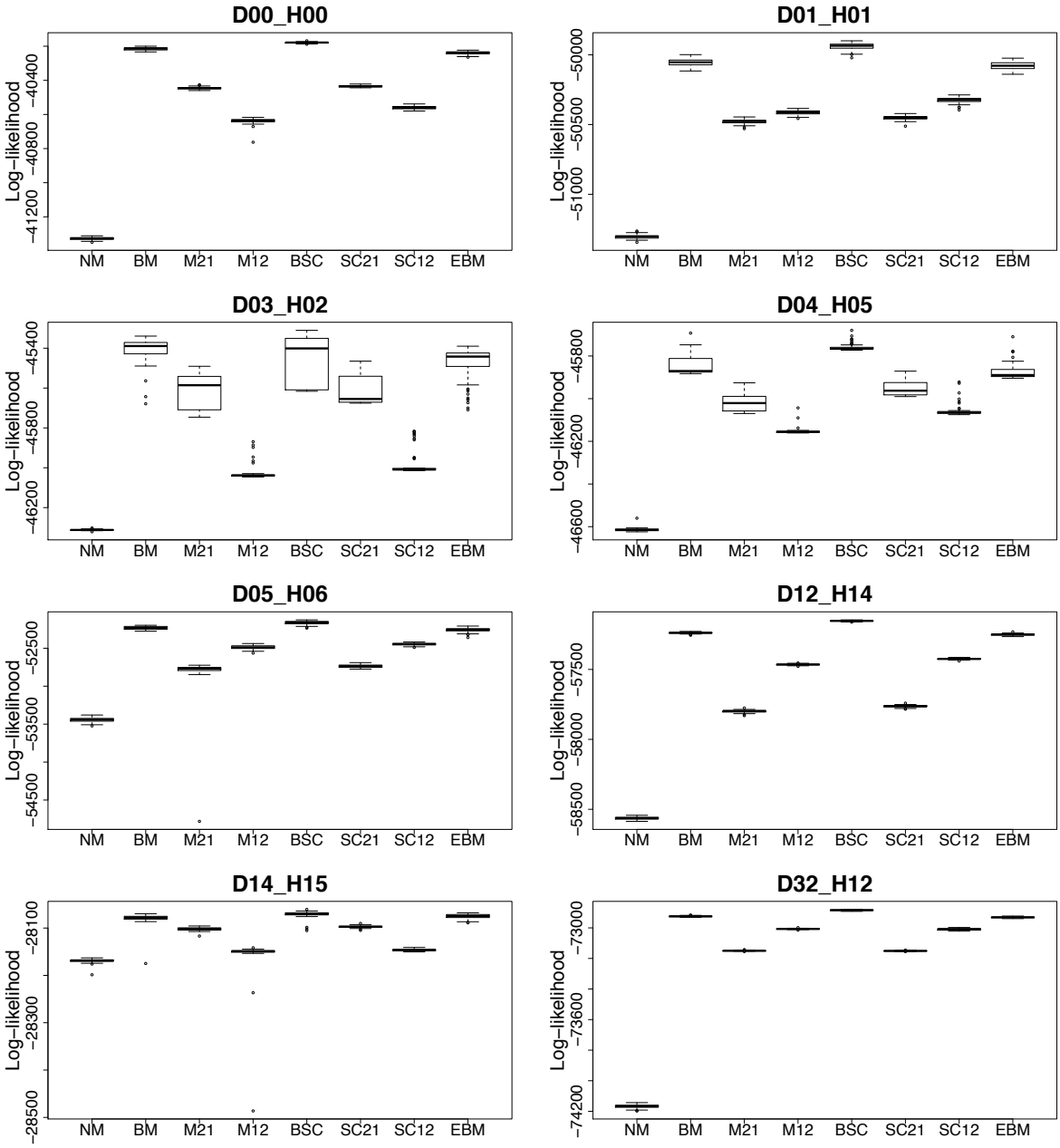


Figure S6 cont.

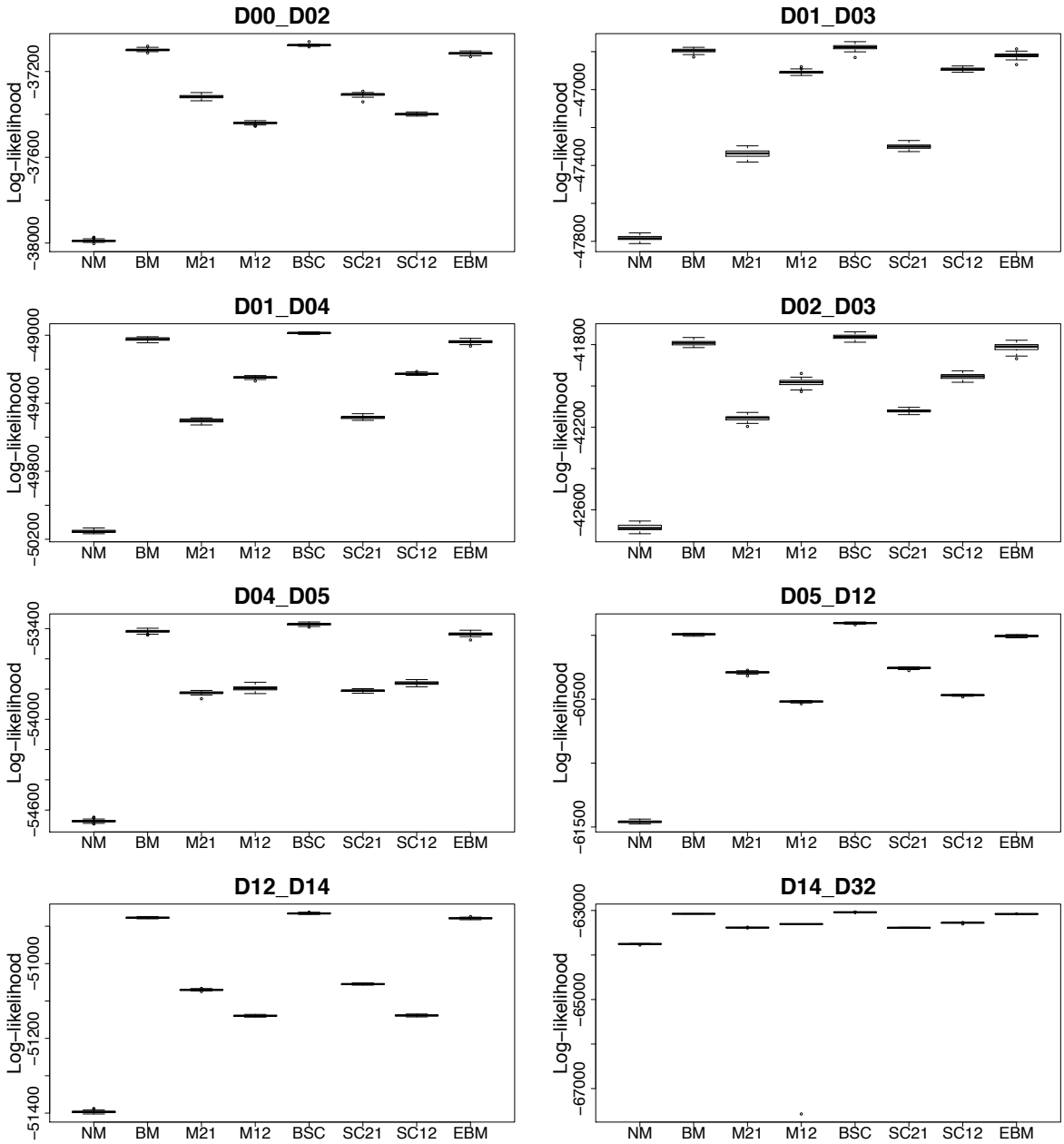


Figure S6 cont.

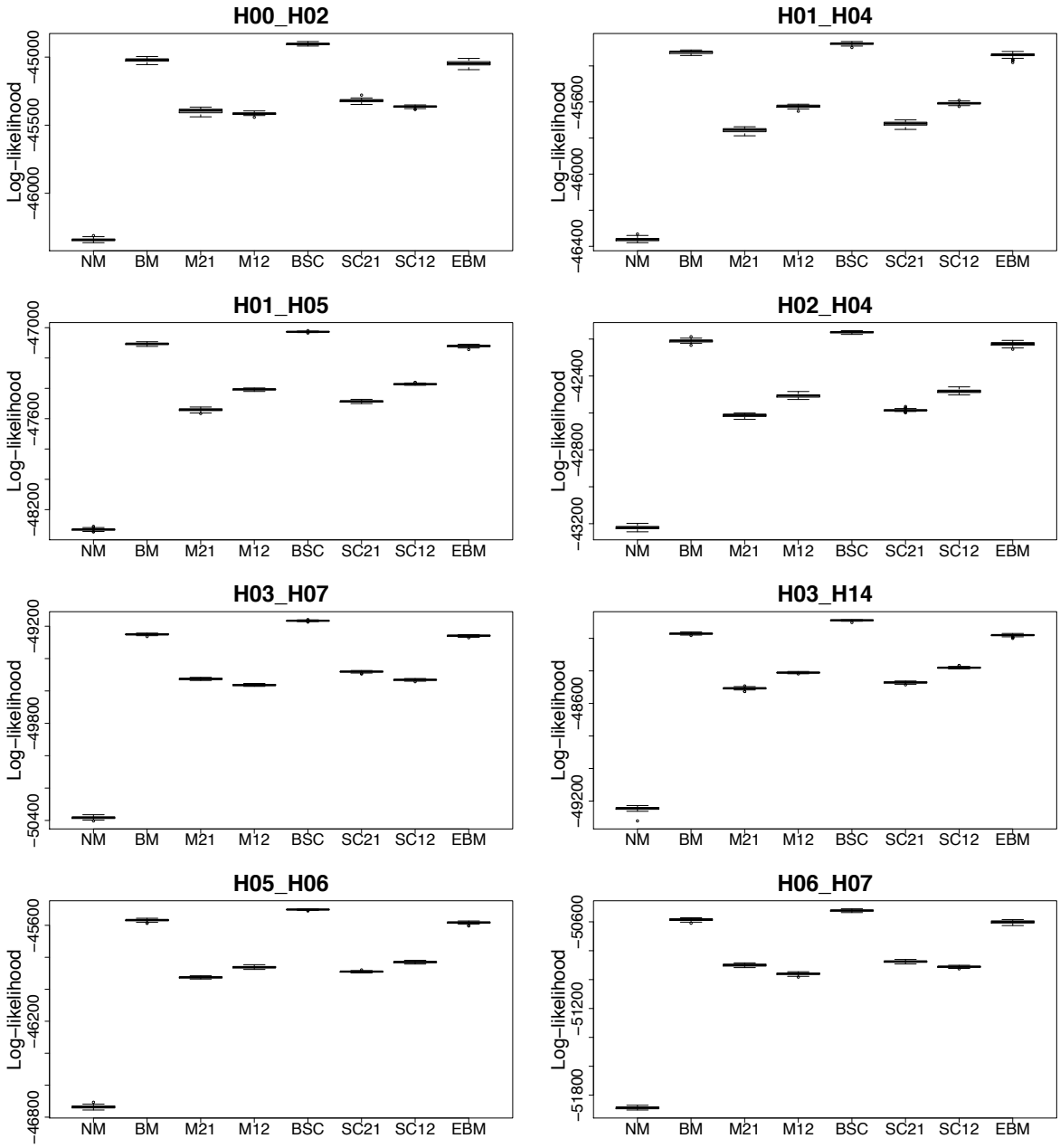


Figure S6 cont.

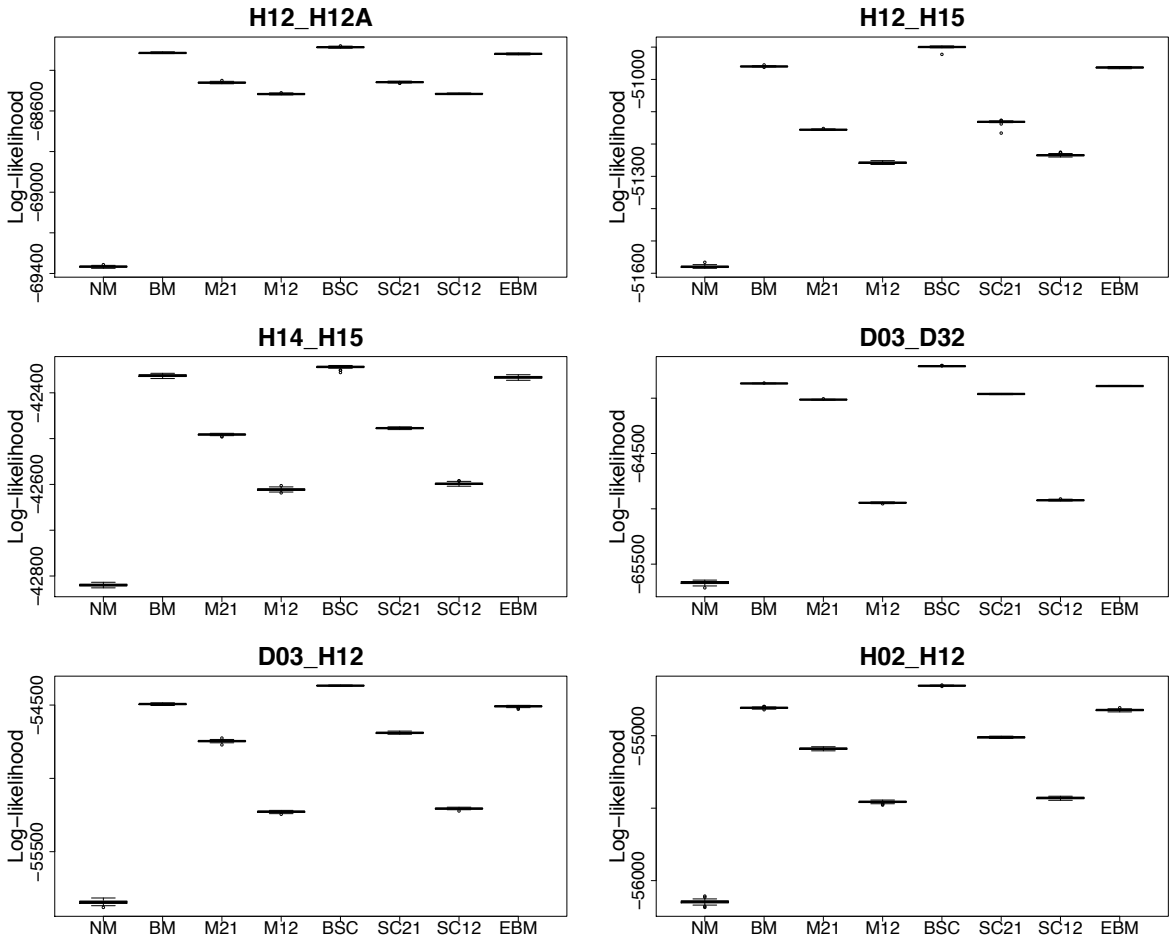


Figure S7. Likelihood values for testing if migration is significantly different from zero

Max L: maximum likelihood for the best run from the best model. *A*: Gene flow from population 2 to 1 is fixed (2Nm = 0.01). *B*: gene flow from population 1 to 2 is fixed (2Nm = 0.01). *C*: gene flow in both directions is fixed (2Nm = 0.01). The asterisk denotes the pair where any of the migration rates is not significantly different from 2Nm = 0.01.

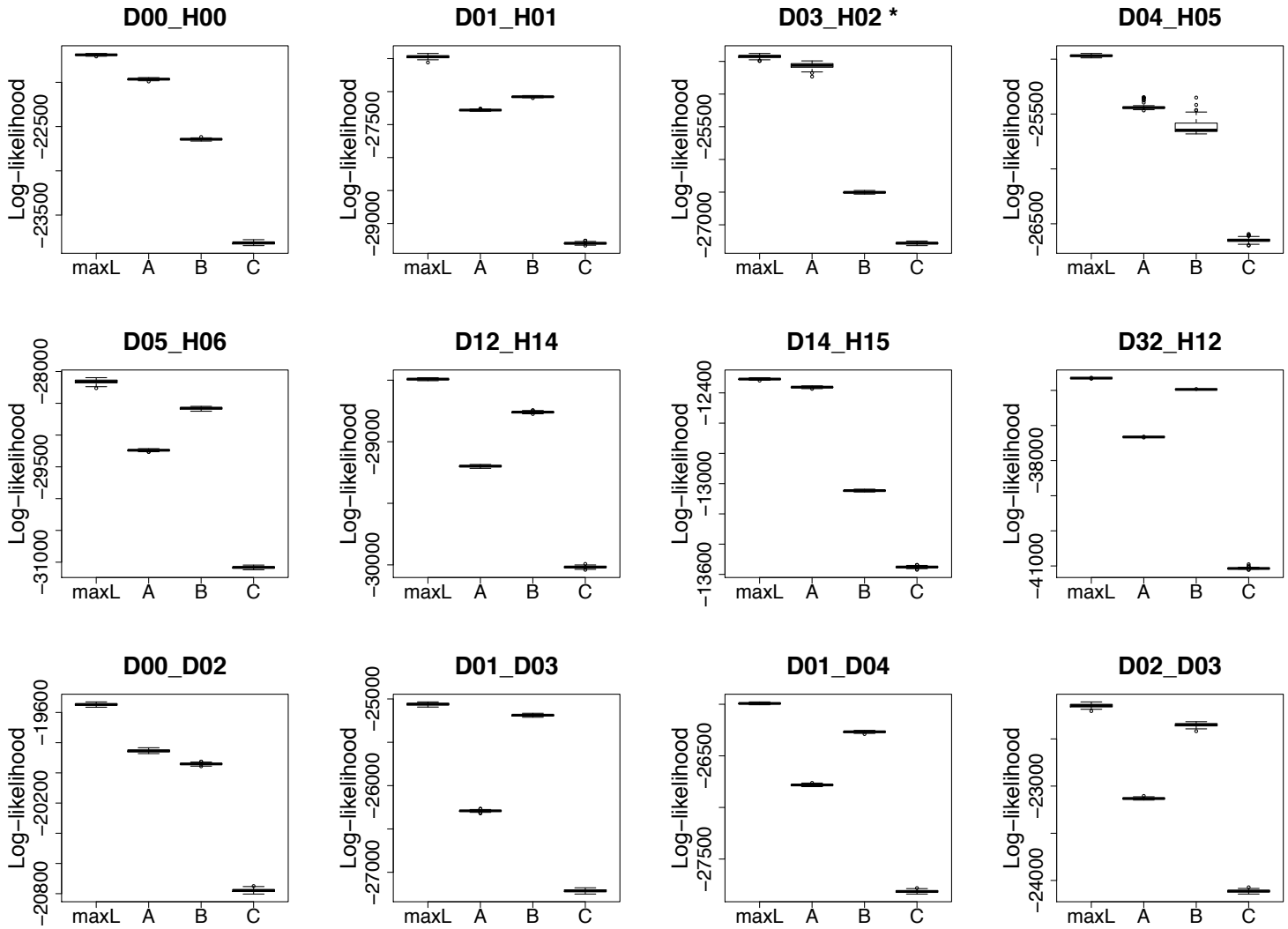


Figure S7 cont.

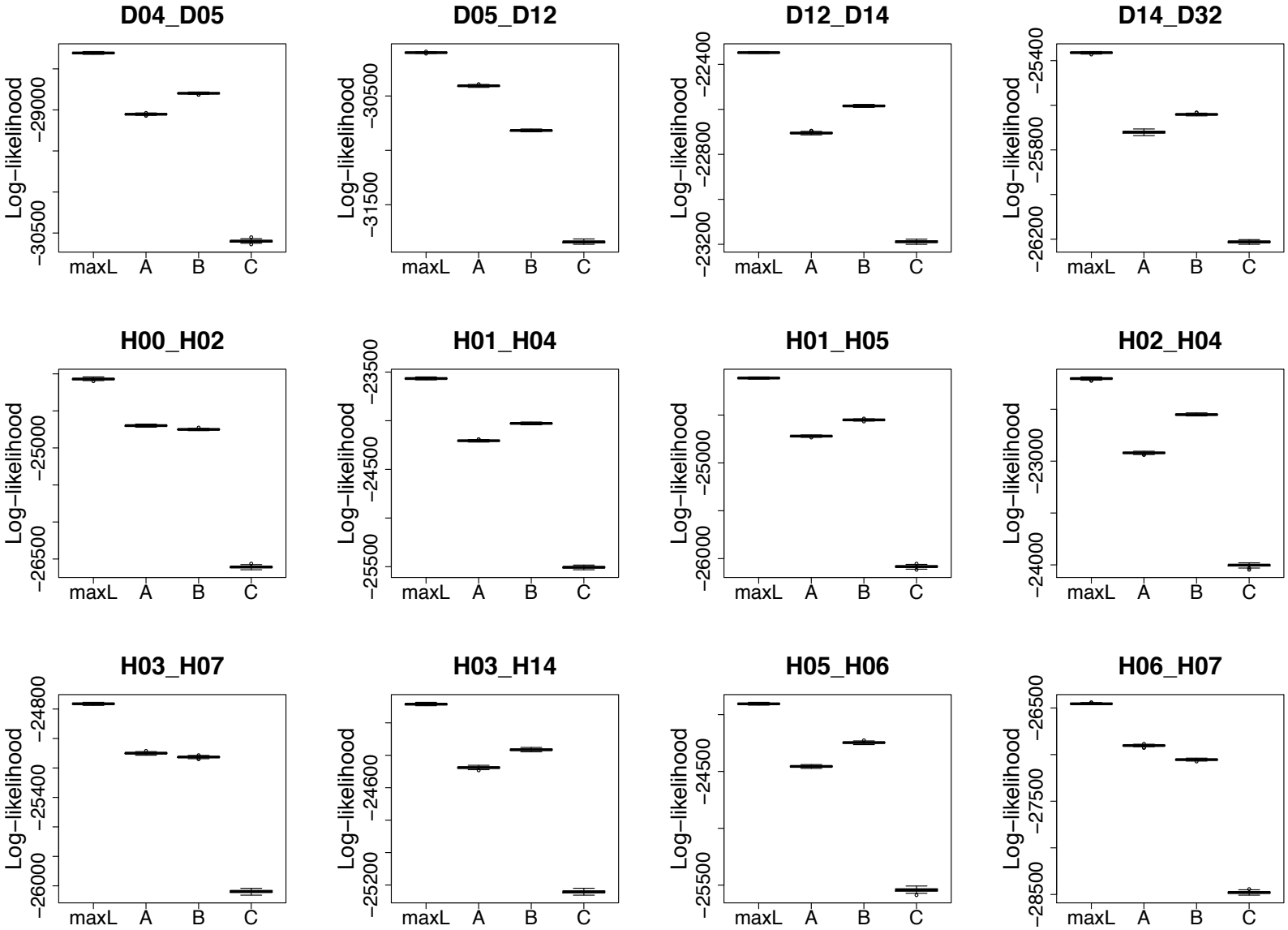
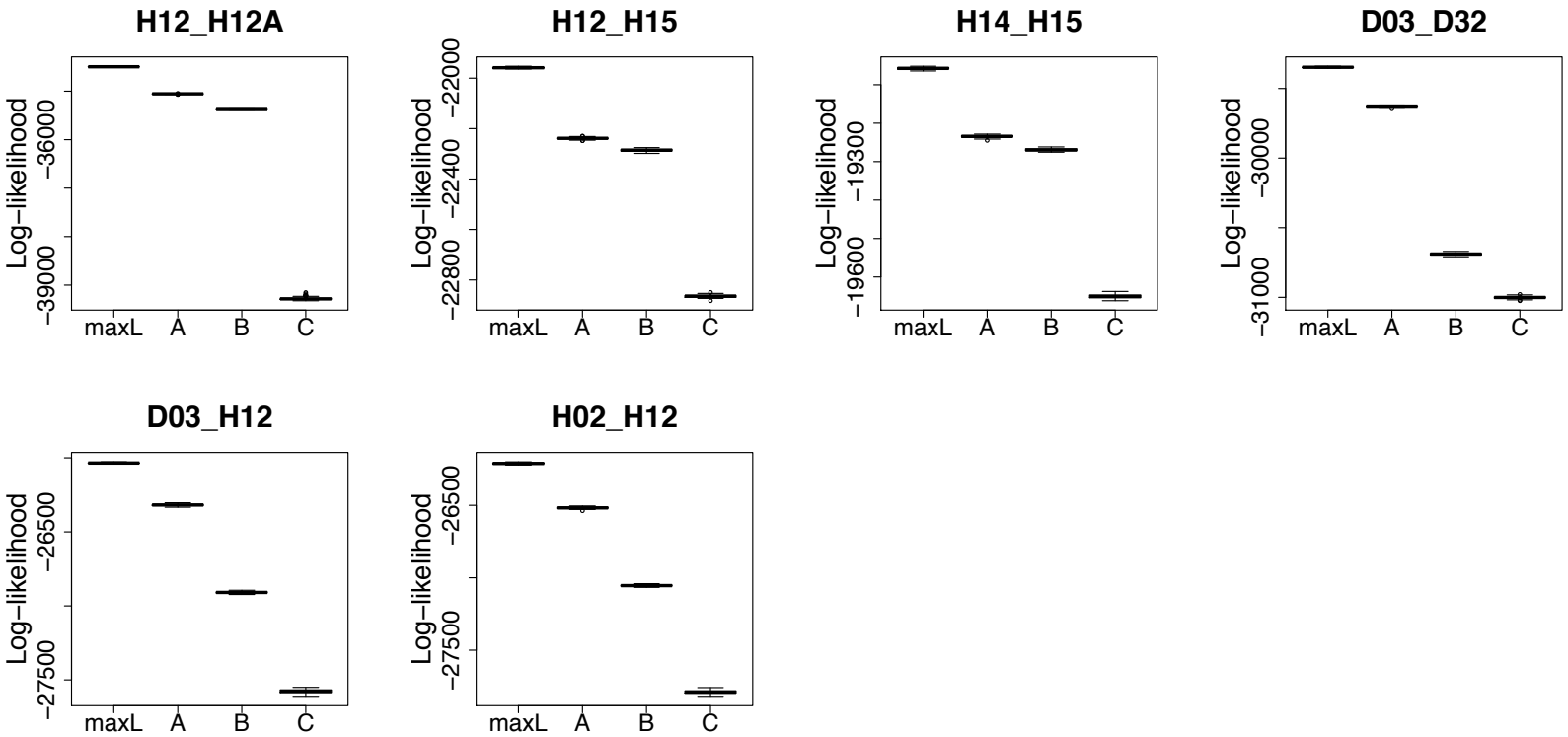


Figure S7 cont.



Supplementary methods

Estimation of the number of monomorphic sites per pair

To estimate the monomorphic sites per pair we first calculated the number of RAD loci by using *PLINK* to thin for one SNP per RAD locus. The total read length of each RAD locus was (on average) 190bp (taking into account the length of the sequencing read after removal of barcodes/indexes). We used the following formula to calculate the number of monomorphic sites per pair:

Monomorphic sites = (read length x number RAD loci) - number variable sites

Here, we may be slightly overestimating the number of monomorphic sites as we are assuming all sites without a called SNP are monomorphic, although some could be actual variants that were not called due to not passing filtering requirements. Nevertheless, the parameter estimates (especially the migration rates) were robust to varying the number of monomorphic sites (data not shown).

SLiM2 code for simulations

```
initialize() {
	if (exists("slimgui")) {
		defineConstant("seed", 1);
		defineConstant("mu", 1e-7);
		defineConstant("r", 1e-8);

		defineConstant("N", 100);
		defineConstant("t1", 10000);
		defineConstant("t2", 5000);
		defineConstant("mig", 0);
		defineConstant("tSec", 0.1);
		defineConstant("outPath", "~/workspace/PopGenSims/OriginScenarios");
	}

	setSeed(seed);
	initializeMutationRate(mu);
	initializeMutationType("m1", 0.5, "f", 0.0);
	initializeGenomicElementType("g1", m1, 1.0);
	initializeGenomicElement(g1, 0, 1e6 - 1);
	initializeRecombinationRate(r);
}

1 {

	// create ancestral population
	sim.addSubpop("p0", N);

	// schedule split and migration events based on parameter values
	t0 = 10*N;
	outGen = t0+t1+t2;

	sim.rescheduleScriptBlock(s1, start=t0+1, end=t0+1);
	sim.rescheduleScriptBlock(s2, start=t0+t1+1, end=t0+t1+1);
	sim.rescheduleScriptBlock(s4, start=outGen, end=outGen);

	if(mig == 0){
		sim.deregisterScriptBlock(s3);
	} else
```

```
	sim.rescheduleScriptBlock(s3, start=asInteger(t0+t1+1+round(t2*(1-tSec))), end=outGen);

}

s1 10 {

	// initial split of ecotypes here
	sim.addSubpopSplit("p10", N, p0);
	sim.addSubpopSplit("p100", N, p0);
	p0.setSubpopulationSize(10);
}

s2 20 {

	// output t1 vcf here
	outgroup = sample(p0.individuals, 1);
	ingroup = sapply(sim.subpopulations[sim.subpopulations != p0], "sample(applyValue.individuals, 30, replace = F);");
	set = c(outgroup, ingroup);
	set.genomes.outputVCF(filePath = paste(c(outPath, "/", "t1.replicate-", seed, ".vcf"), sep=""), outputMultiallelics = F);

	// subsequent split into populations here
	sim.addSubpopSplit("p11", N, p10);
	sim.addSubpopSplit("p101", N, p100);
}

s3 30 {

	// initialize migration here between parapatric divergent ecotypes
	p10.setMigrationRates(p100, mig);
	p100.setMigrationRates(p10, mig);
	p11.setMigrationRates(p101, mig);
	p101.setMigrationRates(p11, mig);
}

s4 40 late() {

	// output final vcf here
	outgroup = sample(p0.individuals, 1);
	ingroup = sapply(sim.subpopulations[sim.subpopulations != p0], "sample(applyValue.individuals, 30, replace = F);");
	set = c(outgroup, ingroup);
	set.genomes.outputVCF(filePath = paste(c(outPath, "/", "t2.replicate-", seed, ".vcf"), sep=""), outputMultiallelics = F);
}
```

R code for the genealogical sorting index (GSI) calculations

```
# Code modified from: Ravinet, M. et al. The genomic landscape at a late stage of stickleback speciation: High genomic divergence interspersed by small localized regions of introgression. PLoS Genetetocs 14, e1007358 (2018).

# ===== Load Dependencies =====
library(ape)
suppressMessages(library(vcfR))
library(geiger)
suppressMessages(library(adegenet))

# ===== Read Input Data =====
args <- commandArgs(trailingOnly = TRUE)
filename <- args[1]

#filename <- "~/Dropbox (OL)/OriginScenarios-results/N-1000.t1-10000.t2-10000.tSec-0.25.mig-1e-06/t2.replicate-1.vcf"
vcf <- read.vcfR(filename, verbose = FALSE)


# ===== Define gsi Function =====
gsi <- function(tr, grp){
  n <- length(grp) - 1
  # only consider internal nodes (tips get index 1:Ntip(tr))
  internal.nodes <- seq(Ntip(tr)+1, Ntip(tr) + Nnode(tr))
  # For each internal node, what are the descendant tips
  node.descendants <- lapply(internal.nodes, function(n) tips(tr, n))

  # ----- Jeff Groh 10 May 2019 -----
  # Previous code contained an error in the following lines:
```

```
  # Which nodes have descendants in the group being considered?
  # required <- sapply(descendants, function(x) any(grp %in% x) )
 # The problem with this is that it selects *all* nodes which contain *any*
 # members of the focal group. However, in the denominator for the gsi calculation,
 # we are only interested in summing the degrees of nodes which belong to the minimum
 # subtree that contains all members of the focal group. The code below fixes this by
 # selecting the correct set of nodes.

 # find root of mimimum subtree containing all members of focal group
 # how many tips of the focal group are descended from each node
 n.focal.members <- sapply(node.descendants, function(x){ length(which(grp %in% x)) })
 # how many total tips are descended from each node
 n.total.members <- sapply(node.descendants, function(x){ length(x) })
 # to be a root of the minimum subtree, a node must contain at least all members of the focal group
 candidate.subtree.roots <- which(n.focal.members >= length(grp))
 # Out of these, the node with the least number of total descendants will be the subtree root
 candidates.total.members <- n.total.members[candidate.subtree.roots]
 winner <- candidate.subtree.roots[which(candidates.total.members == min(candidates.total.members))]
 root.node <- internal.nodes[winner]
 # find all tips which descend from the min subtree root node
 subtree.tips <- tips(tr, root.node)
 # find all nodes whose descendents include any of those tips
 nodes.with.focal.descendants <- sapply(node.descendants, function(x)any(subtree.tips %in% x))
 # but with fewer descendants than that of the subtree root node
 node.depths <- node.depth(tr)[internal.nodes]
 root.depth <- node.depths[winner]
 # select required nodes for calculation
 required.nodes <- nodes.with.focal.descendants == TRUE & node.depths <= root.depth
 # ----- End Correction -----

 # How many connections to those nodes have? (tree is not necessarily
 # dichotomous)
 degree <- table(tr$edge)[ internal.nodes[required.nodes] ]
 #Ape takes one connection off the root node, so if d=2 then treat it as d=3
 # (no other nodes can have d=2)
 obs.gs <- n / (sum( degree - 2 ) + sum(degree==2))
 #minGS (basically same procedure but for  whole tree)
 degree.total <- table(tr$edge)[seq(Ntip(tr)+1, Ntip(tr) + Nnode(tr))]
 min.gs <- n / (sum( degree.total - 2 ) + sum(degree.total==2))
 gsi <- (obs.gs - min.gs) / (1 - min.gs)
 return(gsi)
}


# ===== Calculate GSI From Phylogenetic Tree =====
# Calculate gsi with respect to environment, that is, high gsi should reflect
# apparent monophyly of groups from the same location (multiple origins)
# rather than monophyly of true clades (single origin).
# Also calculate gsi for true clades so these can be compared.
# In vcf output from slim, individuals are organized sequentially as such:
# p0 (1 individual), p10, p11, p100, p101 (30 individuals each)
# where there is gene flow between p10 & p100 and also p11 & p101 (parapatric pairs).
# Create vectors of names of individuals that belong to these groups.
# This will be used as input for the gsi calculation.

all.inds <- colnames(vcf@gt)[-c(1:2)] # this vector starts with i1 (excluding outgroup)
loc1 <- all.inds[c(1:30,61:90)]
loc2 <- all.inds[c(31:60,91:120)]
clade1 <- all.inds[c(1:60)]
clade2 <- all.inds[c(61:120)]

# Calculate gsi for entire chromosome (1Mb)
gen <- as.matrix(vcfR2genlight(vcf))
tr <- root(nj(dist(gen)), outgroup = "i0", resolve.root = TRUE)

gsi.clade1 <- gsi(tr, clade1)
gsi.clade2 <- gsi(tr, clade2)
gsi.loc1 <- gsi(tr, loc1)
gsi.loc2 <- gsi(tr, loc2)


# ===== Output GSI Values =====
cat(paste(c(gsi.clade1, gsi.clade2, gsi.loc1, gsi.loc2), sep="\t"))
cat("\n")
```