**The role of geography, ecology, and hybridisation in the evolutionary history of Canary Island *Descurainia***

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Running Title:

**Abstract**

***Premise of the study***

Oceanic islands offer the opportunity to understand evolutionary processes underlying rapid diversification. Along with geographic isolation and ecological shifts, a growing body of genomic evidence has suggested that hybridisation can play an important role in island evolution. Here we use genotyping-by-sequencing (GBS) to understand the roles of hybridisation, ecology, and geographic isolation in the radiation of Canary Island *Descurainia* (Brassicaceae).

***Methods***

We carried out GBS for multiple individuals of all Canary Island species and two outgroups. Phylogenetic analyses of the GBS data were performed using both super-matrix and gene tree approaches and hybridisation events were examined using D-statistics and Approximate Bayesian Computation (ABC). Climatic data were analysed to examine the relationship between ecology and diversification.

***Key results***

Analysis of the supermatrix dataset resulted in a fully resolved phylogeny. Species networks suggest a hybridisation event has occurred for *D. gilva*, with these results being supported by ABC analysis. Strong phylogenetic signals for temperature and precipitation indicate one major ecological shift within Canary Island *Descurainia*.
***Conclusions***

Inter-island dispersal played a significant role in the diversification of *Descurainia,* with evidence of only one major shift in climate preferences. Despite weak reproductive barriers and the occurrence of hybrids, hybridisation appears to have played only a limited role in the diversification of the group, with a single instance detected. The results highlight the need to use phylogenetic network approaches that can simultaneously accommodate ILS and gene flow when studying groups prone to hybridisation; patterns that might otherwise be obscured in species trees.

**Key Words:** ABBA-BABA; *Descurainia*; speciation; biogeography; diversification; genotyping-by-sequencing (GBS), hybridisation; phylogenetics; species networks

# Introduction

Island systems have played a fundamental role in providing insights into the processes underlying the evolution of plant life (Warren et al., 2015). The Canary Islands, an oceanic archipelago comprised of eight islands, is a hot spot for biodiversity and exhibits high levels of endemism (Reyes-Betancort et al., 2008). Approximately 570 plant species are endemic to the Canary Islands (25% of the entire flora; Guerra et al., 2008), many of which are found in recent and rapidly diversifying lineages, and the archipelago’s flora has been used to investigate evolution, speciation, and adaptation (Juan et al., 2000). Three main processes have been identified to explain the high levels of endemism found across these islands: (1) allopatric speciation arising through geographical isolation, typically after inter-island dispersal between similar habitats (Francisco‐Ortega et al., 2000; Juan et al., 2000); (2) ecological speciation due to speciation into new ecological niches (Silvertown et al., 2005); and (3) hybrid speciation owing to the weak reproductive barriers, dynamic landscapes and the close proximity of related species with different ecological niches (Silvertown, 2004; Fjellheim et al., 2009; White et al., 2018). A significant challenge when constructing the evolutionary history of Canary Island lineages is untangling the relative influence of the different evolutionary processes involved.

Interspecific hybridisation has long been viewed as an important driver of speciation in plants (Rieseberg and Willis, 2007). Nevertheless, despite numerous studies demonstrating hybridisation and gene flow between Canary Island lineages (Brochmann, 1984; Francisco-Ortega et al., 1996; Kim et al., 1996; Saunders and Gibson, 2005; Carine et al., 2007; Fjellgein et al., 2009), most molecular studies have focused only on the relative roles of ecological speciation and inter-island dispersal in generating the high levels of species diversity observed (Jorgensen & Frydenberg, 1999; Barber et al., 2000; Francisco‐Ortega et al*.*, 2002; Allan et al., 2004; Jaén-Molina et al*.*, 2021). Hybridisation as a potential evolutionary force is often difficult to detect, particularly when using small numbers of genetic markers. The low variation uncovered when using a limited number of markers in recent species radiations generally results in poorly resolved phylogenies. Even when phylogenetic incongruence is identified, for example, when chloroplast [cpDNA] and nuclear [nDNA] DNA give different phylogenetic hypotheses (Francisco‐Ortega et al., 1997; Barber et al., 2000; Mort et al., 2002; Jones et al., 2014), the use of only a small number of markers makes it difficult to determine the cause of the incongruence.

Reduced-representation sequencing methods, such as Restriction site Association DNA Sequencing (RAD-seq; Eaton & Ree, 2013; Eaton et al*.*, 2015) and Genotyping-by-Sequencing (GBS; Elshire et al*.*, 2011), use high-throughput sequencing technologies to simultaneously sample hundreds or thousands of loci throughout the genome (Baird et al., 2008; Rowe et al., 2011; Eaton and Ree, 2013; Fernández-Mazuecos et al., 2018). These methods generate large numbers of phylogenetically informative markers, which can overcome limitations presented when using traditional marker methods. As a result, RAD-seq and GBS data sets are providing new insights into the evolution of the Canary Island flora, as demonstrated in *Micromeria* (Lamiaceae; Puppo et al., 2015) and *Argyranthemum* (Asteraceae; White et al., 2018, 2020). Combined with the development of new statistical methods (Green et al., 2010; Patterson et al., 2012; Sankararaman et al., 2014; Vernot and Akey, 2014), studies are also starting to emerge which offer more comprehensive insight into hybridisation in oceanic lineages (Puppo et al., 2015; White et al., 2018, 2020). At present, these developments are reinforcing the notion that gene discordance as a result of hybridisation is not only common but is shaping the plant diversity found across the Canary Islands.

*Descurainia* (Brassicaceae) Webb & Berthel is a genus of 45 species distributed throughout America, Eurasia, and the Canary Islands (Goodson et al., 2011). The seven species in the Canary Islands form a monophyletic group (Goodson et al., 2006) that is distributed across four islands (Fig. 1). Five species (*D. gilva* Svent., *D. gonzalezii* Svent., *D. preauxiana* (Webb) Webb ex O.E.Schulz, *D. artemisioides* Svent., and *D. lemsii* Bramwell.) are single island endemics (SIEs) and two species (*D. millefolia* Webb & Berthel. and *D. bourgaeana* (E.Fourn.) Webb ex O.E.Schulz.) are multi-island endemics (MIEs) found on three and two islands, respectively (Bramwell, 1977; Goodson et al., 2006). The species are found in a range of different habitats and Goodson et al*.* (2006) broadly categorised the ecological zones for Canary Island *Descurainia* as lowland scrub (250 - 700 m) for *D. millefolia*, *D. preauxiana*, and *D. artemisioides*, pine forest (600 – 1,200 m) for *D. gilva*, *D. gonzalezii* and *D. lemsii*, and sub-alpine scrub (1,900 – 2,100 m) for *D. bourgaeana* (Bramwell, 1977; Goodson et al*.,* 2006). Putative hybrids have been documented on Tenerife (*D. bourgaeana* x *D. lemsii*, and *D. gonzalezii* x *D. bourgaeana*; Goodson et al*.,* 2006). Given the distribution of Canary Island *Descurainia* across multiple islands and ecological zones following a single colonisation of the archipelago (Goodson et al., 2006; Goodson et al*.,* 2011), the group offers an opportunity to examine the roles of different evolutionary processes underlying diversification within this archipelago.

Previous phylogenetic analyses of Canary Island *Descurainia* applied nDNA (ITS, the internal transcribed spacer region) and cpDNA sequences and resolved the Euro-Siberian *Descurainia tanacetifolia* as the continental sister species to the island clade (Goodson et al., 2006, 2011). The ITS phylogeny failed to resolve relationships within the Canary Island clade, however, the cpDNA phylogeny revealed two clades. One clade was restricted to Tenerife (*D. gonzalezii*, *D. lemsii* and some *D. millefolia* accessions) and the other comprised taxa from across the four islands (*D. bourgaeana* on Tenerife, *D. artemisioides* and *D. preauxiana* on Gran Canaria, *D. gilva* on La Palma, and *D. millefolia* from La Palma and La Gomera). *Descurainia millefolia* was resolved in both clades and *D. gonzalezii* was found to be polyphyletic in the Tenerife clade.

Goodson et al*.* (2006) suggested that the drivers of diversification in Canary Island *Descurainia* were intra-island ecological speciation and inter-island colonisation. Studies that have subsequently used the Goodson et al. (2006) phylogenetic hypothesis to examine the adaptive radiation of *Descurainia* provide conflicting results on the role of climate and habitats (Herben et al., 2014; Steinbauer et al. 2016). The polyphyly of *D. millefolia* and *D. gonzalezii* revealed by their study could suggest taxonomic revision is needed but also raises questions over the influence of hybridisation on the relationships inferred using chloroplast markers (Goodson et al.*,* 2006). Chloroplast capture can give rise to topologies that do not reflect species relationships, leading to incorrect species trees (Rieseberg and Soltis, 1991). This is backed up by field observations of putative hybrids (Goodson et al*.,* 2006).There is also the potential for gene flow as many species occur in close geographical proximity, with a lack of karyotypic barriers, very similar floral morphologies, and long overlapping flowering periods (Bramwell, 1977).

Here, we use Genotyping-by-Sequencing (GBS) data in conjunction with ecological and distribution data across all seven Canary Island *Descurainia* species and two continental outgroups to resolve the evolutionary history of the group focussing on determining whether hybridisation had played a significant role in the emergence of any taxa. Our objectives are to (1) investigate phylogenetic relationships using both concatenated data and coalescence-based species networks, and using an Approximate Bayesian Computation (ABC) modelling analysis (Cornuet et al., 2008) to examine alternate phylogenies, (2) quantify the extent of hybridisation between taxa using D*-*statistics (Eaton & Ree, 2013), (3) infer the role of ecology in the diversification of Canary Island *Descurainia* using spatial climatic variables, and (4) in so doing, establish the relative role of geographical isolation, hybridisation and ecological divergence in the diversification of the group.

# MATERIALS AND METHODS

## Sampling, DNA extraction, and Genotyping-by-Sequencing (GBS) –

A total of 18 individuals from eight populations were used to represent the seven Canary Island species of *Descurainia* (Appendix S1; see the Supplementary Data with this article). Sampling comprised one individual of *D. lemsii*, five of *D. bourgaeana*, two each of *D. gilva*, *D. gonzalezii*, *D. artemisioides* and *D. preauxiana*, and four of *D. millefolia* (two from La Palma and two from Tenerife). *Descurainia millefolia* populations from La Gomera and *D. bourgaeana* populations from La Palma were not sampled but all other species/island combinations were represented. *Descurainia tanacetifolia* and *D. depressa* were sampled as outgroups to represent the European sister species and a more distantly related South American taxon, respectively.

DNA was isolated from silica-dried leaves collected from wild plant material (collected in 2016; eight samples, permit numbers in the Acknowledgements section) and from fresh leaves of plants grown from seed from the Banco de Germoplasma Vegetal (BGV) seedbank (four samples; Appendix S1). DNA isolations were performed using a modified CTAB protocol (Doyle, 1991). An additional eight DNA samples used in the Goodson et al*.* (2006) study were supplied by R. Jansen (University of Texas).

DNA samples were sequenced using Genotyping-by-Sequencing (GBS) at Novogene (Yuen Long, Hong Kong). DNA was digested with *Mse*l and *Hae*ll and fragments were sequenced for 144 cycles on an Illumina HiSeq. The sampling of 1 to 5 individuals per species for Genotyping-by-Sequencing is expected to prove sufficient to provide powerful resolution for plant phylogenetic studies, whilst proving the depth needed to quantify hybridisation, as demonstrated in Escudero et al. (2014), Anderson et al. (2017), Fernández-Mazuecos et al. (2018), and Pérez-Escobar et al. (2020).

## GBS Assembly –

The GBS data were quality-filtered and assembled using ipyrad v.0.7.23 (Eaton & Ree, 2013; Eaton, 2014). Raw sequence data were demultiplexed for each sample using barcode sequences with no mismatches allowed. Low-quality base scores (< 33) were converted to N and reads with more than five Ns were discarded. Adapter sequences were filtered using the strict setting (2) as recommended for GBS data. The *de novo*-reference assembly method was used to remove reads which mapped to the chloroplast or mitochondrial genomes of *Arabidopsis thaliana* (L.) Heynh. (NCBI Reference Sequence NC\_000932.1 and NC\_001284.2, respectively) whilst assembling unmapped nuclear reads *de novo*. Reads were assembled to identify consensus allele sequences within individuals using three similarity clustering thresholds (80%, 85% and 90%) before clustering consensus allele sequences across samples to identify loci. For the three levels of clustering employed, loci were filtered for a minimum number of samples per locus of 8 or 10, equivalent to 60% and 50% missing data, respectively. A minimum depth of six reads was required for base calling and any locus with a shared heterozygous site in 20% or more of samples was removed as potentially comprising paralogs. A total of six data sets were therefore generated with three clustering thresholds (hereafter referred to as c80, c85, c90) and two minimum samples per locus thresholds (hereafter m8, m10). For each assembly, ipyrad produces a custom “loci” format which was used for all subsequent analyses.

## Phylogenetic Reconstructions 1 – Concatenated Approach –

A supermatrix approach was first used for phylogenetic reconstructions to infer evolutionary relationships and to allow comparison between GBS assemblies. This involves the concatenation of loci from the GBS assembly data set into a single alignment. For each of the six assemblies, the optimal model of sequence evolution was identified using ModelTest-NG [v.0.1.5](https://github.com/ddarriba/modeltest/releases/tag/v0.1.5) (DARRIBA et al., 2020) and a Maximum likelihood (ML) tree generated using RAxML Next-Generation v.0.6.0 (Stamatakis, 2014), with bootstrap support estimated from 1000 replicate searches from random starting trees.

Five of the six GBS data sets gave identical phylogenetic topologies (see results). Of these five, the data set with a clustering threshold of 85% and a minimum sample number of 8 (c85m8) was selected for all subsequent analyses as it had the largest number of SNPs.

## Phylogenetic Reconstructions 2 – Coalescent Approach –

The second phylogenetic approach uses a coalescent-based method which can accommodate reticulated evolutionary histories and incomplete lineage sorting (ILS). Here, we generated species networks modelling both hybridisation and ILS using Phylonet v.3.5 (Than et al., 2008). To prepare the data, the unlinked SNP data set generated by Pyrad was transformed into bi-allelic markers. *Descurainia depressa* was removed from the analysis because of its more distant relationship to the Canary Island ingroup (see results). The function “MLE\_BiMarkers” in Phylonet was used to generate a pseudo-likelihood ML estimation of phylogenetic networks with the “-diploid” and “-pseudo” parameters. The maximum number of reticulation nodes in the phylogenetic networks explored during the search was limited to 8. This number was set to a small number to reduce the overall complexity of a network. The number of runs was set to 50 to reduce memory load. Where multiple individuals of a species were present, these were mapped to a single species to reduce computational time to establish a species tree. The remaining parameters were set to their default settings.

## Approximate Bayesian Computation (ABC) –

We used an ABC approach (Beaumont et al*.*, 2002) to assess the posterior probabilities of the evolutionary scenarios suggested by our analyses (concatenated vs coalescent approaches; see results). DIYABC v.2.1 (Cornuet et al., 2008) was used to compare two scenarios: (1) a fully bifurcating phylogeny representing the topology generated using ML analysis of the concatenated data set; and (2) a scenario including a hybrid origin for *D. gilva* between *D. millefolia* and *D. gonzalezii* as inferred in the coalescent analysis.

The unlinked SNP dataset was transformed into a DIYABC-friendly format using the python script (vcf2DIYABC.py, available from <https://github.com/loire/> [accessed July 2019]).

Population sizes and divergence rates were set between 102 - 107, and admixture rates were 0.001-0.999. A total of 107 simulations were performed for each of the two scenarios. Summary statistics were incorporated to compare the observed and simulated data comparisons, which comprised the mean of genetic diversity, pairwise sample Fst and Nei’s distance, and admixture summary statistics (Hudson et al.*,* 1992; Choisy et al.*,* 2004). The most probable scenario was identified using the posterior probability for each scenario, which was computed using a direct approach and Logistic regression. The scenario probabilities were calculated using 500 simulations for the direct estimate and 10000 for the Logistic regression.

Once the most probable scenario was identified, posterior analyses were carried out to evaluate the robustness of the simulation of the selected scenario with our GBS data set. As DIYABC is a computationally and memory-intensive method, it was necessary to perform the remaining post-simulation analyses on a subset of 1000 simulations. Firstly, the prior distribution of the scenario was performed using a logistic regression, from which false positives and false negatives in the choice of scenario were estimated*.* False positives and negatives were calculated by measuring the proportion of the simulated data set for the best scenario when assigned to the other scenario, and the proportion of data sets simulated under the other scenario that was assigned to the best scenario, respectively. Second, goodness of fit for the selected scenario was performed using DIYABC model-checking analysis. This is to assess how close the simulated data set fits our GBS data set by producing summary statistics and ranking the observed value among the values obtained with simulated data sets.

## Patterson’s D-statistics –

Patterson’sD*-*statistics (also known as ABBA-BABA tests) are used to infer introgression between two lineages in a given phylogeny by testing for shared derived alleles between taxa. ABBA-BABA tests are strictly a tree-based test, from which species are assigned to the topology (((P1, P2), P3), O). P1 and P2 are species that belong to a monophyletic group, P3 corresponds to a taxon from a different ingroup species, and O is the outgroup. TheD*-*statistic measures the asymmetry in the number of alleles supporting “ABBA” and “BABA” patterns of allele distributions (where A is ancestral, and B is derived). If the proportion of alleles is equal (ABBA = BABA), then we cannot rule out ILS as the likely cause of gene incongruence. In contrast, a significant asymmetry between the number of alleles for each topology suggests introgression has occurred between P3 and either P2 (ABBA > BABA) or P1 (BABA > ABBA).

To test the hybridisation event indicated in our species networks (see results), we used Patterson’s D-statistic to test whether there is an excess of shared allele between *D. millefolia* and *D. gilva.* Due to the tree's topology, it was impossible to test whether there is gene flow between *D. gonzalezii* and *D. gilva,* and *D. millefolia.*

SNPs from the c85m8 assembly were used to generate theD*-*statistic using the *baba* tool in ipyrad v.0.9.16 (Eaton and Overcast, 2016). A P value was calculated from the Z-score and adjusted for multiple comparisons using Bonferroni correction.

## Ecological Niche Occupation and Conservatism –

GPS coordinates were taken from 216 Canary Island *Descurainia* herbarium specimens held at the Natural History Museum, London. Climatic variables for these localities were obtained from WorldClim v.2.1 (years 1970-2000; Fick & Hijmans, 2017), using 19 bioclimatic (biolcim) variables derived from temperature and rainfall values at a spatial resolution of 30 seconds (~1 km2). The GIS datasets were used to interpolate climate values for all individuals using ArcMap and the means for each bioclimatic variable per species were calculated. The *pairs* function in R was used to identify correlated variables. Where correlated variables were identified, the one that explained the largest differences between species was retained for further analysis. A phylogenetic PCA (pPCA) was generated with the R package “Phytools” (Revell, 2012), using the phylogeny generated from the analysis of the concatenated data set and the five bioclim variables retained. The averages for each climatic variable were represented in a dotTree using “Phytools”, along with the results of the phylogeny PCA (PC1 and PC2).

We tested for phylogenetic signal in each trait using Blomberg’s *K* statistic, as implemented with the function “phylosig” in the R package Phytools. K values closer to zero correspond to a random or convergent pattern of evolution, while values greater than 1 indicate a strong phylogenetic signal and conservatism of traits.

# RESULTS

## GBS Assembly –

Between 0.46 and 2.14 million reads were generated for each sample. After QC and removal of poor-quality reads, six data sets were assembled from the GBS raw reads (Table 1). Between 113,107 and 122,724 loci were generated in each assembly (Table 1). An increase in the clustering threshold (c; 80%, 85%, and 90%) resulted in a greater number of loci. Increasing the minimum sample coverage (m) from 8 to 10 resulted in a considerable decrease in the number of total SNPs and Parsimony informative (PI) SNPs available (Table 1).

## Phylogenetic Reconstructions 1 – Concatenated Approach –

The GTR+I+G model of nucleotide evolution was found to be the best model for all six assemblies (Appendix S2) and was employed for maximum likelihood (ML) phylogeny reconstruction.

All phylogenies resolved two main clades (BS = 100%), “clade A” and “clade B” (Fig. 2a, Appendix S3). Clade A is composed of *D. preauxiana*, *D. millefolia* and *D. artemisioides* and in five of the six phylogenies (i.e., excluding c90m8), *D. millefolia* is resolved as monophyletic (BS = 54-82%). *D. millefolia* is sister to *D. artemisioides*, with *D. preauxiana* resolved as sister to this pair. Within *D. millefolia*, Tenerife populations are resolved as paraphyletic with respect to the La Palma accessions that are resolved as monophyletic (BS = 100%). For the data set with the alternate topology (c90m8), *D. millefolia* is resolved as polyphyletic with one individual nested within *D. artemisioides* (BS = 48%).

The clade B topology was consistent for all six assemblies and is composed of subclades B1 (*D. gilva* and *D. gonzalezii*) and B2 (*D. bourgaeana* and *D. lemsii*), both of which have maximum support (BS = 100%). In B1, both *D. gonzalezii* and *D. gilva* are resolved as monophyletic (BS > 96%). Within Clade B, *D. lemsii* is found nested within *D. bourgaeana*, although the position is variable and weakly supported (BS = 42-79%).

## Phylogenetic Reconstructions 2 – Coalescent Approach –

A total of 13,797 unkinked SNPs were used as input data for Phylonet. The phylogenetic network with the lowest log-likelihood score from all 50 runs resulted in a network with one reticulation (Fig. 2b). The resulting topology was similar to that of the concatenated approach but *D. gilva* is suggested to be of hybrid origin between *D. gonzalezii* (clade B) and *D. millefolia* (clade A).

## Approximate Bayesian Computation (ABC) –

The c85m8 SNP data set was filtered, so that there was at least one individual per species at each SNP site (as required by DIYABC), after which 1722 SNPs remained. Scenario 2, in which *D. gilva* is of hybrid origin between *D. millefolia* and *D. gonzalezii*,had the highest posterior probability (0.786, 95% confidence interval 0.43 – 1.00; Table 2). Scenario 1, which represented the bifurcating phylogeny without hybridisation, had a much lower posterior probability (0.214; confidence interval 0.00 – 0.57).

The summary statistics calculated from the scenario 2 simulated data set were found to be close to the summary statistics calculated with our GBS data set (Appendix S4). The estimation of error rates provided confidence in scenario choice; the false positive and false negative error rates were estimated to be 10.2% and 0.1%, respectively.

Our analysis also indicated that the parental contributions to the genetic composition of the hybrid *D. gilva* were not equal (Appendix S4). There was a large bias in contribution with *D. gonzalezii* contributing 98% (95% CI: 98.4-99.6) and *D. millefolia* 2%. The parameter estimation for the time of this hybridisation event was 3.7 ± 2.1 Mya.

## Patterson’s D-statistics –

Patterson’s D-statistic examining gene flow between *D. gilva* (P3) and *D. millefolia* (P2) resulted in a significant D-statistic (BABA > ABBA) when both *D. artemisioides* and *D. preauxiana* were used as P1 (Table 3; P < 0.005, after multiple correction).

## Ecological Niche Occupation and Conservatism –

After filtering the most highly correlated variables, five BioClim variables were included in the ecological analyses: Annual Temperature, Temperature Seasonality, Annual Precipitation, Precipitation in the Wettest Quarter, and Precipitation in the Coldest Quarter (Appendix S5). The first two axes, PC1 and PC2, of the phylogenetically corrected PCA explained 84% and 16% of the variance in climatic variables across the seven species (Fig. 2c, Table 4). Precipitation in the Coldest Quarter (-0.99) and Annual Mean Temperature (0.98) have the highest loads for PC1. *Descurainia bourgaeana* (-3.45) was on one extreme of PC1, followed by *D. gilva*, *D. lemsii* and *D. gonzalezii,* all demonstrating high average precipitation, whereas *D. artemisioides* (3.44), *D. millefolia* (2.77) and *D. preauxiana* (2.77) are on the opposite end of the PC1 axis and represent higher annual mean temperature.

Temperature seasonality (0.67) and annual precipitation (-0.43) represented the highest loads for PC2 with *D. gilva* (-1.59) and *D. preauxiana* (0.72) on the extremes of this axis.

The *K* statistic indicated a strong phylogenetic signal for all bioclimatic variables (*K* > 1; Table 4). Annual mean temperature, annual precipitation and precipitation in the wettest quarter were significant (p < 0.05), indicating these traits have non-random phylogenetic signal.

# DISCUSSION

The Genotyping-by-Sequencing (GBS) analysis presented provides new insights into phylogenetic relationships among Canary Island *Descurainia* (Brassicaceae) and the evolutionary processes that underpin the group’s diversification. Phylogenetic analyses of the concatenated data set revealed two major and well-resolved clades, with ABBA-BABA tests and species networks both supporting a role for hybridisation in the diversification of the group. Our results suggest that a hybridisation event has occurred for *D. gilva*. Further, the results suggest that diversification of the group has involved a single shift in climatic preferences associated with a shift in elevation, with the low-elevation Clade A species occupying high temperature and low precipitation environments and the higher-elevation Clade B species occupying lower temperature and higher precipitation environments. The study, therefore, indicates the importance of geographic isolation, hybridisation and ecological diversification in driving the diversification of Canary Island *Descurainia*.

## Inferring Species Relationships Using Coalescent versus Concatenated Methods –

Previous studies have suggested that phylogenies based on concatenated GBS data sets suffer strong systematic bias because high statistical support for multiple alternative topologies depending on clustering threshold values used in the sequence analysis is often found (Fernández-Mazuecos et al., 2018). Here, our GBS assemblies show congruent and well-supported topologies across five of six data sets. The incongruent data set differed only in the resolution of *D. millefolia.* Phylogenetic analysis of all concatenated data sets revealed two major clades (A and B) and two sub-clades within B (B1 and B2) with maximum bootstrap support. This suggests the different clustering and minimum sample coverage thresholds used have little impact on phylogenetic inferences, proving confidence in the results.

In the topology resolved in five of the six analyses, *D. bourgaeana* was the only species revealed as non-monophyletic, with *D. lemsii* nested within this species, although branch lengths in this clade were short and relationships not well supported. The occurrence of paraphyly such as this is often explained by taxonomic error, ILS, or hybridisation (Holder et al., 2001). Morphologically, *D. bourgaeana* and *D. lemsii* may be distinguished since the former has decurrent leaves and ascending siliquae, whereas the latter lacks decurrent leaf-segments and has erect siliquae (Bramwell, 1977). However, hybridisation has played a role in Canary Island *Descurainia* more generally (see below) and putative hybrids of *D. bourgaeana* and *D. lemsii* have been observed in the wild (Goodson et al., 2006; ACJ personal field observations). Due to the topology of our phylogeny, it was not possible to test for hybridisation (versus ILS) involving these two taxa with Patterson’s D-statistics. Given that neither ILS nor hybridisation can be ruled out, further morphological and genetic studies with greater sampling of *D. lemsii* are necessary to resolve the status of these two taxa.

The topology of our concatenated phylogenywas incongruent with the previous phylogenetic reconstructions of Goodson et al*.* (2006) based on 44 cpDNA characters from seven loci. Specifically, our analysis did not support the polyphyly of *D. gonzalezii* and *D. millefolia* found by Goodson et al. (2006), instead both were resolved as monophyletic. The Goodson et al*.* phylogeny may be impacted by chloroplast (cp) capture (the transfer of cpDNA between species due to hybridisation). Given that our results indicate hybridisation in the evolutionary history of the Canary Islands *Descurainia*, cpDNA capture likely explains the significant differences between the results of Goodson et al*.* and those presented here. Further, our phylogenetic reconstructions were generated using a significantly larger number of genetic markers due to our use of our GBS datasets generated through next-generation sequencing technology. For instance, our ML phylogeny was generated using 76,875 SNPs (c85m8 GBS assembly; Figure 2.2), which significantly exceeds the 44 cpDNA characters employed in the Goodson et al. (2006) study. Our datasets provide a far more powerful phylogenetic signal to resolve the relationships within Canary Island *Descurainia* and likely explains the conflicting topologies between studies.

In recent years, evolutionary networks have been explored as an alternative method for phylogenetic reconstructions (McCluskey and Postlethwait, 2015). Since reduced-representation data sets (i.e., RADseq and GBS) sample data from hundreds to tens of thousands of loci across the entire genome, these sequencing methods can be applied to generate multilocus species trees. While Phylonet has been shown to be a promising tool in estimating hybridisation between taxa (Hibbins and Hahn, 2021), few studies have attempted to use reduced-representation data sets to estimate evolutionary networks (Eaton and Ree, 2013; Blanco-Pastor et al., 2019). Several issues have been highlighted with this approach, notably the short length of each locus means that there is variable phylogenetic information between loci and the data sets may be subjected to phylogenetically structured patterns of missing data (Salichos and Rokas, 2013). However, a coalescent analysis of reduced-representation data allows for variation across loci in a genealogy, whereas a concatenated approach assumes a shared phylogenetic history for all genes (Rokas et al., 2003). As a result, a coalescent approach can be useful for understanding hybridisation.

Here, the non-reticulated species network demonstrated a similar topology to that of the concatenated analysis. However, we found evidence that the most likely evolutionary scenario for Canary Island *Descurainia* is reticulate andincludes inter-species gene flow. The reticulated phylogenies generated by Phylonet were also supported by D-statistics and DIYABC models. This implies that the concatenated approach, despite the larger number of loci included and the high bootstrap support, has masked incongruence between loci within the data set.

Our analyses support a hybridisation event for *D. gilva*, between the *D. gonzalezii* and *D. millefolia* lineages. This is at odds with the hypothesis of Bramwell (1977), who, based on morphological similarities, considered *D. gilva* to be a vicariant of *D. bourgaeana*, a species which also occupies high altitudes.

Our ABC analysis indicated there is a large bias in putative parental contributions, with 98% contribution from *D. gonzalezii* and 2% from *D. millefolia.* Inter-island hybridisation has been documented in other Canary island endemic plant lineages (van Hengstum et al., 2012; Puppo et al., 2015; White et al., 2020). However, it seems most likely that *D. gilva* is rather a vicariant form of *D. gonzalezii* which has been exposed to gene flow from *D. millefolia* upon establishing itself on La Palma. It is notable that *D. gilva and D. gonzalezii* are somewhat ecologically distinct; *D. gilva* habitats exhibit Annual Precipitation and Precipitation in the Wettest Quarter > 80% higher than *D. gonzalezii* habitats (Fig. 2b; Table S4). Our work therefore suggests that introgressive hybridisation, despite only representing a minor fraction of the hybrid genome, was enough for a lineage to establish in a new niche, as has been reported in *Senecio* (Kim et al., 2006) and *Helianthus* (Whitney et al., 2006).

## Diversification and the role of Ecology and Geography –

Inter-island dispersal and ecological shifts are the two processes most commonly invoked to explain the high levels of diversity seen within Canary Island lineages (Jorgensen and Frydenberg, 1999; Francisco‐Ortega et al., 2002; Mort et al., 2002; Allan et al., 2004; Jones et al., 2014). Previous phylogenetic analysis of Canary Island *Descurainia* suggested that inter-island dispersal was more influential than ecological speciation (Goodson et al.*,* 2006)*,* with three ecological shifts inferred alongside multiple inter-island colonisation events. Our phylogeny also supports the importance of geographic speciation in the evolution of Canary Island *Descurainia*. Within clade B1, there is a dispersal event from Tenerife to La Palma for *D. gilva,* and in clade A there are likely two dispersal events, one from Gran Canaria to Tenerife, followed by another from Tenerife to La Palma.

Climatic variation within the Canary Islands has often been seen as a major driver of diversification within endemic plant lineages (Irl et al., 2015; Harter et al., 2015). Climatic variation, especially along elevation gradients, promotes ecological shifts and speciation (Hua and Wiens, 2013), for example, precipitation gradients have played a significant role in the radiation of *Aeonium* (Crassulaceae; Harter et al., 2015). Our results indicate that for *Descurainia,* precipitation and temperature are strongly linked phylogenetically. We observe only one shift in climatic preferences, supporting the idea of niche conservatism between closely related *Descurainia* species. Taxa in each of our two clades occupy similar ecological zones. *Descurainia gonzalezii, D. lemsii* and *D. bourgaeana* in clade B are species that inhabit Tenerife with similar environmental pPCA axes. They are largely allopatric, suggesting intra-island geographic diversification without large ecological divergence. Similarly, *D. artemisioides* and *D. preauxiana,* both endemic to Gran Canaria, occupy similar habitats but are largely allopatric: *D. artemisioides* is restricted to the northwest of the island and *D. preauxiana* is more widespread but they do not overlap geographically. Inter- and intra-island isolation within similar habitats has played a more significant role in the diversification of the group than ecological shifts based on the data to hand. However, we acknowledge that other factors, for example, wind exposure and soil types should be examined.

The phylogenetic framework of Goodson et al. (2006) has been used to investigate the role of adaptive divergence in the evolution of Canary Island *Descurainia.* Steinbauer et al. (2016) examined climatic niche differentiation between pairs of species existing in sympatry with that for pairs of species in allopatry for a range of Canary Island radiations, including *Descurainia*. They concluded that *Descurainia* showed niche conservatism for temperature. In contrast, Herben et al. (2014) proposed that morphological traits related to water availability were not phylogenetically linked and therefore suggested adaptive divergence to differing habitats based on water availability. However, both these studies used the Goodson et al. (2006) phylogenetic framework, which conflicts with our own phylogeny.

Whilst several Macaronesian plant radiations have been subject to molecular phylogenetic analysis, resolution of relationships and support for those relationships are often limited and many groups would benefit from further investigation with more extensive molecular sampling. Where reduced representation sequencing approaches have been used, for example to investigate the Macaronesian endemics *Argyranthemum*, *Micromeria* and *Lavatera* (Villa-Machío et al., 2020; White et al., 2020), better resolved phylogenies have resulted and more complex patterns of relationships than earlier assessments suggested have been revealed.

Our results suggest that ecological shifts are not entirely absent from the diversification of Canary Island *Descurainia*. A significant ecological distinction is found between the two clades, and our pPCA results show that species from clade A are found in warm and arid habitats and species from clade B are found cooler, wetter habitats. Upslope migrations, involving adaptation to higher altitudes, are a common observation in Canary Island lineages, for example in *Helianthemum* (Cistaceae) and *Echium* (Albaladejo et al., 2021; Graham et al., 2021). This fits with the finding that the initial colonisation of the Canary Islands is often at low altitude where more diverse habitats are available, followed by diversification upslope to more specialised high-altitude habitats (Steinbauer et al., 2012). However in *Descurainia*, the sister group of the Canary Islands clade, *D. tanacetifolia* (Goodson et al., 2006), is a species restricted to montane regions of northern Iberia and the Alps. Given our data, we cannot rule out ‘downslope’ migration from cooler wetter habitats to warmer, drier habitats. A more comprehensive analysis of the evolution of climatic preferences in the genus would be necessary to provide insights into this.

# CONCLUSIONS

Despite only analysing a few individuals per species, we produced a new multilocus framework for Canary Island *Descurainia* whichindicates that hybridisation has occurred within this group, likely explaining species polyphyly identified in an earlier study (Goodson et al., 2006). Inter-island dispersal, within-island isolation and to a lesser extent, ecological shifts and hybridisation, are all implicated in the diversification of Canary Island *Descurainia*. The findings of this study reinforce the notion that hybridisation is one of the drivers contributing to the complex history of Canary Island flora. It also highlights how phylogenetic analyses of island lineages should employ multiple analytical approaches to test for alternative scenarios.

# Acknowledgements

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Author Contributions

ACJ, MCarine and MChapman designed the study; MChapman extracted DNA, OWW assembled the raw GBS data with ACJ performing the rest of the analyses; ACJ wrote with manuscript with contributions from all authors.

# Data Availability

Climate data for Canary Island *Descurainia* individuals has been uploaded to FigShare (https://doi.org/10.6084/m9.figshare.22270987.v1) and raw Genotyping-by-Sequencing to the NCBI SRA (BioProject number PRJNA894797).

# Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix S1:** Twenty Canary Island Descurainia individuals from eight species, and their origin, which were used within this study. Field-collected leaf material was dried in silica gel and voucher specimens were deposited at the Natural History Museum (BM), London. Origin abbreviation as follows: GC = Gran Canaria, TEN = Tenerife, LP = La Palma, SP = Spain, SA = South America**.**

**Appendix S2:** Most likely Maximum-Likelihood model for all six GBS assemblies using ModelTest-NG.

**Appendix S3:** Maximum-Likelihood phylogenies using 18 individuals of Canary Island Descurainia and 2 outgroups from six assembly parameters using GBS data. Bootstrap values are next to nodes in red. Branch length bar above phylogeny. Individual ID numbers are represented in Appendix S1.

**Appendix S4:** The mean, median, mode and quantiles of posterior distribution samples for effective population sizes (parameter N), time (t) and admixture (r) for the scenario tested in ABC analysis generated by DIYABC (Cornuet et al., 2008).

**Appendix S5:** Averages for bioclimatic variables for each 7 species across 217 individuals of Canary Island *Descurainia*.

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# TABLES

Table 1: Summary of the results of filtering and clustering of GBS raw sequences using ipyrad (Eaton, 2014). GBS reads included 18 individuals from seven Canary Island *Descurainia* species and two continental *Descurainia* individuals.Loci = unique GBS DNA cluster; PI = Parsimony informative; SNPs = PI SNPs and autapomorphies; PI SNPs = minor allele in >1 sample; PI uSNPs = unlinked PI SNP

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Assembly** | **Clustering Threshold (%)** | **Minimum Taxon Coverage** | **N loci** | **SNPs** | **PI SNPs** | **PI uSNPs** |
| c80m8 | 80 | 8 | 113,107 | 69,339 | 29,037 | 12,779 |
| c80m10 | 80 | 10 | 113,107 | 41,235 | 18,002 | 7,201 |
| c85m8 | 85 | 8 | 118,553 | 75,603 | 31,714 | 13,797 |
| c85m10 | 85 | 10 | 118,553 | 45,347 | 19,794 | 7,828 |
| c90m8 | 90 | 8 | 122,724 | 76,875 | 32,488 | 14,652 |
| c90m10 | 90 | 10 | 122,724 | 45,526 | 19,981 | 8,174 |

Table 2: Posterior probability of parameters, and their confidence intervals, for each four scenarios tested with ABC in DIYABC (Cornuet et al., 2008). The corresponding phylogeny or network for each scenario is found in Figure 2.

|  |  |  |  |
| --- | --- | --- | --- |
| **Scenario** | **Test Description** | **Posterior Probability** | **95% Confidence Interval** |
| 1 | Bifurcating phylogeny | 0.214 | [0.000, 0.574] |
| 2 | *D. gilva* is a hybrid origin between parents *D. millefolia* and *D. gonzalezii*.  | 0.786 | [0.427, 1.000] |

Table 3: Patterson’sD*-*statistic (ABBA-BABA), which compares *D. gilva* (gil; P3) with *D. millefolia* (mil; P2) and its sister taxa (P1), either *D. artemisioides* (art) or *D. preauxiana* (pre). *Descurainia tanacetifolia* and *D. depressa* were selected as the outgroups (O).D*-*statistics in bold represent significant P values at < 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Test** | **D stat** | **Z** | **ABBA** | **BABA** | **# loci**  | **P value** |
| art (P1) mil (P2) gil (P3)  | -0.121 | 3.929 | 1031.256 | 1313.998 | 5,817 | **0.000** |
| pre (P1) mil (P2) gil (P3) | -0.132 | 3.221 | 610.708 | 796 | 3,530 | **0.001** |

Table 4: The loadings of each trait for the first two axes from the pPCA and K statisticunder a Brownian motion model of evolution for our five bioclimatic variables. Traits which are significant where P < 0.05 have a non-random phylogenetic signal. K*-*values greater than 1 indicate a strong phylogenetic signal and phylogenetically conservation.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Bio Variable** | **PC1 (84%)** | **PC2 (16%)** | **K**  | **K P value** |
| Annual Mean Temp (BIO1) | 0.989 | -0.131 | 1.365 | **0.045** |
| Temp Seasonality (BIO4) | -0.738 | 0.672 | 1.447 | **0.037** |
| Annual Precipitation (BIO12) | -0.898 | -0.432 | 1.150 | 0.116 |
| Precipitation in Wettest Quarter (BIO16) | -0.935 | -0.346 | 1.395 | **0.041** |
| Precipitation in Coldest Quarter (BIO19) | -0.992 | 0.087 | 1.530 | 0.053 |

# FIGURE LEGENDS

Figure : Map of the Canary Islands (age of islands in brackets; Schmincke, 1976) and the distribution of each island's seven endemic Canary Island *Descurainia*.

Figure : (A) Maximum-likelihood phylogenetic tree inferred from GBS data set (c85m8) for 18 Canary Island *Descurainia* individuals and two continental relatives. Numbers represent bootstrap values as inferred from 1000 bootstraps (BS) repetitions. Branch lengths are represented by the bottom bar. The continental relatives, *D. depressa* and *D. tanacetifolia,* are the outgroups. ID numbers next to taxa names represent samples in Table S1, and shapes represent the island of origin. (B) Schematic representing the phylogenetic network generated by Phylonet under Pseudo-likelihood when max reticulates is set to eight. Dots represent the average value of bioclimatic variable and pPCA loads for each species (c) Phylogenetic principal component analysis (pPCA) plots of PC1 and PC2 from five bioclimatic variables. Arrows in bold indicate a significant phylogenetic signal (*K* > 1 at P < 0.05).