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Disentangling Complex Histories of Hybridisation: The Genomic Consequences of Ancient and Recent Introgression in Channel Island Monkeyflowers

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ABSTRACT

Hybridisation is a common feature of evolutionary radiations, but its genomic consequences vary depending on when it occurs. Since reproductive isolation takes time to accumulate, hybridisation can occur at multiple points during divergence. Previous studies suggested that the taxonomic diversity in evolutionary radiations can help infer the timing of past gene flow events. Here, we assess the power of these approaches for revealing when gene flow occurred between two monkeyflower taxa (*Mimulus aurantiacus*) endemic to the Channel Islands of California. Coalescent simulations reveal that conventional four-taxon tests may not be capable of fully distinguishing between recent and ancient introgression, but genome-wide patterns of phylogenetic discordance vary predictably with different histories of hybridisation. Using whole-genome sequencing and phylogenetic tests for introgression across the *M. aurantiacus* radiation, we identify signals of both ancient and recent hybridisation that occurred between the island taxa and their ancestors. In addition, we find widespread selection against introgressed ancestry, consistent with polygenic barriers to gene flow. However, we also identify localised signals across the genome that may indicate adaptive introgression. This study highlights the power and challenges of trying to disentangle complex histories of hybridisation. More broadly, our results illustrate the multiple roles that gene flow can play in evolutionary radiations: hybridisation can expose genetic incompatibilities that contribute to reproductive isolation while also likely facilitating adaptation by transferring beneficial alleles between taxa. These findings underscore the dynamic interplay between the timing of hybridisation and natural selection in shaping evolutionary trajectories within radiations.

1 | Introduction

Phenotypic and genetic differences that evolve between populations can lead to the accumulation of reproductive barriers and the origin of new species (Mayr 1942; Coyne and Orr 2004). Determining how reproductive isolation develops through time is critical for understanding the process of speciation (Matute and Cooper 2021). Although previously believed to be rare, hybridisation between emerging species is now known to be common (Roux et al. 2016; Martin and Jiggins 2017; Malinsky et al. 2018; Stankowski et al. 2019; Liu et al. 2022). Depending on when it occurs, hybridisation and subsequent gene flow between diverging lineages (i.e., introgression) can have various evolutionary consequences. For example, introgression can expose genetic incompatibilities that promote speciation (Coughlan and Matute 2020); it can swamp out divergence (Todesco et al. 2016); or in some cases, it can lead to the transfer of beneficial alleles across taxonomic boundaries (Suarez-Gonzalez et al. 2018). Thus, understanding the evolutionary history and genomic consequences of introgressive hybridisation can help reveal the processes contributing to divergence and speciation (Harrison and Larson 2016; Ravinet et al. 2017).

In their classic study, Coyne and Orr (1989) found that reproductive isolation accumulated with levels of sequence divergence between multiple pairs of *Drosophila* species, a finding that has been supported in various other groups of divergent taxa (Moyle et al. 2004; Mérot et al. 2017). Given that reproductive isolation takes time to evolve, hybridisation can occur at multiple points throughout the divergence of any pair of species (e.g., Martin et al. 2013; Malinsky et al. 2018; Meier et al. 2019, 2023). Determining the relative timing of hybridisation events is key to understanding how speciation progresses, as reproductive barriers that accumulate through time will impact the potential for future gene flow (Stankowski and Ravinet 2021).

Although various modelling and simulation approaches have attempted to reveal the timing of past gene flow events, there remain serious challenges to accurately parameterise the models (Momigliano et al. 2021). Another option that has been proposed is to take advantage of the taxonomic diversity available in evolutionary radiations. By performing tests for gene flow among the taxa in a radiation, it may be possible to estimate the relative timing of past introgression. Martin et al. (2013) used the diversity among *Heliconius* butterflies to demonstrate a history of continuous hybridisation throughout the divergence of currently sympatric species pairs. Malinsky et al. (2018) expanded on this approach by performing tests for introgression between all sets of taxa in the Lake Malawi cichlid radiation, enabling researchers to evaluate which lineages showed evidence of hybridisation and when it was likely to have occurred across the phylogeny of the group. They identified evidence of extensive ancient hybridisation between the ancestors of modern cichlid lineages, as well as recent hybridisation among closely related species found in similar ecological niches. Nelson et al. (2021) explored patterns of phylogenetic discordance across the genome to infer the presence of recent and ancient gene flow in monkeyflowers. These studies highlight how the taxonomic diversity present in evolutionary radiations may be useful for identifying the relative timing of past gene flow events. In this study, we investigate the power of these approaches for revealing the timing of introgression between island and mainland taxa in a different radiation of monkeyflowers.

In addition to characterising the history of gene flow, understanding the evolutionary consequences of this hybridisation can provide clues about the process of speciation. In most cases, when functionally relevant genetic variation is transferred into a foreign genetic background, it will decrease fitness, resulting in the removal of these alleles due to negative selection (Mallet 2005). These introgression-resistant loci are termed “barrier loci,” and as time proceeds, they should accumulate across the genome, leading to increased reproductive isolation (Wu 2001; Feder et al. 2012). Positive selection following introgression also can result in the preservation and eventual fixation of transferred alleles (i.e., adaptive introgression) (Suarez-Gonzalez et al. 2018), which in some cases directly results in reproductive isolation (Pardo-Diaz et al. 2012). Thus, the varying outcomes of introgression can have important implications for the speciation process.

Other processes not directly related to speciation also can be impacted by introgression. For example, it has been hypothesised that the reduced genetic diversity and lower effective population size typical in island taxa should result in the accumulation of higher deleterious genetic load relative to their mainland relatives (Barrett 1996). However, introgression between related island endemics may contribute to the maintenance of genetic diversity (Carlquist 1966). This is because gene flow between a pair of species that recently experienced population bottlenecks can restore genetic variation and mitigate the negative effects of any deleterious genetic load that has accumulated (Schumer et al. 2018; Liu et al. 2022). Finally, post-divergence gene flow can have no fitness consequences, in which case allele frequencies will be influenced primarily by genetic drift (Schumer et al. 2018).

To distinguish among these evolutionary processes, we expect certain genome-wide relationships to emerge. Natural selection operating against deleterious introgressed ancestry will result in its removal from populations, the extent of which will be modulated by variation in the local recombination rate (Schumer et al. 2018). Specifically, selection will be highly efficient at removing blocks of introgressed ancestry when they occur in regions of low recombination, as deleterious alleles are less likely to be separated from neutral or beneficial variation (Aeschbacher et al. 2017; Brandvain et al. 2014). This is expected to be more pronounced under highly polygenic architectures of reproductive isolation, as more of the genome acts as a barrier to gene flow (Martin et al. 2019). As a result, selection against gene flow will result in a positive relationship between introgression and recombination rate. Similarly, because gene flow opposes divergence, we would expect a negative relationship between introgression and genetic divergence (Martin et al. 2013, 2019). Alternatively, localised genomic regions that retain extensive admixture are excellent candidates for adaptive introgression (Martin and Jiggins 2017), but additional tests are necessary to distinguish adaptive from neutral processes (Moest et al. 2020). Finally, hybridisation between island taxa may lead to similar patterns of genetic diversity and effective population size compared to their mainland relatives.

In this study, we take advantage of the diversity in the *Mimulus aurantiacus* species complex to investigate the evolutionary history and genomic consequences of introgression between a pair of taxa restricted to the Channel Islands off the coast of California. The *M. aurantiacus* complex is a radiation of seven closely related, woody shrub subspecies distributed throughout California that display extensive phenotypic variation in their floral and vegetative traits and diverged from their sister species approximately 1 million years ago (Tulig 2000; Tulig and Nesom 2012; Chase et al. 2017; Stankowski et al. 2019). However, despite extensive phenotypic differentiation, there is evidence of hybridisation between many of the taxa (Streisfeld and Kohn 2005; Stankowski et al. 2019; Short and

Streisfeld 2023). Indeed, ancient introgression resulted in the repeated evolution of red flowers, a trait that has been shown to contribute to both pollinator adaptation and speciation in this group (Stankowski and Streisfeld 2015; Short and Streisfeld 2023; Sobel and Streisfeld 2015; Stankowski et al. 2017).

The Channel Islands are currently inhabited by two subspecies of *M. aurantiacus* that are known to hybridise (Wells 1980; Chase et al. 2017). The red-flowered *parviflorus* is endemic to the islands (Tulig 2000; Tulig and Nesom 2012) but is listed as rare by the California Native Plant Society (2023). Subspecies *longiflorus* has larger, yellow flowers and is found both on the Channel Islands and throughout mainland southern California (Tulig 2000, Tulig and Nesom 2012). Its widespread distribution on the mainland sets up an interesting contrast with the island, as the mainland form of *longiflorus* cannot actively hybridise with the island endemic *parviflorus*, but it is able to mix with other taxa that it comes into contact with across southern California (Grant 1993). On Santa Cruz Island, both taxa occur in sympatry, where they tend to inhabit dry, hillside habitats in the chaparral (Beeks 1962).

We used whole-genome sequence data and coalescent simulations to address three primary objectives. First, we estimated the evolutionary relationships of samples collected from the island relative to the rest of the radiation. Then, we combined simulations with genome sequence data to reveal the power and limitations of available approaches to determine the history of hybridisation among taxa in this radiation. Finally, we assessed the genome-wide impacts of this introgression to determine which processes have likely contributed to these patterns. Our results reveal that patterns of phylogenetic discordance across the genome can provide a predictable means to distinguish among evolutionary scenarios, which indicate that both recent and ancient introgression have shaped the genomic landscapes of the island taxa. In addition, there appears to be extensive selection against gene flow between them, as well as a region with a potential signal of adaptive introgression, implying that gene flow between these taxa may have been beneficial for their shared existence on the island. Overall, these results indicate the power—and limits—of evolutionary radiations for disentangling complex histories of hybridisation.

2 | Materials and Methods

2.1 | Genome Sequencing and Variant Calling

Leaf tissue was collected from 27 plants across four locations on Santa Cruz Island, California, USA (Table S1), consisting of red-flowered *parviflorus*, yellow-flowered *longiflorus*, and their putative hybrids. Tissue was dried in silica in the field, and DNA was isolated using the Zymo Plant and Seed DNA kit following the manufacturer's instructions. Sequencing libraries were prepared according to Gaio et al. (2022), with slight modifications. Bead-linked transposase from the Illumina Nextera XT Kit was used for initial tagmentation, generating insert sizes in the range of 400–1200 bp. Multiplexed libraries were sequenced on the Illumina Novaseq 6000 using paired-end 150 bp reads at the University of Oregon's Genomics Core Facility.

New sequences from Santa Cruz Island were combined with previously generated whole-genome sequences from various *M. aurantiacus* subspecies from Stankowski et al. (2019) and Short and Streisfeld (2023), resulting in a final dataset containing 74 individuals. Raw reads were filtered using *fastp* to remove reads with uncalled bases or poor quality scores (Chen et al. 2018). The retained reads were then aligned to the *M. aurantiacus* reference assembly (Stankowski et al. 2019) using *BWA* version 0.7.17 (Li and Durbin 2009). An average of 90.56% of reads aligned (range: 75.45%–95.33%), and the average sequencing depth was 10× per individual (range: 6–18×). PCR duplicates were marked using *Picard* (https://broadinstitute.github.io/picard/). Variant calling was performed following Stankowski et al. (2019). We then phased the VCF using *BEAGLE* (Browning and Browning 2007) and were further filtered for biallelic SNPs using *vcftools* (Danecek et al. 2011). The final dataset contained 12,749,566 SNPs across all 74 samples. Finally, we ran *UnifiedGenotyper* with the EMIT_ALL_CONFIDENT_SITES option to output all variant and invariant genotyped sites.

2.2 | Admixture, Principal Component Analysis, and Phylogenetic Analysis

Prior research has consistently revealed four monophyletic clades delineating the taxa in *M. aurantiacus* (Stankowski and Streisfeld 2015; Chase et al. 2017; Stankowski et al. 2019; Short and Streisfeld 2023). Clade A consists entirely of subspecies *grandiflorus*; clade B shows *parviflorus* and *aridus* as sister taxa; clade C consists entirely of *aurantiacus*; and the remaining taxa (*longiflorus*, *calcyinus*, and the three lineages of *puniceus*) from southern California comprised the diverse clade D. To determine how the newly sequenced island samples were related to the other taxa in the complex, we used *Admixture* (Alexander et al. 2009) to estimate ancestry proportions from all samples, with the number of clusters (K) set between 2 and 11. We ran the cross-validation error function in *Admixture* and reported the results based on the value of K with the lowest cross-validation error. Because of the potential for ongoing hybridisation between *parviflorus* and *longiflorus* on Santa Cruz Island (Wells 1980) and the phenotypic similarity between *longiflorus* from the island and mainland (Tulig 2000), we ran *Admixture* again using only these samples to identify putatively unadmixed individuals. Plants from the island that showed no evidence of admixture at $K = 2$ were used to assign individuals to subspecies. To further assess the clustering patterns among these samples, we also performed a principal component analysis (PCA) in *Plink* using the 31 samples from the island and the 7 *longiflorus* from the mainland. Admixed individuals were removed from further analysis, as they likely represented contemporary hybrids, which were not appropriate to include in the phylogenetic tests we used to estimate introgression.

To determine the phylogenetic relationships of samples from the island and mainland, we generated a maximum-likelihood consensus tree using *IQ-TREE* v1.6.12 (Nguyen et al. 2015). We used a concatenated dataset consisting of 12,749,566 biallelic SNPs that did not include island samples that showed evidence of admixture in the above analyses (resulting in 63 individuals, see Section 3). We used 1000 bootstrap replicates to estimate support values at each node.

2.3 | Tests for the Timing of Admixture

To test for genome-wide evidence of introgression, we used *dsuite* (Malinsky et al. 2021) to calculate the *f4*-ratio (Reich et al. 2009) for all possible trios of ingroup taxa, using *Mimulus clevelandii* as the outgroup. The *f4*-ratio is derived from the *D*-statistics and their relatives, which measure asymmetries in the numbers of sites with ABBA and BABA patterns (where A and B are ancestral and derived alleles, respectively) across a phylogeny with three ingroup taxa and an outgroup that has the relationship (((P1, P2), P3) O). A significant excess of either pattern gives a nonzero value of *D*, which is taken as evidence that gene flow has occurred between P3 and one of the sister taxa. The *f4*-ratio then measures the proportion of the P2 genome that is derived from P3 and thus provides an estimate of the admixture proportion (Reich et al. 2009). To estimate how introgression varied across the genome, we calculated another version of the admixture proportion (known as f_d; Martin et al. 2015) in 100 kb non-overlapping windows using the *ABBABABAwindows.py* Python script (https://github.com/simonhmartin/genomics_general). Similar to the *f4*-ratio, f_d searches for asymmetries in the number of ABBA and BABA sites across the genome; however, it has been optimised specifically for use in genomic windows. For the f_d analysis, mainland *longiflorus* was set as P1, Island *longiflorus* was P2, *parviflorus* was P3, and *M. clevelandii* was the outgroup (also referred to as Test 1, below).

The *fbranch* statistic was designed to evaluate which taxa in an evolutionary radiation showed evidence of introgression and to assess the relative timing of that gene flow (Malinsky et al. 2018, 2021). Specifically, given the *f4*-ratios calculated for all possible trios among a set of related taxa, *fbranch* uses the inferred phylogenetic relationship among these taxa, as well as variation in the phylogenetic distance between the sister taxa used as P1 and P2, to assign introgression to specific branches on a phylogenetic tree. The idea behind this test is the assumption that alleles introgressed into the common ancestor of P1 and P2 should be present in roughly equal proportions in both descendent lineages, making it difficult for any phylogenetic test to distinguish shared ancestral variation from introgression (Martin et al. 2013; Short and Streisfeld 2023). However, by gradually increasing the phylogenetic distance between the two sister taxa used in these tests, *fbranch* should be able to detect introgression that occurred at various points farther back in time (Malinsky et al. 2021). Indeed, Malinsky et al. (2018) used *fbranch* to conclude that there was extensive ancient hybridisation between the ancestors of modern cichlid lineages, as well as recent hybridisation among closely related species found in similar ecological niches.

Despite the availability of this test, *fbranch* (or any 4-taxon test of introgression) might present challenges in distinguishing recent from ancient introgression (Figure S1). Although *fbranch* tests all possible trios of ingroup taxa in a phylogeny, it does not account for shared genetic variation between the P3 taxon and its relatives. For example, if P1 and P2 are more diverged from each other than the P3 taxon is from its immediate relatives, then variation that arose in the ancestor of P3 and its sister taxa would be shared in all descendant taxa. Thus, if gene flow occurred recently only between P3 and P2, we would detect a signal of introgression between P2 and all the relatives of P3 (Figure S1). Alternatively, the same shared pattern of introgression could be caused by ancient gene flow that occurred prior to the divergence of these sister taxa. Consequently, the same signals of introgression obtained from 4-taxon tests can arise from distinct evolutionary histories, making it potentially difficult to determine whether introgression was recent or ancient.

To test the ability of *fbranch* to distinguish between the recent and ancient introgression, we performed neutral, coalescent simulations in msprime v. 1.2.0 (Baumdicker et al. 2022). Specific details of each simulation can be found in the supplemental methods, but we began by testing three divergence times and three migration rates across three different demographic scenarios (Figure S2). We evaluated these scenarios using a common phylogenetic history that matched the evolutionary relationships found in the *M. aurantiacus* species complex (see Section 3). The specific scenarios included: (i) "recent introgression," where gene flow occurred between *parviflorus* and Island *longiflorus* after the split between mainland and Island *longiflorus*; (ii) "ancient introgression," where gene flow occurred between the ancestor of *parviflorus* and the ancestor of clades C and D; and (iii) "recent and ancient introgression," which combined scenarios i and ii (Figure S2). *fbranch* was run separately for all divergence time and simulation scenario combinations, and then the different migration rates were tested for each demographic model using a single divergence time (see supplemental methods). The simulated results were compared to the results from *fbranch* calculated from the empirical sequence data. In addition, we ran a fourth demographic scenario where no introgression occurred (to account for the effects of incomplete lineage sorting [ILS]), which was used in the following section.

2.4 | The Relationship Between Genome-Wide Phylogenetic Discordance and Introgression

To further describe the history of introgression with *parviflorus*, we explored the patterns of phylogenetic discordance across the genome using *TWISST* (Martin and Van Belleghem 2017). Given a set of samples from multiple ingroup taxa, *TWISST* builds trees in genomic windows and then calculates the support for that topology among all possible topologies, which is referred to as the topology weighting. If all samples from each taxon are reciprocally monophyletic, then that topology is given a weighting of 1.0 for that window. The topological discordance among samples within a taxon will result in lower topology weightings for that window. We used *TWISST* to identify variation in the relationships among *aridus*, *parviflorus*, Island *longiflorus*, and mainland *longiflorus* in 100 kb genomic windows, with *M. clevelandii* as the outgroup. With four ingroup taxa, this resulted in 15 possible topologies, which were then partitioned into those that supported: (a) the species tree, where *aridus* and *parviflorus* were sisters and reciprocally monophyletic relative to Island *longiflorus* and mainland *longiflorus*; (b) a set of recent introgression trees, where *parviflorus* and Island *longiflorus* were sisters to one another relative to *aridus* and mainland *longiflorus*; and (c) an ancient introgression tree, where *parviflorus* was sister to mainland and Island *longiflorus* relative to *aridus*. By quantifying variation in the support for these topologies across the genome, we should be able to identify how often ancient and recent introgression contributed to phylogenetic discordance. This is because adding a fourth ingroup taxon that is sister to P3 should allow us to distinguish between the sharing of ancestral variation and signals caused by the introgression of lineage-specific alleles.

However, topological discordance can also be influenced by ILS. To test if these discordant topologies could be caused by ILS, we performed 10 iterations of each of the simulated demographic scenarios described above, including the scenario with no introgression. We ran TWISST on each simulated dataset to count the number of windows that showed complete support for the recent or ancient introgression topologies (i.e., a topology weighting of 1.0). Under ILS alone, we would expect to find support for both the recent and ancient introgression topologies in the simulation where no gene flow was allowed. To further confirm that these topology weightings were impacted by introgression, we calculated F_{ST} between *parviflorus* and Island *longiflorus* in all 100 kb non-overlapping windows that showed complete support for the species tree, the recent introgression tree, or the ancient introgression tree. Windows that matched the species tree or the ancient introgression tree were expected to show greater divergence between *parviflorus* and Island *longiflorus* than genomic regions reflecting the recent introgression. This is because genomic windows where there has been recent introgression should have more allele sharing (and thus lower F_{ST}) between the pair of hybridising taxa than genomic windows supporting the species tree (where there is no history of gene flow) or ancient introgression (where the signal may have been lost over time due to either selection or drift). F_{ST} was calculated using the *popgenwindows.py* Python script (https://github.com/simonhmartin/genomics_general), with only the variant sites included. Finally, to distinguish between recent and ancient introgression, we compared the empirical distribution of tree topologies to the four simulated scenarios to determine which demographic history most closely resembled our observed results.

2.5 | The Genomic Consequences of Introgression

Introgression can have multiple consequences on patterns of genetic variation across the genome. For example, under a model where selection against gene flow is polygenic, we expect a positive relationship between introgression and recombination rate and a negative relationship between introgression and genetic differentiation (Brandvain et al. 2014; Schumer et al. 2018; Liu et al. 2022). To estimate the relationship between introgression and recombination rate, we partitioned the f_d values calculated in 100 kb windows into quantile bins of recombination rates that were calculated previously in 500 kb windows by Stankowski et al. (2019). We then calculated the mean and 95% confidence intervals for f_d within each recombination rate quantile bin and fit a linear model, with f_d as the dependent variable and the recombination rate quantile bins as the independent variable. We then used the *emmeans* package (Lenth 2019) to perform pairwise comparisons of the estimated marginal mean f_d values for the different quantile bins from the linear model. We used Spearman's correlation coefficient to assess the relationship between f_d and F_{ST}, calculated in the same 100 kb windows.

In addition, we determined whether genetic diversity among the hybridising island taxa varied relative to the mainland taxa. Specifically, we asked whether the nucleotide diversity (π) and effective population size in both island subspecies were similar to the other taxa in the complex. We calculated π in 100 kb windows using *PIXY* version 1.2.5 (Korunes and Samuk 2021), with both variant and invariant sites included. To identify differences in the effective population sizes of these taxa, we used PSMC (Li and Durbin 2011) to estimate the effective population size through time for all samples from the island taxa and their most closely related mainland relatives. In accordance with Stankowski et al. (2023), we assumed a generation time of 2 years and a mutation rate of 7×10^{-9} for these calculations.

2.6 | Identifying Signatures of Adaptive Introgression

Extensive heterogeneity in admixture across the genome raises the possibility that adaptive introgression has maintained foreign ancestry in particular regions. To determine whether regions of elevated f_d display signatures of adaptive introgression between *parviflorus* and Island *longiflorus*, we calculated the frequency of Q95 sites found in windows across the genome (Racimo et al. 2017; Feng et al. 2024). Q95 sites are defined as those that are fixed (at a frequency of 1.0) in the donor taxon, near fixation (at a frequency greater than the 95th percentile) in the recipient taxon, and absent in the taxon that is sister to the recipient. Thus, any derived alleles that are present at high frequency in a recipient taxon that are also absent in its sister taxon but are fixed in a more distantly related donor taxon are candidates for adaptive introgression. Although other possibilities might generate an increase in the frequency of these Q95 sites (i.e., drift and bottlenecks), the Q95 statistic has been shown to be a powerful and reliable approach for detecting adaptive introgression (Racimo et al. 2017). Moreover, genomic regions that display an excess of Q95 sites are even more likely to have been targets of positive selection because selection will have correlated effects on linked sites. By contrast, other tests that rely on polymorphism or linkage disequilibrium are often less effective at detecting positive selection in introgressed regions due to known modifications of the haplotype structure and patterns of genetic diversity (Suarez-Gonzalez et al. 2018; Moest et al. 2020; Setter et al. 2020; Feng et al. 2024). Thus, by identifying an elevated frequency of Q95 sites in 100 kb windows across the genome, we can identify genomic regions that may have been adaptively introgressed.

To identify Q95 sites, we first identified all derived SNPs that were polymorphic among samples. Derived SNPs were defined as those where one nucleotide was fixed in *M. clevelandii* but a different nucleotide was present on at least one chromosome from samples of mainland *longiflorus*, Island *longiflorus*, *parviflorus*, or *aridus*. We then determined the number of derived sites that were fixed in *parviflorus*, absent in mainland *longiflorus*, and present in at least one haplotype in Island *longiflorus*. These represented sites that were introgressed from *parviflorus* into Island *longiflorus*. The 95th percentile of the allele frequency distribution among introgressed sites in Island *longiflorus* (=0.875) was used as a cutoff to identify adaptively introgressed sites (i.e., Q95 sites)

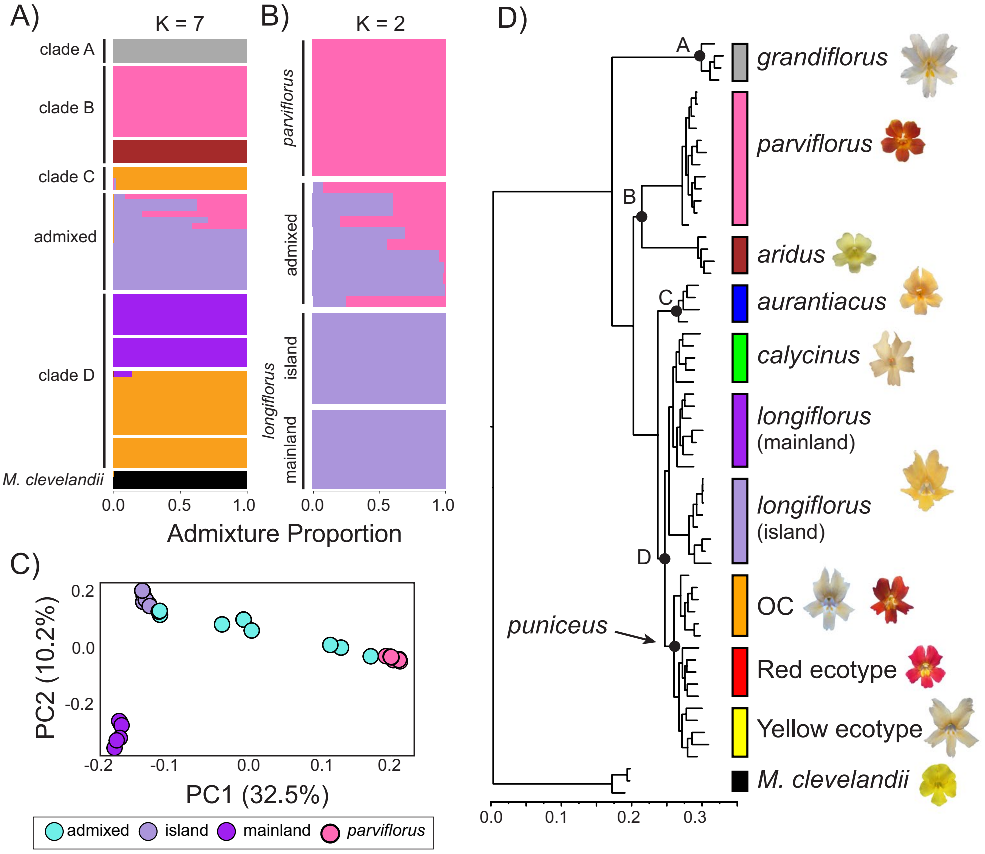

FIGURE 1 | The relationships among the taxa in the *Mimulus aurantiacus* species complex. (A) The ancestry proportions from *Admixture* at $K = 7$ for all the subspecies and their sister species, *Mimulus clevelandii*. (B) Ancestry proportions at $K = 2$ from a run that included only samples from the island as well as *longiflorus* from the mainland. (C) Plot of the first two principal components from the island samples and mainland *longiflorus*. The percent variation explained by each axis is reported. (D) Maximum-likelihood consensus tree showing evolutionary relationships across the radiation, with representative photographs of each taxon's flower. The four major clades of the radiation are labelled with letters. The tips corresponding to each taxon are indicated by a coloured bar. All nodes defining clades and subspecies relationships were supported with 100% bootstrap support, with only four nodes towards the tips consisting of less than 100% support (minimum support = 0.67).

(Racimo et al. 2017, Feng et al. 2024). For each 100 kb window, we then divided the number of Q95 sites by the total number of derived SNPs that were found on at least one chromosome in Island *longiflorus* to estimate the frequency of Q95 sites in each window.

To identify signatures of adaptive introgression that were transferred from Island *longiflorus* into *parviflorus*, we identified derived SNPs that were fixed in Island *longiflorus*, present on at least one haplotype in *parviflorus*, but were absent in *aridus*. In this case, the 95th percentile of the allele frequency distribution in *parviflorus* included only sites fixed in *parviflorus*. We then divided the number of Q95 sites by the total number of derived SNPs that were present on at least one chromosome in *parviflorus* in 100 kb windows.

To further explore signatures of introgression for the region with the highest frequency of Q95 sites across the genome, we calculated f_d, d_{xy}, F_{ST}, π, and the frequency of Q95 sites in 10 kb windows with 1000 bp steps. We calculated π and d_{xy} using *PIXY* version 1.2.5 (Korunes and Samuk 2021), with both variant and invariant sites included, and F_{ST} was calculated using the *popgenwindows.py* Python script described above. Introgression will result in reduced d_{xy} and F_{ST} between a pair of hybridising taxa because the taxa share alleles in this region. By contrast, there should be a corresponding increase in genetic divergence between the recipient and its sister taxa.

3 | Results

3.1 | Evolutionary Relationships and Hybridisation on Santa Cruz Island

Admixture assigned samples to ancestry groups that were largely consistent with previous analyses of the phylogenetic history of these subspecies (Figure 1A, Figure S3) (Chase et al. 2017; Stankowski et al. 2019; Short and Streisfeld 2023). At $K = 7$ (the K value with the lowest cross-validation error), we found that the newly sequenced Santa Cruz Island samples contained a mixture of individuals with both pure and admixed ancestry, suggesting the occurrence of ongoing hybridisation between *parviflorus* and *longiflorus* on the island (Figure 1A). By re-running Admixture at $K = 2$ with only *parviflorus* and *longiflorus*, we identified 12 "unadmixed" *parviflorus* and 15 "unadmixed" *longiflorus* (with q-scores greater than 0.99; Figure 1B). Of these 15 *longiflorus*, 8 were found on Santa Cruz Island and were subsequently defined

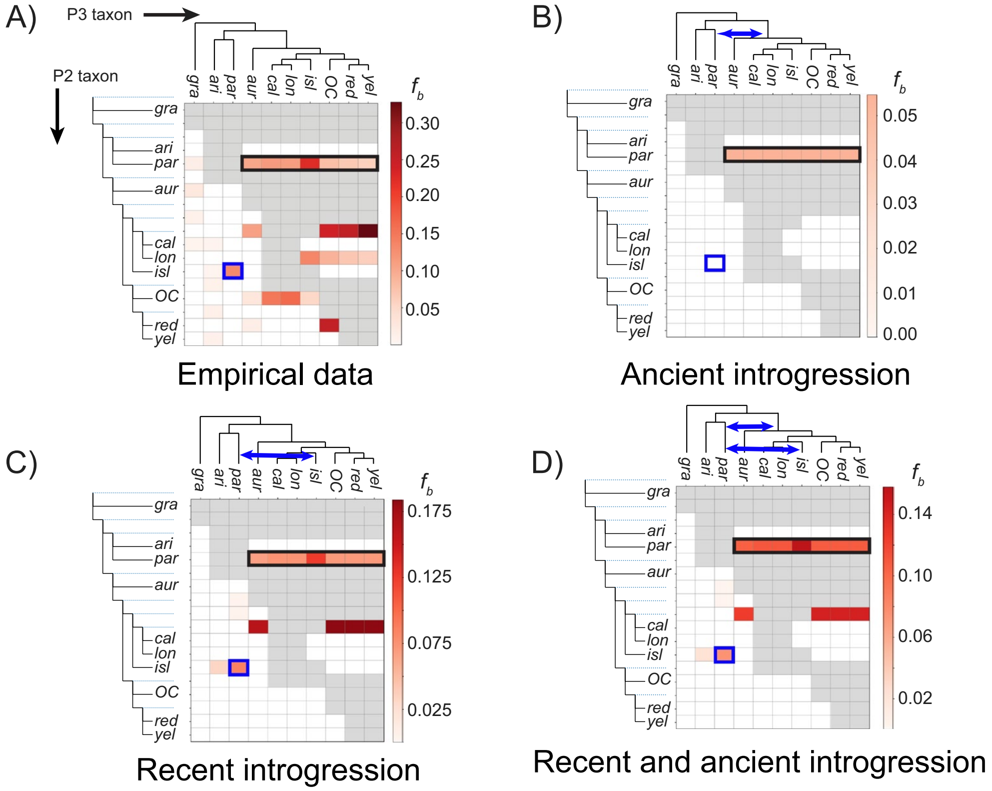

FIGURE 2 | Assessing recent and ancient hybridisation in the *Mimulus aurantiacus* species complex using *fbranch* on empirical and simulated data. (A) Genome-wide patterns of introgression using the *fbranch* statistic (*fb*) from the empirical sequence data. This analysis uses knowledge of the relationships among taxa and the results of the *f4*-ratio (calculated using all possible trios of ingroup taxa) to estimate the relative position of hybridisation on a species tree. The colour gradient represents the values of *fb*, with darker colours indicating greater evidence of introgression. Each box represents a particular *f4*-ratio test using the taxon on the top of the matrix as P3 and the taxon (or its ancestor; represented as blue dotted lines) depicted in the phylogeny to the left as P2. Grey boxes indicate tests that cannot be performed based on the available taxa and their phylogenetic relationships. (B–D) Simulated data using 11 populations and the same phylogenetic history as the empirical data under 3 demographic histories. For simplicity, population names in the simulations match the *Mimulus* taxon names. (B) A scenario with only ancient introgression; (C) a scenario with only recent introgression; and (D) a scenario with both recent and ancient introgression. The relative timing of the hybridisation events used in each simulation is shown via blue arrows on the phylogeny. More details can be found in Figure S2. Test 1 calculations of the *f4*-ratio are highlighted in each scenario with a blue box and were performed using the mainland *longiflorus* as P1, Island *longiflorus* as P2, and *parviflorus* as P3. Test 2 calculations of the *f4*-ratio are highlighted with a black box and were performed using *aridus* as P1, *parviflorus* as P2, and the various taxa from clades C and D as P3.

as Island *longiflorus* samples. These samples were clearly separated from mainland *longiflorus* in the PCA, where "unadmixed" Island *longiflorus* and *parviflorus* samples clustered separately along PC1, mainland *longiflorus* separated on PC2, and admixed samples were distributed between them (Figure 1C). The 11 admixed samples from Santa Cruz Island were removed from subsequent analyses, as they represented contemporary hybrids that were not appropriate for the phylogenetic tests of introgression we performed.

The consensus species tree was consistent with previous analyses and again revealed the same four primary clades (Stankowski and Streisfeld 2015; Chase et al. 2017; Short and Streisfeld 2023). Within the diverse clade D, Island *longiflorus* was sister to both mainland *longiflorus* and *calycinus* (Figure 1D). To confirm this relationship, an additional run of *Admixture* was performed at $K = 3$, revealing three distinct ancestry groups that corresponded to Island *longiflorus*, mainland *longiflorus*, and *calycinus* (Figure S4).

3.2 | Tests for Recent and Ancient Hybridisation

Our calculations of the *fbranch* statistic revealed evidence of widespread introgression, particularly among the very recently diverged taxa within clades C and D (Figure 2A). In addition, we identified evidence of hybridisation between *aurantiacus* and *grandiflorus*, as well as between *aridus* and the clade D taxa, confirming previous results by Stankowski et al. (2019) and Short and Streisfeld (2023). Moreover, we found a signal of introgression between the two island taxa when mainland *longiflorus* was set as P1, Island *longiflorus* was P2, and *parviflorus* was P3 (referred to as Test 1; Figure S1). However, when the test was reversed, so that *aridus* was P1, *parviflorus* was P2, and Island *longiflorus* was P3 (referred to as Test 2; Figure S1), we observed a strong signal of introgression between *parviflorus* and all the taxa from clades C and D, with the largest value between *parviflorus* and Island *longiflorus* (Figure 2A). These contrasting patterns could have multiple explanations (Figure S1).

To distinguish among these scenarios, we performed coalescent simulations in msprime, where we allowed only recent introgression, only ancient introgression, or a combination of both ancient and recent introgression. Results were qualitatively the same regardless of the different divergence times or migration rates that we tested (Figures S5 and S6). Therefore, we compared the empirical data to simulations with a migration rate of 0.1 and a total divergence time of 500,000 generations, which roughly corresponds to the previously estimated ~1-million-year divergence time between *M. aurantiacus* and *M. clevelandii*, assuming a 2-year generation time (Stankowski et al. 2019).

In the "ancient introgression" simulation, there was no signal of introgression between the island taxa in Test 1 (when mainland *longiflorus* was P1, Island *longiflorus* was P2, and *parviflorus* was P3; Figure 2B). This is expected when only ancient introgression is allowed, because Test 1 can only identify gene flow that occurred after Island and mainland *longiflorus* split from each other. However, we did find evidence for introgression between the island taxa using Test 1 in both the "recent" and "combined" simulations, as well as in the empirical data (Figure 2A,C,D). This signal implies the presence of recent introgression between the island taxa that occurred after Island and mainland *longiflorus* split.

By contrast, in Test 2 (when *aridus* was P1, *parviflorus* was P2, and Island *longiflorus* was P3), there was evidence for weak gene flow between *parviflorus* and all taxa from clades C and D in the "ancient introgression" simulation (Figure 2B). This is expected if introgression occurred in the ancestor of clades C and D (Figure S1). In the "recent and ancient introgression" scenario, we found this same pattern among all clade C and D taxa, but the signal was considerably stronger than in the "ancient introgression" scenario (Figure 2D). Moreover, the *fbranch* statistic was notably higher between *parviflorus* and Island *longiflorus* than among the rest of the taxa from clades C and D. This result closely resembles our empirical findings. However, in the "recent introgression" scenario, where gene flow occurred only after mainland and Island *longiflorus* split from their ancestor, we still detected the same broad pattern of introgression between *parviflorus* and all the clade C and D taxa, but the signal was weaker than in the "combined" simulations (Figure 2C). Qualitatively, these results suggest that *fbranch* alone cannot distinguish between ancient and recent introgression.

3.3 | Ancient and Recent Hybridisation Contribute to Genome-Wide Phylogenetic Discordance

To further explore the history of introgression between Island *longiflorus* and *parviflorus*, we used *TWISST* to define tree topologies that supported the "species tree," a set of "recent introgression trees" where Island *longiflorus* and *parviflorus* were sister, and an "ancient introgression" topology where *parviflorus* was sister to both mainland and Island *longiflorus* (Figure 3A). By scanning the genome for the distribution of the various tree topologies, we found widespread evidence of phylogenetic discordance in our sequence data (Figure 3B). We identified 553 windows with topology weightings of 1.0 for the species tree, 61 windows that supported the recent introgression trees, and 419 windows that matched the ancient introgression tree (Figure 3C) (a remaining 915 windows did not match any of these topologies or had topology weightings less than 1.0, which we refer to as "unresolved").

In addition to introgression, ILS can also contribute to genome-wide variation in phylogenetic discordance. However, across 10 iterations of the "no introgression" simulation, we found no windows with a topology weighting of 1.0 for either the recent or ancient introgression topologies, implying that any effects of ILS on topological discordance at this scale were likely minor (Figure 3D). Moreover, in our empirical data, we found a considerably reduced median F_{ST} between the island taxa in genomic windows that supported the recent introgression topologies relative to the species tree or ancient introgression tree (Figure 3E), as expected if these taxa recently shared genetic information due to introgression.

To assess whether ancient and recent introgression influenced the evolution of these island taxa, we examined the distribution of topologies in our simulations and compared them to the empirical distribution (Figure S7). In the "ancient introgression" simulation, no windows showed complete support for the recent introgression topology—consistent with the fact that introgression occurred prior to the split between the Island and mainland *longiflorus* (Figure 3D). In contrast, despite only allowing recent introgression in the "recent introgression" simulation, we still detected a substantial number of topologies consistent with ancient introgression. As discussed below, this likely results from extensive allele sharing between Island and mainland *longiflorus*. We also found a similar number of topologies supporting recent introgression in both the "combined" and "recent introgression" simulations. However, in the "combined" simulation—where both ancient and recent admixture were allowed—there was a clear increase in the number of windows fully supporting the ancient introgression topology (Figure 3D). This pattern aligns with expectations that ancient admixture resulted in this discordant phylogenetic pattern. Since our empirical data also show this same pattern (Figure S7), the most likely explanation is that there has been recent introgression between Island *longiflorus* and *parviflorus* on Santa Cruz Island, as well as ancient hybridisation between the ancestor of *parviflorus* and the common ancestor of clades C and D.

3.4 | The Genomic Consequences of Recent and Ancient Hybridisation

We identified extensive variation in the extent of introgression across the genome (Figure 4A). Values of f_d in 100 kb windows ranged from 0 to 0.92. To determine the evolutionary processes responsible for this extreme heterogeneity in introgression across the genome, we compared f_d with levels of genetic divergence and recombination rates. We found an overall positive relationship between recombination rate and mean f_d (Figure 4B). Specifically, mean f_d in the lowest recombination rate quantile was significantly lower than all four of the remaining higher quantiles (Table S2). We also identified a strong negative relationship between f_d and F_{ST} ($r = -0.77$; Figure 4C), implying that

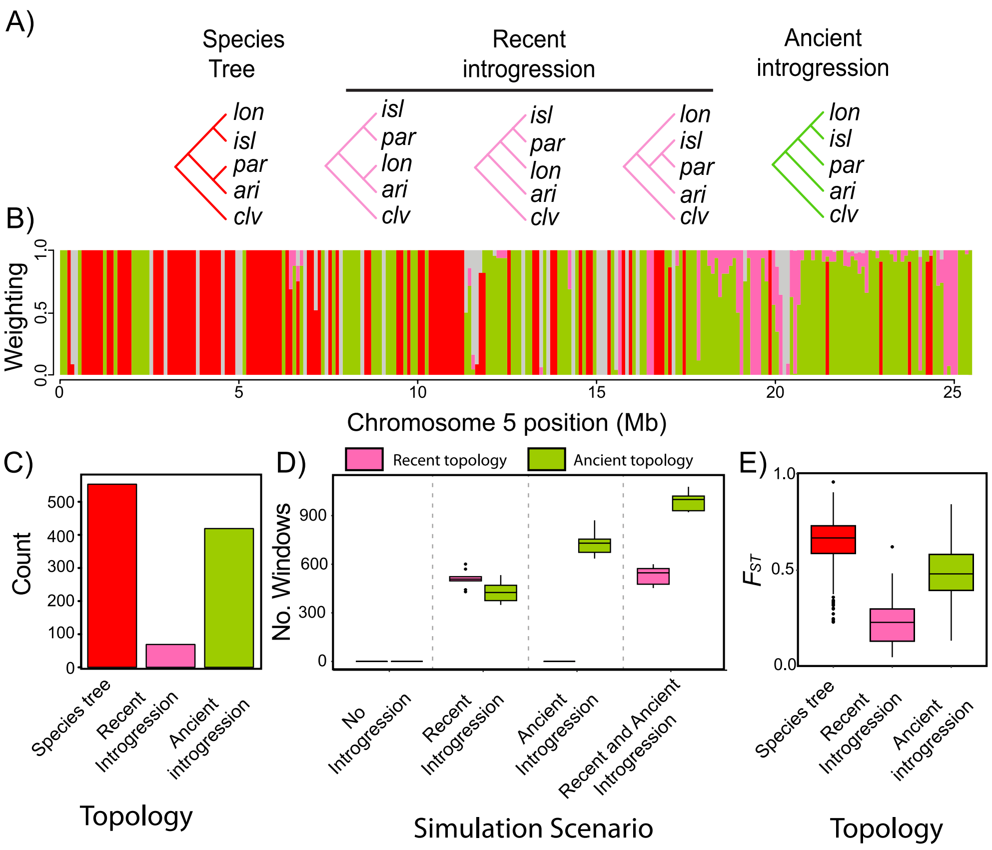

FIGURE 3 | Genome-wide variation in levels of phylogenetic discordance. (A) Topologies that represent the species tree (red), recent introgression trees (pink), and the ancient introgression tree (green). (B) Representative variation in topology weighting across chromosome 5. Given a set of samples from multiple ingroup taxa, *TWISST* builds trees in genomic windows and then calculates the support for that topology among all possible topologies, which is referred to as the topology weighting. If all samples from each taxon are reciprocally monophyletic, then that topology is given a weighting of 1.0 for that window. Topological discordance among samples within a taxon will result in lower topology weightings for that window. Red bars indicate support for the species tree, pink bars indicate support for the recent introgression trees, green bars indicate support for the ancient introgression tree, and grey bars indicate no support for these topologies. (C) The number of 100 kb windows across the genome containing a topology weighting of 1.0 for the species tree, recent introgression trees, and the ancient introgression tree. (D) Box plots of the number of simulated 100 kb windows that show a topology weighting of 1.0 for the recent introgression topologies (pink) or the ancient introgression topology (green) among 10 iterations of each of the demographic scenarios tested. (E) Box plots of F_{ST} between Island *longiflorus* and *parviflorus* in genomic windows that show complete support for the species tree, recent introgression topologies, or the ancient introgression topology. Shown in (D) and (E) are box plots, with horizontal black lines indicating the median, box heights representing the lower and upper quartiles, and whiskers corresponding to 1.5 times the interquartile range.

negative selection has likely purged deleterious foreign genetic variation across the genome.

Average nucleotide diversity (π) was very similar among taxa, including the island endemics (Figure 5A). Furthermore, we found no evidence for recent or historical reductions in effective population size in the island taxa (Figure 5B). However, we did observe a small increase in effective population size in *parviflorus* and Island *longiflorus* estimated around 100,000 years ago, suggesting that hybridisation between these subspecies may have started around that time.

3.5 | Support for Localised Adaptive Introgression

Regions of elevated f_d surrounded by windows of low admixture raise the possibility of adaptive introgression. We calculated the frequency of Q95 sites in 100 kb windows across the genome (Figure S8). As might be expected, most windows displayed no increases in the frequency of Q95 sites. However, we did identify clear pileups of elevated Q95 ratios in a few regions (Figure S8), with one region in particular where two adjacent windows on chromosome 5 had more than 40% of derived sites that matched the Q95 pattern (24.7–24.9 Mbp). These same windows had f_d values of 0.73 and 0.74 (Figure 4).

By zooming in on this region on chromosome 5, we identified a strong signal of introgression that spanned approximately 300 kb (Figure 6A). This region of elevated f_d was substantially higher than the genome-wide average f_d. Furthermore, we identified reduced d_{xy} and F_{ST} between *parviflorus* and Island *longiflorus* and a clear increase in the frequency of Q95 sites transferred from Island *longiflorus* into *parviflorus* (Figure 6). In contrast, we did not see a signal of elevated Q95 when considering introgression in the other direction. In addition, we identified elevated F_{ST} and d_{xy} between *parviflorus* and *aridus*, but no clear

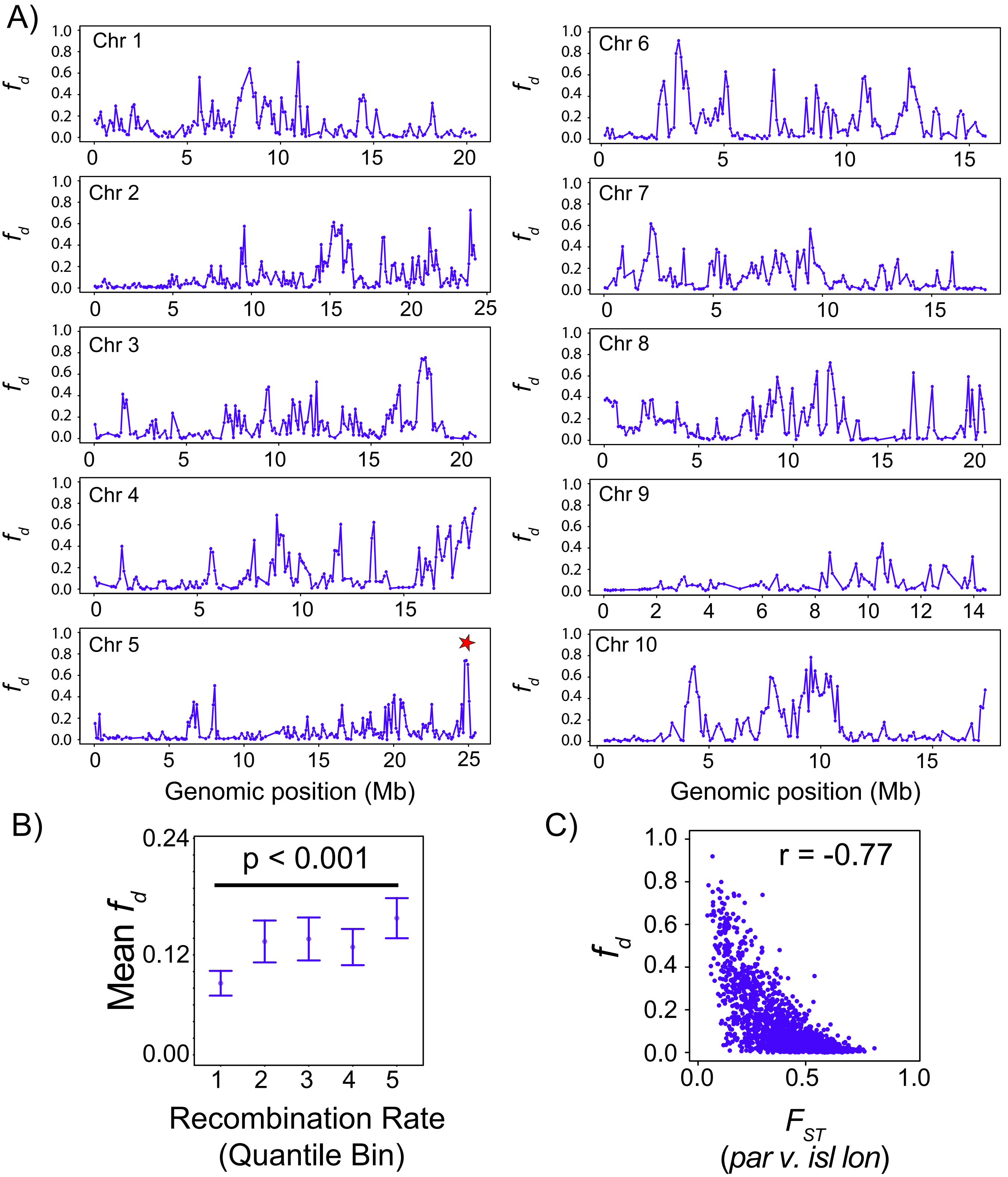

FIGURE 4 | Genome-wide variation in introgression. (A) Raw f_d values plotted across the 10 chromosomes of the *Mimulus aurantiacus* genome. The red star above the peak of f_d on chromosome 5 corresponds to the putative region of adaptive introgression described in Figure 6. (B) Mean and 95% confidence intervals for f_d calculated among quantile bins of recombination rate. Quantile bins of recombination rate are as follows: 1 = 0–0.824 cM/Mb; 2 = 0.824–1.51 cM/Mb; 3 = 1.51–2.33 cM/Mb; 4 = 2.33–3.66 cM/Mb; 5 = 3.66–10.9 cM/Mb. (C) Scatterplot showing the relationship between f_d and genetic differentiation (F_{ST}) between Island *longiflorus* and *parviflorus*, with the correlation coefficient between the statistics in the upper right-hand corner.

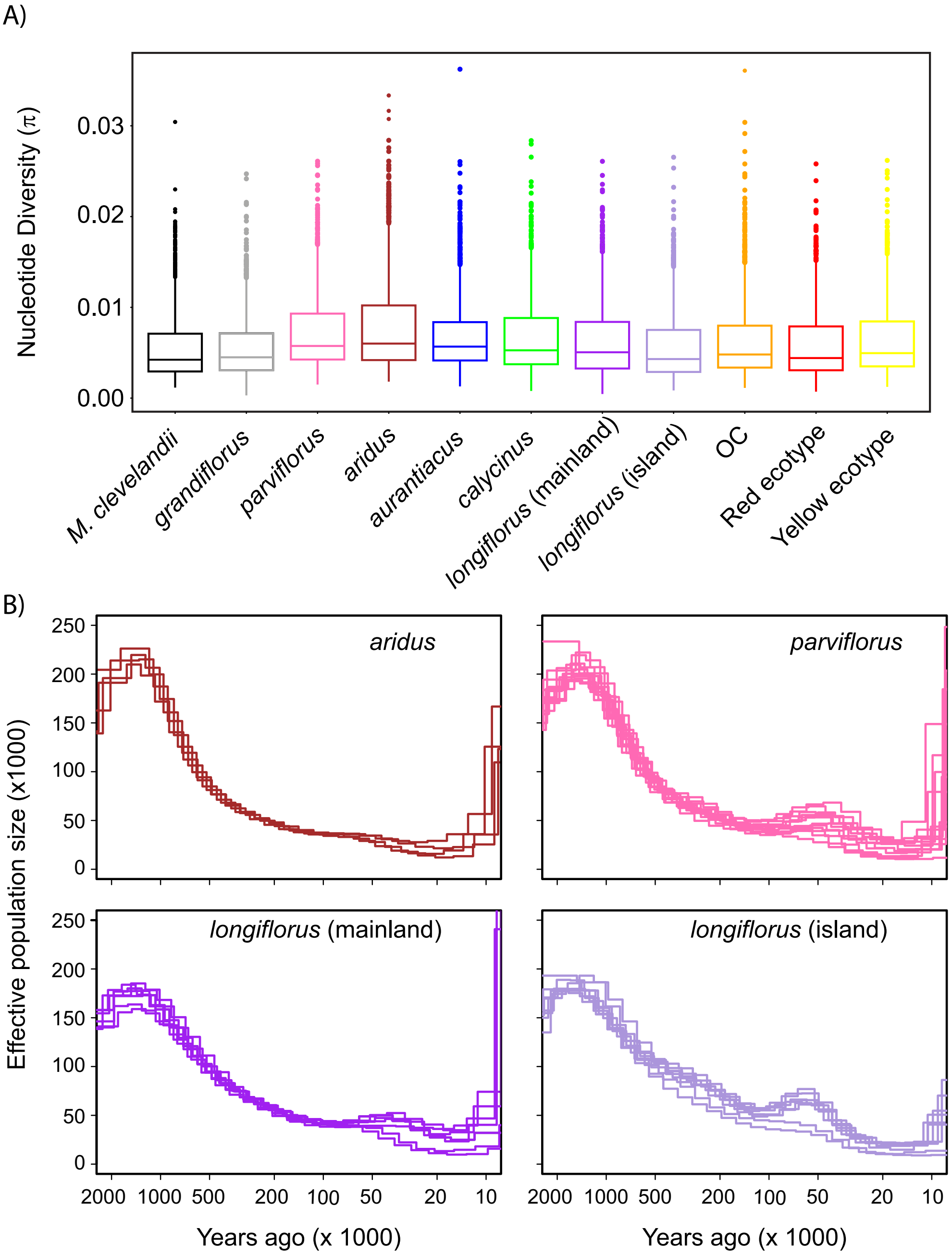

FIGURE 5 | Levels of genetic diversity and effective population size are similar in island and mainland taxa. (A) Box plots of nucleotide diversity (π) calculated in 100kb windows across the genome for each taxon in the *Mimulus aurantiacus* complex and its sister species, *Mimulus clevelandii*. Horizontal black lines indicating the median, box heights representing the lower and upper quartiles, whiskers correspond to 1.5 times the interquartile range, and points indicate outliers. (B) Estimates of effective population size through time from PSMC for the island taxa and their closest mainland relatives. Each line corresponds to a different sequenced individual in our dataset.

increase in F_{ST} or d_{xy} between Island and mainland *longiflorus* (Figure 6B,C), providing further support that introgression in this region occurred from Island *longiflorus* into *parviflorus*. However, we observed no clear decrease in π for either island taxon, but note that this region of the genome has unusually low diversity relative to the genome-wide average (Figure 6D). Based on the available annotation from the reference assembly (Stankowski et al. 2019), there were 37 genes in this interval (Table S3).

4 | Discussion

Hybridisation can occur throughout the evolutionary history of a radiation. Understanding the history of hybridisation can help resolve the long-term impacts of introgression on adaptation and speciation. In this study, we leveraged the diversity within the *M. aurantiacus* species complex to test for evidence of introgressive hybridisation at different points in the group's history. While previous studies have suggested that tests of admixture can distinguish between recent and ancient introgression (Malinsky

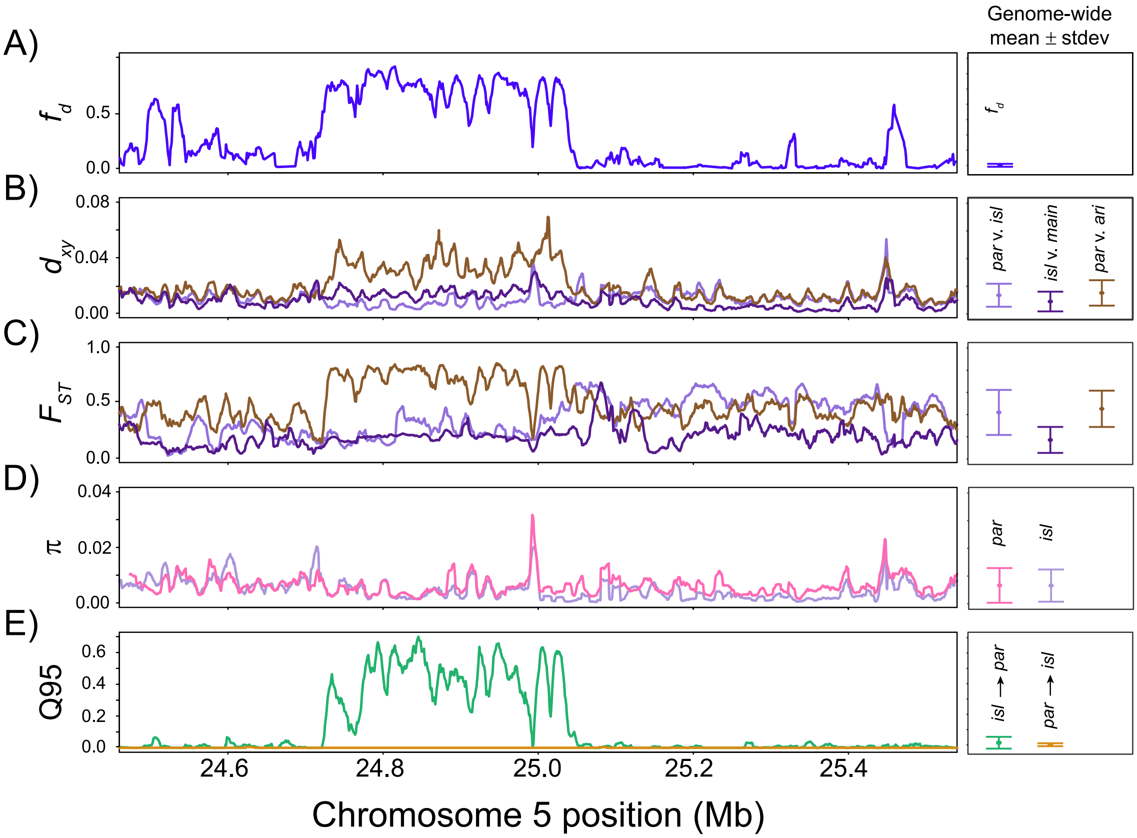

FIGURE 6 | Hybridisation may lead to adaptive introgression from Island *longiflorus* into *parviflorus*. On the left, the admixture proportion (f_d), genetic divergence (d_{xy}), genetic differentiation (F_{ST}), genetic diversity (π), and the frequency of Q95 sites are plotted across an 800 kb region on chromosome 5 identified as a candidate region using the Q95 statistic (Figure S7). On the right of each plot is the genome-wide mean ± standard deviation of each statistic, calculated in 10 kb windows and 1000 bp steps across all 10 chromosomes. (A) f_d values from Test 1 indicate a defined region of elevated introgression. (B) d_{xy} between *parviflorus* and *aridus* (brown) is clearly elevated across this region. However, d_{xy} between *parviflorus* and Island *longiflorus* (light purple) and d_{xy} between Island and mainland *longiflorus* (purple) are similar to genome-wide levels. (C) F_{ST} between *parviflorus* and Island *longfilorus* (light purple) is reduced in this region, and F_{ST} between Island and mainland *longiflorus* (purple) is similar to genome-wide levels. F_{ST} between *parviflorus* and *aridus* (brown) displays a clear increase in this region as compared to genome-wide levels. (D) No clear decrease in π was observed for either *parviflorus* (pink) or Island *longiflorus* (light purple). (E) A clear increase in the number of alleles that are shared between Island *longiflorus* and *parviflorus* but absent in *aridus* (green) was observed within this region. In contrast, no increase in the number of alleles that are shared between *parviflorus* and Island *longiflorus* but absent in mainland *longilforus* (yellow) was observed within this region, indicating unidirectional introgression from Island *longiflorus* into *parviflorus*.

et al. 2018, 2021; Short and Streisfeld 2023), we conducted simulations to evaluate this hypothesis. We demonstrated that recent hybridisation alone can produce signals that resemble those expected when both ancient and recent introgression have occurred, a phenomenon that is especially likely to occur due to the short divergence times found in evolutionary radiations (Pang et al. 2025, e.g., Malinsky et al. 2018). To further explore these patterns, we constructed phylogenetic trees across the genome using two sister pairs of ingroup taxa and an outgroup, which revealed that genome-wide patterns of topological discordance vary in a predictable manner across different histories of hybridisation.

When comparing our sequence data to the simulated results, we found that the genome-wide patterns of topological discordance observed in the *M. aurantiacus* radiation are best explained by a combination of recent hybridisation between the island taxa and ancient hybridisation between their ancestors. Estimating the evolutionary consequences of this hybridisation revealed evidence for widespread selection against recent gene flow, as well as multiple genomic regions that were nearly fixed for introgressed alleles, suggesting the possibility of adaptive introgression. These findings highlight the complex interplay between recent and ancient hybridisation in shaping the evolutionary trajectories of taxa within adaptive radiations, underscoring the importance of considering both timeframes in studies of introgression and speciation.

4.1 | Distinguishing Recent and Ancient Introgression

By studying the history of hybridisation between island taxa, we demonstrate the power and limitations of evolutionary radiations for estimating the relative timing of hybridisation events. Martin et al. (2013) showed that increasing the sequence divergence between sister taxa in a four-taxon test improves the detection of older introgression. Malinsky et al. (2018) advanced this by developing the *fbranch* statistic to estimate the relative timing of hybridisation across phylogenies. In this study, we applied these methods to a pair of island endemic taxa and showed that recent hybridisation can produce signals similar to ancient events, likely driven by the sharing of ancestral variation between taxa.

Our whole-genome sequencing results using *fbranch* revealed a signal of hybridisation between the island taxa in Test 1. In addition, we see evidence of hybridisation between *parviflorus* and all taxa in clades C and D in Test 2. However, the strongest signal of hybridisation in Test 2 was between Island *longiflorus* and *parviflorus*. One interpretation for these results is that there was recent introgression between the island taxa, as well as ancient hybridisation between the ancestor of *parviflorus* and the common ancestor of clades C and D. However, it has been shown that ancient hybridisation with an extinct lineage can lead to false signals of hybridisation between the extant taxa (referred to as "ghost introgression"; Pang and Zhang 2024, Zhang et al. 2024). Moreover, variation in relative branch lengths among clades can limit our ability to identify introgression because recently diverged taxa share more alleles than taxa that are more highly diverged (Hibbins and Hahn 2022). Thus, because *aridus* and *parviflorus* diverged prior to the taxa in clades C and D, we must consider additional explanations for our finding that all clade C and D taxa showed evidence of introgression with *parviflorus*. For example, because Island *longiflorus* and its relatives continue to share alleles that arose in the ancestor of clades C and D, even if introgression occurred recently from Island *longiflorus* into *parviflorus*, all clade C and D taxa would still produce a signal of admixture with *parviflorus* when they are set as P3 in Test 2 (Figure S1). Alternatively, this signal could be caused by ancient introgression alone between their ancestors.

To attempt to distinguish between these scenarios, we performed coalescent simulations using the estimated evolutionary relationships of the taxa. The results from *fbranch* revealed that ancient introgression can be detected reliably in simulations that included only older hybridisation. However, simulations of recent hybridisation produced a pattern that was qualitatively similar to the "combined" scenario that allowed both recent and ancient hybridisation. Although Malinsky et al. (2021) noted that allele sharing could create false signals of hybridisation using *fbranch*, they did not explore how variation in introgression histories or divergence times could affect these signals.

Although we are unable to distinguish between recent introgression combined with allele sharing and other explanations based on qualitative patterns from *fbranch*, the different magnitudes of introgression that we observed may provide clues about which histories of hybridisation were most likely. Specifically, we found a higher value of *fbranch* in Test 2 for the simulation where both recent and ancient introgression occurred compared to the simulation of only recent introgression. While the effects of allele sharing between Island *longiflorus* and its relatives in clades C and D would contribute to this signal in both demographic scenarios, the "combined" simulation also includes the effects of ancient introgression, explaining the stronger *fbranch* values for Test 2.

By building phylogenetic trees across genomic windows from simulated data, we were able to obtain additional evidence that distinct signals of topological discordance could be attributed to different histories of gene flow. Specifically, we found that simulations of ancient gene flow identified windows that contained complete support for the ancient introgression topology but not the recent introgression topology. By contrast, when only recent introgression was permitted, we found a nearly equal number of windows that supported both topologies. Even though we expected that Island *longiflorus* and *parviflorus* would frequently occur as sisters under this demographic scenario (i.e., resulting in the recent introgression topology), since mainland and Island *longiflorus* continue to share substantial genetic variation, the result was an excess of windows where mainland and Island *longiflorus* remained sisters but *parviflorus* was sister to them (i.e., matching the ancient introgression topology). This likely resulted in an underestimate of the number of windows that supported the recent introgression topology. However, simulations that combined both recent and ancient introgression led to nearly twice as many windows that supported the ancient introgression topology. This increase must have been caused by the addition of ancient gene flow in the simulation. Thus, the greater divergence between *parviflorus* and *aridus* relative to clades C and D, plus the addition of a fourth taxon sister to the Island *longiflorus*, make it possible to detect introgression that occurred deeper in the past. This same idea was used by Pease and Hahn (2015) to identify the directionality of gene flow in their D_{FOIL} statistic by using two pairs of sister taxa with different relative divergence times. Similarly, Nelson et al. (2021) used patterns of phylogenetic discordance to identify evidence of both recent and ancient introgression between *Mimulus lewisii*, *Mimulus cardinalis*, and their relatives.

Applying this framework to our empirical data, we found patterns that were most consistent with the combined scenario of ancient and recent hybridisation. Indeed, we found substantial topological discordance, such that the ancient introgression topology occurred across the genome more than six times as frequently as the recent introgression tree. We also found nearly equal numbers of windows that supported the species tree and the ancient introgression tree. Based on the results from the simulations, these empirical findings suggest that the ancient introgression topology includes the combined signal of recent introgression between the island taxa, allele sharing between Island *longiflorus* and its relatives, as well as ancient introgression that occurred in their ancestors. While the simulations we employed probably did not precisely match the demographic history of these island taxa, the simple scenarios we used aligned reasonably well with the empirical data, adding confidence to our conclusions. Nevertheless, future work in this area should continue to test additional demographic scenarios that vary the timing of hybridisation, the relative branch lengths between lineages, mutation and recombination rates, the directionality of introgression, and the effects of selection. Understanding how hybridisation has changed through time will allow us to test critical hypotheses about the accumulation of reproductive isolation across the speciation continuum (Stankowski and Ravinet 2021).

4.2 | The Genomic Consequences of Hybridisation

Hybridisation has had heterogeneous effects on the genomes of *parviflorus* and Island *longiflorus*. Genome-wide patterns of introgression revealed strong evidence of selection against foreign alleles, as demonstrated by the positive relationship between recombination rate and f_d. This suggests that deleterious alleles introduced via hybridisation were more efficiently purged in regions of low recombination, where linkage prevents their separation from beneficial or neutral variants (Schumer et al. 2018). Such patterns of polygenic selection against gene flow imply the presence of widespread reproductive barriers that maintain species boundaries (Martin et al. 2019). However, reproductive isolation is not complete between *parviflorus* and Island *longiflorus*, as numerous contemporary hybrids were detected in our samples (Figure 2B,C), implying that gene flow is ongoing.

In addition, we identified localised genomic regions where introgressed alleles appear to have been retained due to positive selection, suggesting the potential for adaptive introgression. A notable example is a roughly 300 kb region on chromosome 5, which was fixed for alleles in both *parviflorus* and Island longiflorus but absent from *aridus*. This region also exhibited elevated f_d, as well as reduced F_{ST} and d_{xy}, which are all consistent with introgression. The maintenance of this extensive signal of introgression suggests a role for non-neutral processes. However, the haplotype structure and patterns of genetic diversity can be altered in introgressed regions, making it challenging to detect signatures of positive selection unless there is a priori knowledge of specific genes that affect adaptive phenotypes (Suarez-Gonzalez et al. 2018; Moest et al. 2020; Short and Streisfeld 2023). Thus, an increased frequency of Q95 sites, which defines sites that are uniquely shared between donor and recipient, can provide reliable evidence in support of adaptive introgression, though drift remains an alternate explanation (Racimo et al. 2017). In addition, we detected multiple, linked sites with elevated Q95, which is consistent with positive selection having led to this increased frequency. Although there are no obvious candidate genes in this region (Table S3), there is still much to learn about the ecology of this island system.

Indeed, the ongoing gene flow between *parviflorus* and Island *longiflorus*, coupled with their sympatry and ecological overlap, presents an interesting opportunity to study the maintenance of reproductive barriers in the face of hybridisation. For example, the effects of hybridisation on the retention of adaptively introgressed regions, in conjunction with the purging of deleterious alleles, reveal a potentially interesting dynamic between the evolution of reproductive isolation and adaptation (Martin and Jiggins 2017; Martin et al. 2019; Short and Streisfeld 2023). These processes may be further influenced by unique aspects of the island environment, such as limited dispersal opportunities and high ecological specialisation (Franks 2010). Future work in this area should focus on quantifying hybrid fitness, characterising the ecological factors driving selection on introgressed alleles, identifying the genetic basis of these adaptive traits, and determining whether they have contributed to the evolution of reproductive barriers between these co-occurring lineages.

4.3 | Hybridisation and Its Implications for Island Taxa

The evolutionary dynamics of *parviflorus* and Island *longiflorus* have been shaped by their unique geography and history of hybridisation. Island taxa often have reduced effective population sizes and genetic diversity, which would increase their susceptibility to genetic drift and deleterious load (Barrett 1996). However, our analyses revealed similar patterns of nucleotide diversity and effective population size in these taxa compared to their mainland relatives. This could be explained by their history of introgression, which may have restored the necessary genetic variation to mitigate the effects of drift. Additional sampling from the other Channel Islands where the two taxa do not occur in sympatry will be necessary to test this hypothesis and to determine if hybridisation can play a role in maintaining genetic diversity and adaptive potential in small, isolated populations.

Although *parviflorus* is currently endemic to the Channel Islands, our results suggest that the ancestor of *parviflorus* also may have been present on the mainland of California at some point in the past, where it hybridised with the common ancestor of clades C and D. It has been argued that all plant species currently found on the Channel Islands are descended from mainland California ancestors (Axelrod 1965; Thorne 1969; Schoenherr et al. 2003). Furthermore, many of the woody plant species restricted to the Channel Islands were found on the mainland in the past, where they appear to have been driven to extinction by changing climate conditions (Axelrod 1965). Many of the Channel Island endemic species are believed to have migrated from the mainland during the Pleistocene glacial period when sea levels were lower (Johnson 1978; Muhs et al. 2015; Mychajliw et al. 2020). During this period, Santa Cruz Island was connected to Santa Rosa and Anacapa Islands, and the distance between these islands and the mainland was roughly 6–10 km (Johnson 1978; Muhs et al. 2015), making dispersal to the islands possible. Furthermore, consistent with what we report here in *Mimulus*, there is also evidence of ancient and recent introgression between a pair of Channel Island endemic oak species (Ortego et al. 2018; Mead 2023; Mead et al. 2024). One of the island species appears to be a relic of a now extinct species that was present on the mainland, and the other is widely distributed on both the mainland and the Channel Islands. Indeed, hybridisation between plant species on the Channel Islands appears to be common (Thorne 1969), which is consistent with a general finding of increased hybridisation between closely related island endemics (Carlquist 1966; Reatini and Vision 2023). This raises the possibility that hybridisation may be a common feature among closely related Channel Island endemics.

4.4 | Broader Implications for Evolutionary Radiations

This study highlights the power and challenges of evolutionary radiations for disentangling complex histories of hybridisation. By leveraging the taxonomic diversity within the *M. aurantiacus* species complex, and combining this with coalescent simulations, we demonstrated how recent and ancient introgression can leave distinct genomic signatures, providing information about the processes driving speciation and adaptation. Our findings underscore the importance of integrating phylogenetic, genomic, and computational approaches to unravel the multifaceted consequences of hybridisation.

Overall, the patterns of hybridisation observed in *Mimulus* contribute to a growing body of evidence that gene flow plays a dual role in evolutionary radiations (Marques et al. 2019). While hybridisation can uncover deleterious incompatibilities, it can also facilitate adaptation by transferring beneficial alleles across taxa. These combined effects highlight the dynamic interplay between hybridisation and natural selection, shaping the evolutionary trajectories of taxa within radiations.

Author Contributions

A.W.S. and M.A.S. designed the study, conducted all analyses, and wrote the manuscript.

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Disclosure

Benefit-sharing statement: Benefits generated: Benefits from this research accrue from the sharing of our data and results on public databases as described above.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw sequencing reads were downloaded from the Short-Read Archive (SRA) from bioproject ID: PRJNA549183. New sequencing reads generated here have been uploaded and added to bioproject ID PRJNA1149754. VCF files and population genomic data have been deposited to Dryad (DOI: 10.5061/dryad.cfxpnvxjw). The reference assembly and annotation are available at mimubase.org. Computer scripts used for population genomic analyses are available on Github at: https://github.com/awshort/Channel_Islands_monkeyflower_hybridization. Samples were collected from Santa Cruz Island according to the Scientific Research and Collecting Permit from the National Parks Service (CHIS-2024-SCI-0008).

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Supporting Information

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