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# Genomic landscapes of divergence among island bird populations: Evidence of parallel adaptation but at different loci?

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## Abstract

When populations colonise new environments, they may be exposed to novel selection pressures but also suffer from extensive genetic drift due to founder effects, small population sizes and limited interpopulation gene flow. Genomic approaches enable us to study how these factors drive divergence, and disentangle neutral effects from differentiation at specific loci due to selection. Here, we investigate patterns of genetic diversity and divergence using whole-genome resequencing (>22× coverage) in Berthelot's pipit (*Anthus berthelotii*), a passerine endemic to the islands of three north Atlantic archipelagos. Strong environmental gradients, including in pathogen pressure, across populations in the species range, make it an excellent system in which to explore traits important in adaptation and/or incipient speciation. First, we quantify how genomic divergence accumulates across the speciation continuum, that is, among Berthelot's pipit populations, between sub species across archipelagos, and between Berthelot's pipit and its mainland ancestor, the tawny pipit (*Anthus campestris*). Across these colonisation timeframes (2.1 million–ca. 8000 years ago), we identify highly differentiated loci within genomic islands of divergence and conclude that the observed distributions align with expectations for non-neutral divergence. Characteristic signatures of selection are identified in loci associated with craniofacial/bone and eye development, metabolism and immune response between population comparisons. Interestingly, we find limited evidence for repeated divergence of the same loci across the colonisation range but do identify different loci putatively associated with the same biological traits in different populations, likely due to parallel adaptation. Incipient speciation across these island populations, in which founder effects and selective pressures are strong, may therefore be repeatedly associated with morphology, metabolism and immune defence.

**KEYWORDS**
birds, craniofacial evolution, divergence landscape, genomic islands, immune defence, parallel adaptation, speciation

## 1 | INTRODUCTION

Genetic differentiation among populations accumulates over time due to a combination of differing adaptive and neutral processes (Feder et al., 2013; Seehausen et al., 2014). The speed at which divergence occurs and the resulting genomic landscape depend on the strength of these selective and neutral forces (including drift, mutation and gene flow) occurring within and among populations (Nosil et al., 2009). Across the genome, loci under differential selection in single or multiple populations are expected to diverge first with neutrally evolving genomic regions typically differentiating more slowly (Walsh et al., 2019). Demographic history, such as previous bottlenecks or inbreeding, may also lead to radical changes in the genome, but the effects of these events are expected to occur more evenly throughout the genome (Kimura, 1991; Nei, 2005; Quilodrán et al., 2020). Genomic approaches provide an opportunity to study the genetic landscape of divergence among populations, enabling the relative importance of differing evolutionary forces driving divergence to be determined (Bergström et al., 2022; Estandía et al., 2023; Ravinet, Faria, et al., 2017; Stajich & Hahn, 2005).

Upon colonisation of new environments, populations may be exposed to novel selective pressures, which may result in rapid ecological and phenotypic divergence between populations (Lamichhaney et al., 2015; Walsh et al., 2019). Selection may then act on standing genetic variation or de novo mutations (Estandía et al., 2023). When a locally beneficial allele arises at a locus, positive selection may cause it to rapidly increase in frequency in one population, resulting in a local selective sweep, while in the other population that allele may be lost through drift or purifying selection, or remain at low frequencies (Hejase et al., 2020; Ravinet, Faria, et al., 2017). During the initial stages of divergence genetic differentiation is expected to be localised with peaks of divergence around selected loci, often referred to as 'genomic islands of divergence' (Burri et al., 2015; Ellegren et al., 2012; Nadeau et al., 2012; Nosil et al., 2009). Such areas can be responsible for the accumulation of genetic and phenotypic differences between populations, which may play a fundamental role in speciation (Ruegg et al., 2014; Via & West, 2008). As well as genomic islands of divergence, highly conserved genomic regions – where differentiation is far below background levels – have been identified in a range of species (Hofer et al., 2012; Ravinet, Yoshida, et al., 2017; Sendell-Price et al., 2020; Van Doren et al., 2017). These 'genomic valleys of divergence' may occur because the same allele is favoured in both populations (parallel or balancing selection) (Nielsen, 2005; Roesti et al., 2012; Spurgin & Richardson, 2010) or linked neutral loci are favoured through background selection (Cvijović et al., 2018), leaving a distinct signature of reduced genetic diversity in both populations (Roesti et al., 2014). Furthermore, reduced recombination and background selection can also result in the formation of genomic islands through linkage to selected loci (Noor & Bennett, 2009). Individual-based modelling can be used to further determine if observed patterns align with expectations for non-neutral divergence or if they likely result from stochastic events (Ali et al., 2023; Sendell-Price et al., 2020).

Linkage disequilibrium may facilitate divergence hitchhiking of neutral (and weakly selected or deleterious) loci in proximity to strongly selected loci, resulting in broad genomic islands surrounding these loci (Maynard-Smith & Haigh, 1974; Nosil et al., 2009; Nosil & Feder, 2012; Via, 2012). Broad peaks of divergence often form as a result of recent selection, where recombination has not yet acted upon the regions around the selected loci. Over time recombination erases the effect of divergence hitchhiking, by reducing linkage, which may result in sharp peaks surrounding selected loci (Nosil et al., 2009). However, recombination does not act evenly across the genome and population processes including limited gene flow and small effective population sizes ($N_e$) are associated with reduced recombination rate, which in turn maintains large regions of divergence (Feder & Nosil, 2010). Discerning and dating the combination of evolutionary processes that have shaped genetic diversity between populations is complex, particularly since the same pattern has the potential to evolve as a result of differing evolutionary scenarios. Therefore, studies need to combine observations of the divergence landscape with knowledge of past and present population processes to determine the relative roles of drift and selection in the divergence of a particular genomic region (Nosil & Feder, 2012; Ravinet, Faria, et al., 2017).

Newly established populations may also be strongly influenced by founder effects, which result in a loss of genetic diversity (Barton & Charlesworth, 1984; Berry, 1986; Harrison, 1991). The characteristics of divergence over time are then mediated by the level and timing of gene flow between populations (Delmore et al., 2020; Räsänen & Hendry, 2008; Ravinet, Faria, et al., 2017), although new species can emerge across differing levels of genetic isolation (Bay & Ruegg, 2017; Kirkpatrick & Ravigné, 2002; Li et al., 2010; Martin et al., 2013). Genetic drift may also act particularly strongly in small, genetically isolated, populations which can result in the rapid loss or fixation of genetic variants (Alleaume-Benharira et al., 2006). The impact of such processes may be exaggerated by sequential founder events and the cumulative effects of drift through a chain of colonisation events (Clegg et al., 2002; Prugnolle et al., 2005; Tomozawa et al., 2014).

The search for genes associated with selection has often involved using genomic patterns of $F_{ST}$ – a measure of the relative difference in allele frequencies between populations. However, since the mechanisms underlying differentiation between populations can be complex, contrasting different measures of genetic diversity can be helpful for inferring differing modes of divergence between populations (Delmore et al., 2018; Irwin et al., 2018; Osmond & Coop, 2020), and a range of diversity statistics can be used (Reviewed in Wolf & Ellegren, 2017), as well as individual-based modelling to disentangle neutral and selective drivers of divergence (Ali et al., 2023).

Island populations of Berthelot's pipit (*Anthus berthelotii*) and its mainland sister species, the tawny pipit (*Anthus campestris*), provide an excellent system to explore genomic patterns of divergence and speciation across divergence timescales and known colonisation events (Armstrong et al., 2018; Illera et al., 2007; Martin et al., 2023;

Spurgin et al., 2014). The ancestor of these two species colonised the Canary Islands from mainland Africa ca. 2.1 million years ago (Mya) and dispersed independently to both the Madeiran and Selvagens archipelagos ca. 50 thousand years ago (kya) and 8 kya respectively (Martin et al., 2023). Previous research suggests colonisation is associated with reduced genetic diversity through founder effects and an absence of post-colonisation gene flow across the Berthelot's pipit range (Gonzalez-Quevedo et al., 2015; Spurgin et al., 2014). This is confirmed by signatures of inbreeding – runs of homozygosity (ROH) > 1 Mb – across the recently colonised archipelagos (Martin et al., 2023). Across the species' range, strong genetic structure exists between, but not within, Berthelot's pipit populations at the archipelago level (Armstrong et al., 2018; Martin et al., 2021).

Importantly, across the Berthelot's pipit system there are strong selection gradients, including climate, habitat and pathogen regimes (Illera et al., 2016). For example, different populations have considerable, and temporally consistent, variation in the prevalence of avian pox and malaria (Illera et al., 2008; Spurgin et al., 2012), which has enabled previous studies of host–pathogen evolution (Gonzalez-Quevedo et al., 2014, 2015; Sheppard et al., 2022). Such pathogens can exert strong selective pressures on avian populations (Liao et al., 2017). There are also significant morphological differences across the system, with reduced body and bill size in Berthelot's pipit compared to the tawny pipit, and archipelago-level variation in bill morphology and body size in Berthelot's pipit (Armstrong et al., 2018; Spurgin et al., 2014). Berthelot's pipit is classified into two subspecies: *Anthus berthelotii berthelotii* inhabits the Canary Islands and Selvagens, while *Anthus berthelotii madeirensis*, which inhabits the Madeiran islands, is characterised by longer bill lengths (Arctander et al., 1996; Illera et al., 2007) and larger body size (Spurgin et al., 2014). The relative role of selection and founder effects in shaping divergence for this trait is currently not clear (Armstrong et al., 2018).

Reduced representation sequencing (RAD-seq) markers have previously been used to investigate selection across the Berthelot's pipit range, both at broad (Armstrong et al., 2018) and fine (Martin et al., 2021) geographical scales. The strongest signatures of selection were identified between archipelagos, compared to between island populations within archipelagos. Loci near to or within genes associated with immunity, metabolism and bill length were identified as being divergent. However, it is not clear to what extent patterns of diversity in these genomic regions are shaped by drift and selection, and it is likely many divergent loci in the genome have gone undetected as a result of unsequenced genomic regions.

To uncover loci of importance for divergence and adaptation, we use whole-genome resequencing to assess genomic landscapes of divergence across sequentially colonised archipelagos by the Berthelot's pipit and its ancestor. Our specific aims were: (1) to determine how divergence has accumulated across the genome between Berthelot's pipits and its mainland relative the tawny pipit (ca. 2.1 Mya), as well as between more recently divergent archipelago populations of Berthelot's pipit (colonised ca. 50 and 8 kya); (2) to identify genomic islands and valleys of divergence and (3) to understand how drift and selection have interacted to shape variation across these genomic regions. To generate hypotheses about potential adaptive phenotypes, we identify candidate genes in regions under selection.

## 2 | METHODS

### 2.1 | Avian sampling, genome resequencing and variant calling

Berthelot's pipit was sampled across the three Macaronesian archipelagos of its range, to which it is endemic (the Canary, Madeiran and Selvagens archipelagos; see Figure 1). These islands have formed as a result of volcanic activity 1–26 million years ago (Florencio et al., 2021) and support a range of habitats across which the Berthelot's pipit is relatively abundant (Garcia-del-rey & Cresswell, 2007). We used Berthelot's pipit blood samples ($n = 11$) from individuals across six island populations (spanning the three archipelagos) to maximise the geographical range of samples and to account for differences in habitat, pathogen exposure and demographic history variation across the species (Table 1). One male and female sample was selected for each population, and all individuals had no detected malaria infection when screened for *Haemoproteus* and *Plasmodium* spp. or evidence of pox lesions (Illera et al., 2008). In addition, we sequenced one sample from the Berthelot's pipit mainland sister species, the tawny pipit (*Anthus campestris*, sampled Latitude: 17.991703°, Longitude: – 146 16.016672°). This allowed a range of divergence levels and colonisation timeframes to be compared within Berthelot's pipit and its sister species (Table 1). Blood samples were stored in absolute ethanol (800 μL) at 4°C and DNA was extracted using a salt extraction protocol (Richardson et al., 2001), and individuals were molecularly sexed (Griffiths et al., 1998).

We used a draft Berthelot's pipit reference genome assembly, generated by Armstrong et al. (2018), to align genome-wide sequence reads and to call genomic variants (see below). This assembly was generated using a male bird from Porto Santo in the Madeiran archipelago and was selected based on having low genome-wide heterozygosity as determined by previous RAD-seq analyses (Armstrong et al., 2018). This genome was assembled de novo from paired-end Illumina reads (2 × 125 bp on HiSeq 2500 sequencer) using DISCOVAR (Weisenfeld et al., 2014). The resulting draft assembly has a total size of 1.15 Gb (94.3% of the length of the Zebra finch genome), with a BUSCO score (Simão et al., 2015) of 64% (vertebrate-specific single-copy orthologues) and contig N50 of 355 kb. For full details see Armstrong et al. (2018).

To perform whole-genome resequencing and variant calling against the Berthelot's pipit genome assembly, low input transposase-enabled libraries were sequenced across four lanes using paired-end Illumina sequencing on a HiSeq 4000 (2 × 150 bp), generating high genome read coverage (>20× per individual; Table S1). Raw reads were merged at the individual level, trimmed (Phred quality score > Q30) and aligned to the draft Berthelot's pipit genome assembly using BWA-mem v0.7.12 (Li, 2013). Potential duplicate reads

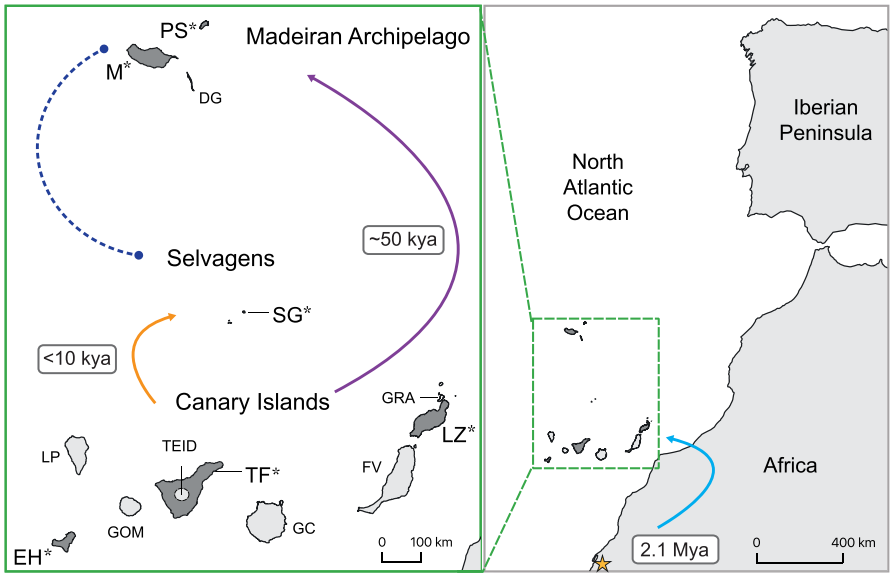


**FIGURE 1** Map of the island endemic Berthelot's pipit populations across its Macaronesian range and its ancestor, the tawny pipit, sampling location in Mauritania (see star). Populations included in this study are denoted with an asterisk and the island shaded dark grey. Solid lines with arrows indicate population comparisons, with the timing and direction of colonisation events highlighted (Martin et al., 2023). The blue dotted line indicates comparison between Selvagens and Madeiran populations after these were colonised in two independent events from the Canary Islands. Canary Island populations: El Hierro (EH), La Palma (LP), La Gomera (GOM), El Teide (TEID) mountain population on Tenerife (>2000 m a.s.l.), Tenerife (TF), Gran Canaria (GC), Fuerteventura (FV), Lanzarote (LZ), La Graciosa (GRA). Madeiran populations: Madeira (M), Porto Santo (PS) and Deserta Grande (DG). Selvagens: Selvagem Grande (SG).

**TABLE 1** Sampling information for divergence comparisons across populations of Berthelot's and tawny pipit.

| Divergence comparison | Population locations | Estimated divergence timeframe | Bottleneck severity | *n* retained loci |
|---|---|---|---|---|
| Tawny pipit, sister species/Canary Islands | Mauritania, Mainland Africa/LZ, TF & EH | 2.1 Mya[a] | Weak. Genome-wide reduction in diversity | 10,829,660 |
| Canary Islands/Madeiran archipelago | LZ, TF & EH/M & PS | 50 kya[a] | Strong. Founder effect, contemporary population recovery | 5,590,607 |
| Canary Islands/Selvagens archipelago | LZ, TF & EH/SG | 8–10 kya[a] | Strong. Founder effect, contemporary inbreeding | 5,266,205 |
| Madeiran archipelago/Selvagens archipelago | M & PS/SG | 50 kya[a] | Very strong. Two independent bottlenecks | 3,733,990 |

*Note*: Population codes from: (1) Canary Islands: Lanzarote (LZ), Tenerife lowland (TF), El Hierro (EH); (2) Madeiran archipelago: Madeira (M), Porto Santo (PS) and (3) Selvagens archipelago: Selvagem Grande (SG). *n* retained loci = autosomal and Z mapped loci retained for each population comparison.

[a]Divergence timeframes from Martin et al. (2023).

were then marked using Picard tools *MarkDuplicates* in GATK v4.1 (McKenna et al., 2010), and binary alignment files (.bam) were validated prior to variant calling. GATK *HaplotypeCaller* was then used in GVCF mode to call genomic variants, with the Berthelot's pipit draft genome assembly using as a reference to call variants against. Unmapped or poor-quality reads (with a root-mean-squared read mapping quality < 25) were discarded. To account for errors in read mapping, variants were then filtered for read strand bias (Fisher's exact test > 60 and Strand Odds Ratio > 3) and quality by depth (QD < 2) using GATK. Joint genotyping was then performed across samples, to reduce call error rate, using GATK's *GenomicsDBImport* and *GenotypeGCVF* tools. Contig-level Variant Call Format (VCF) files were combined using GATK *SortVcf*, with variants mapped to contigs less than 500 bp removed.

To allow chromosome-level genomic divergence to be assessed and visualised, genomic variants were mapped to relative chromosome positions of the Zebra finch (*Taeniopygia guttata*) genome assembly bTaeGut1_v1.p (NCBI Assembly GCA_003957565.1) (Warren et al., 2010) using the SatsumaSynteny module within Satsuma (Grabherr et al., 2010), which performs well on fragmented genome assemblies (Liu et al., 2018). Since our draft Berthelot's pipit reference genome is a highly fragmented

assembly, this collinearity mapping allowed pseudochromosome locations to be determined which could be used for assessing genomic landscapes of divergence and to make use of gene identities in the well annotated Zebra finch genome. To visually assess the collinearity of the Berthelot's pipit and Zebra finch genome assemblies, we used the D-GENIES dot-plot tool (Cabanettes & Klopp, 2018) with the default options. This showed that high synteny exists between these genomes, although percentage identity is relatively low for most contigs (25%–75%). Despite this, only a very few short regions of the Berthelot's pipit genome are misassembled/misplaced, many of which are on the only assembled sex chromosome (Figure S1). Satsuma determines the order, location and orientation of Berthelot's pipit contigs relative to Zebra finch chromosomes. This output was used to reassign contig locations of genomic variants to pseudochromosome locations using custom R scripts (RStudio Team, 2016).

The VCF files were generated to assess (1) divergence through speciation between the tawny and Berthelot's pipit and (2) divergence among Berthelot's pipit populations across archipelagos. For the first dataset 'All Pipits' variants were joint called across the Berthelot's and tawny pipit individuals. For the second dataset 'Berthelot's' variants were joint called across only the Berthelot's pipit individuals. Both datasets were filtered in VCFtools v0.1.15 (Danecek et al., 2011); removing unmapped sites (--not-chr 0), indels (--remove-indels), sites with >2 alleles (--max-alleles 2), sites with more than four failed genotype calls (--max-missing-count 4) and variants with accuracy <99.9% (--minQ 30). Sites were further filtered to remove those at which the mean read depth < 10 or more than twice genome-wide average (Li, 2014) (>45 for 'All Pipits', >44 for 'Berthelot's') (--min-meanDP 10, max-meanDP 45/44). Quality and coverage statistics for both datasets are provided in Table S1.

## 2.2 | Population comparisons

We compared pairwise divergence between the tawny and Berthelot's pipit, and then among three dyads of Berthelot's pipit archipelago populations (Table 1, Figure 1). The dyads chosen for comparison varied in their temporal, spatial and morphological divergence, and in terms of selective pressures as outlined below:

### 2.2.1 | Tawny pipit versus Canary Islands

The ancestor of Berthelot's pipit initially colonised the Canary Islands ca. 2.1 Mya (Martin et al., 2023; Voelker, 1999) with founder effects resulting in a genome-wide reduction in genetic diversity compared to the tawny pipit (its sister species). There is no evidence of subsequent gene flow. The tawny pipit is a Palearctic migrant. After ending the breading season Western European populations winter mainly in sub-Saharan Africa, while breeding Asian populations winter in the Arabian Peninsula and Southwest Asia (Tyler & Christie, 2020). In contrast, Berthelot's pipit is an island resident. Habitat types and disease prevalence varies substantially across the Berthelot's pipit Canary Islands range. Both species are exposed to avian malaria, avian pox and other pathogens (tawny pipit; Calero-Riestra & Garcia, 2016, Berthelot's pipit; Illera et al., 2008).

### 2.2.2 | Canary Islands versus Madeiran archipelago

Colonisation of the Madeiran archipelago from the Canary Islands by Berthelot's pipit is estimated to have occurred ca. 50 kya (Table 1, Figure 1; Martin et al., 2023), and resulted in a strong population bottleneck. Berthelot's pipit from the Madeiran archipelago are classified as a separate subspecies *A. berthelotii madeirensis* (Hartert, 1905). The Madeiran populations show the longest bill lengths (Illera et al., 2007) and larger body size (Spurgin et al., 2014). Considerable variation in disease prevalence occurs within and among these two islands archipelagos (Illera et al., 2008).

### 2.2.3 | Canary Islands versus Selvagens archipelago

The Selvagens was populated by Berthelot's pipit through colonisation from the Canary Islands ca. 8–10 kya (Table 1, Figure 1; Martin et al., 2023). Small island size (ca. 3 $km^2$), geographic isolation and strong founder effects have resulted in low genetic diversity in the Selvagens' population and strong signatures of inbreeding (Martin et al., 2023; Spurgin et al., 2014). While there is considerable variation in disease prevalence across the Canary Islands, no disease has been detected on the Selvagens (Illera et al., 2008).

### 2.2.4 | Madeiran archipelago versus Selvagens archipelago

Berthelot's pipit populations across these two archipelagos are separated by ca. 50 thousand years and two independent bottleneck events, with no evidence of post-colonisation gene flow between the archipelagos (Illera et al., 2007; Martin et al., 2021; Spurgin et al., 2014). While Berthelot's pipit within the Selvagens are not infected with avian pox or malaria, there are strong differences in disease prevalence between islands within the Madeiran archipelago (Illera et al., 2008).

## 2.3 | Differentiation landscapes and genomic islands

We calculated pairwise $F_{ST}$ between the tawny pipit and the Canary Island Berthelot's pipit population (one comparison) using the 'All Pipits' dataset, and between the three Berthelot's pipit archipelago populations using the 'Berthelot's' dataset in VCFtools (Table S1). Variation across the genome was visualised using Manhattan plots.

To identify highly divergent genomic regions between population comparisons, pairwise $F_{ST}$ values were *Z*-transformed. Since population history shapes genetic variation between populations, baseline levels of divergence vary significantly between each of the population comparisons (see Section 3). Within each comparison, we classified windows as divergent if their mean $F_{ST}$ was more than five standard deviations greater than the genome-wide mean ($_ZF_{ST} > 5$) and in the top 1% of SNP windows, which is a conservative approach to identifying outliers (Choi et al., 2020; Han et al., 1999; Lamichhaney et al., 2015; Walsh et al., 2019). For each population comparison, $F_{ST}$ was calculated in 50 kb non-overlapping windows. Genomic linkage typically extends 25–35 kb in the three archipelago populations of Berthelot's pipit (Martin et al., 2021). We used a 50-kb window for genomic island detection because it provided sufficiently fine resolution across the genome while representing 184–532 sites per window. Finally, windows containing <30 sites were removed prior to conducting analysis (~1% of windows). We considered the Z chromosome separately due to known differences in evolutionary pressures across sex chromosomes (Ellegren, 2011), and poor pseudochromosome assembly quality (Figure S1). It is also important to note that the exact number of genomic islands of divergence per chromosome may differ if there was a chromosome-level pipit assembly.

By investigating genomic variation across the tawny pipit and Berthelot's pipit speciation event, it was also possible to identify highly conserved (low divergence) genomic regions which may have a role in parallel adaptation between the species (Van Doren et al., 2017). To do this we applied the same $_ZF_{ST}$ approach, instead identifying windows with mean $F_{ST}$ less than 5 standard deviations below the genome-wide mean.

## 2.4 | Detailed characterisation of variation in divergence peaks

To identify regions putatively under selection in elevated regions of differentiation, we compared values of Tajima's *D* and nucleotide diversity ($\pi$) in and outside of outlier windows (i.e. assessing whether regions of elevated differentiation had corresponding dips in Tajima's *D* and $\pi$). We calculate 50 kb-windowed Tajima's *D* and $\pi$ using VCFtools, for each population dyad to allow direct comparison. Tajima's *D* can be used to test for deviations from neutral evolution (values near or equal to zero), with positive values indicative of balancing selection or sudden population contraction, and negative values indicative of a recent selective sweep or population expansion following a recent bottleneck (Tajima, 1989). Nucleotide diversity ($\pi$) is defined as the number of nucleotide differences per site between sequences within a population. Estimating $\pi$ across the genome may reveal population-level diversity within genomic regions, which can be used together with Tajima's *D* to make inferences about potential evolutionary forces acting within regions of interest.

To identify genes located within divergent windows, we viewed 50 kb regions of interest using the Zebra finch genome (v. bTaeGut1_v1.p) in NCBI Genome Data Viewer v. 4.8. (www.ncbi.nlm.nih.gov/genome/gdv/browser). Patterns of divergence across peak regions were assessed across the three archipelago dyadic comparisons. Where several windows exceeded this threshold within a genomic island, we assessed the distribution of $F_{ST}$ within peaks, and where appropriate highlight the most likely candidate genes under selection.

## 2.5 | Simulations of divergence

We performed individual-based simulations of genomic divergence, under neutral and selective processes using the 'glads' R package (Quilodrán et al., 2020). These simulations consequently provide a comparison of neutral and non-neutral divergence patterns, which we used to compare to our empirical genome-wide $F_{ST}$ distributions.

The glads model, with an additive genotype–phenotype map, simulated the divergence of two populations – parameterised to represent the demography and colonisation history of Berthelot's pipit in the Canary Islands and Selvagens archipelagos. Each population comprised of 300 biallelic SNPs, with 50 loci under selection and the remainder neutral (i.e. did not contribute to the additive phenotype, but were influenced by neutral evolutionary processes such as drift and recombination). Simulations excluded post-divergence gene flow (Martin et al., 2021), with population sizes set at 4000 and 400 diploid individuals for the Canary Islands and Selvagens respectively (Spurgin et al., 2014). A recombination rate of 3 cM/MB was used, based on the genome-wide average of the collared flycatcher (*Ficedula albicollis*) for a 75-Mb chromosome. Mutation and migration were not included in the simulation. Assuming a divergence time of 8000 years and a generation time of 2.2 years (Martin et al., 2023), 3636 generations were simulated. The simulation was repeated independently 100 times for both parallel and divergent selection, maintaining the same starting parameters, and ensuring distinct genetic identities for individuals (i.e. genotype for the 300 loci) at the onset of each independent simulation.

# 3 | RESULTS

## 3.1 | Whole-genome resequencing

Illumina sequencing resulted in 1,030,115,042 paired-end reads, providing mean individual read coverage of 23.6 X ± 2.6 s.d. when mapped to the Zebra finch's 1.1 Gb genome (Warren et al., 2010). Variants were called, mapped to the Zebra finch assembly and quality filtered, resulting in 11,788,225 mapped SNPs in the 'All Pipits' dataset and 5,934,934 in the 'Berthelot's' dataset. Individuals had low levels of missing data (<5%). Final depth of coverage for the quality filtered SNPs was high (Jiang, Jiang, et al., 2019; Sims et al., 2014),

with a mean 24.3 X for the 'Berthelot's' dataset and 24.7 X for the 'All Pipits' dataset (Table S1).

### 3.2 | Distributions of $F_{ST}$ between comparisons

Autosomal mean $F_{ST}$ was high between the tawny and Berthelot's pipit (Figure 2; Martin et al., 2023) but low between Berthelot's pipit archipelago populations separated by one colonisation event (i.e. Canary Islands vs. Madeira, Canary Islands vs. Selvagens). The distribution of $F_{ST}$ was positively skewed between the Canary Islands and both subsequently colonised archipelagos (Madeira and Selvagens) but approaches a normal distribution between the tawny and Canary Islands Berthelot's pipit. Between single colonisation events, standard deviations of windowed $F_{ST}$ values were low, ranging 0.041–0.070. The spread of divergence scores was much greater between the Selvagens and Madeiran archipelago (s.d. = 0.139), which are separated by two independent colonisation events (Figure 2).

Like the autosomes, the Z chromosome showed increasing divergence over longer timeframes between single colonisation events, with highest divergence between the tawny pipit and Berthelot's pipit and lowest between the most recently separated Berthelot's pipit archipelago populations (i.e. the Canary Islands and Selvagens; Figure S2a). Between the tawny and Berthelot's pipit, mean $F_{ST}$ divergence across the Z chromosome exceed that observed across the autosomes, while the converse was found across Berthelot's pipit archipelago populations (Table 2).

### 3.3 | Overall correlations between genomic landscapes of divergence

Divergence at genomic loci in tawny-Berthelot's pipit comparisons was significantly but weakly correlated with divergence at these loci between Berthelot's archipelago comparisons (Figure 3 and Figure S3, Pearson's correlation: $r = .02–.15$, $p < .01$). As expected,

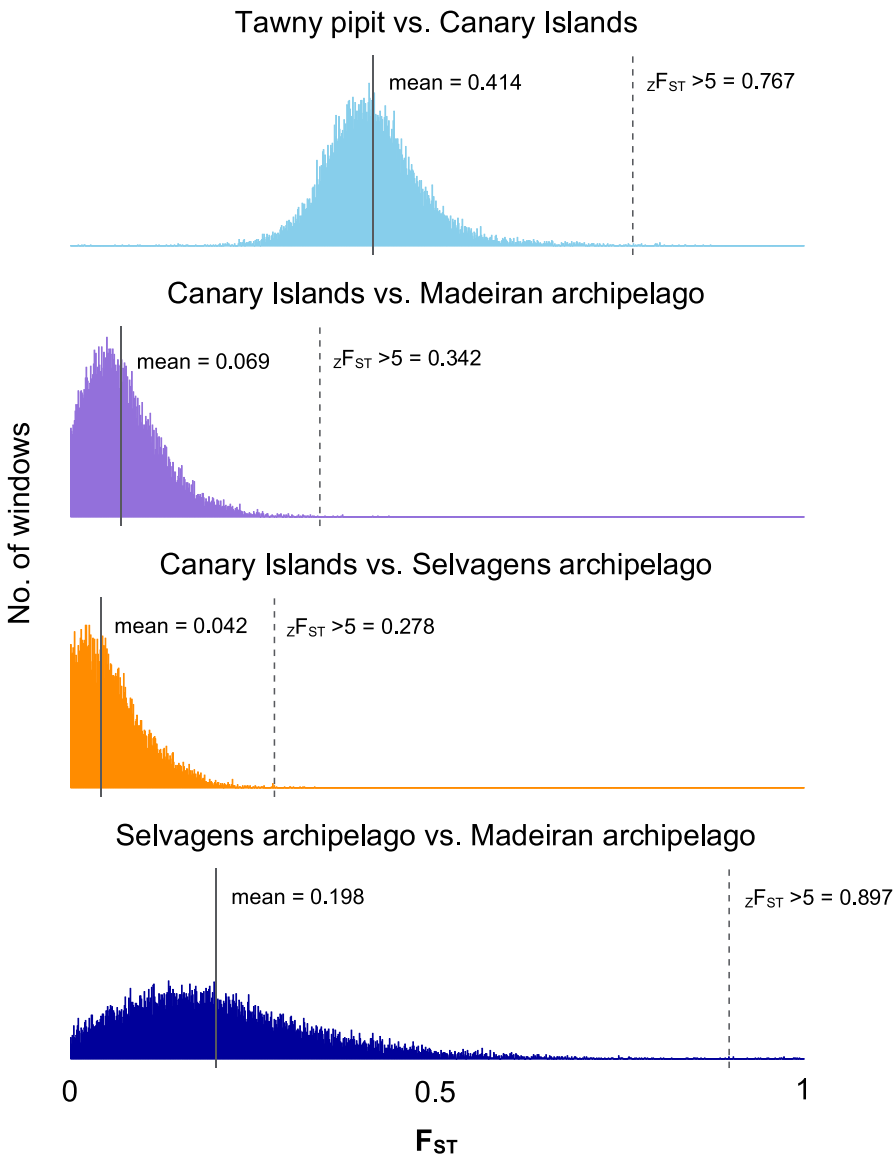


FIGURE 2 Distribution of pairwise genomic differentiation, $F_{ST}$, across Berthelot's and tawny pipit comparisons calculated in 50 kb autosomal windows. Positions of means (solid line) and $_zF_{ST}>5$ threshold (dotted black line) are highlighted.

TABLE 2 Genomic differentiation, $F_{ST}$, between Berthelot's pipit and tawny pipit, and among Berthelot's pipit archipelagos.

| Comparison | Autosomal mean $F_{ST}$ | Z Chr mean $F_{ST}$ | Autosomal $_{Z}F_{ST} > 5$ | Top 1% SNPs | *n* windows $_{Z}F_{ST} > 5$ |
|---|---|---|---|---|---|
| Tawny pipit, sister species/Canary Islands | 0.414 | 0.427 | 0.767 | 0.637 | 27 |
| Canary Islands/Madeiran archipelago | 0.069 | 0.063 | 0.342 | 0.229 | 9 |
| Canary Islands/Selvagens archipelago | 0.042 | 0.034 | 0.278 | 0.186 | 10 |
| Selvagens archipelago/Madeiran archipelago | 0.198 | 0.171 | 0.897 | 0.609 | 22 |

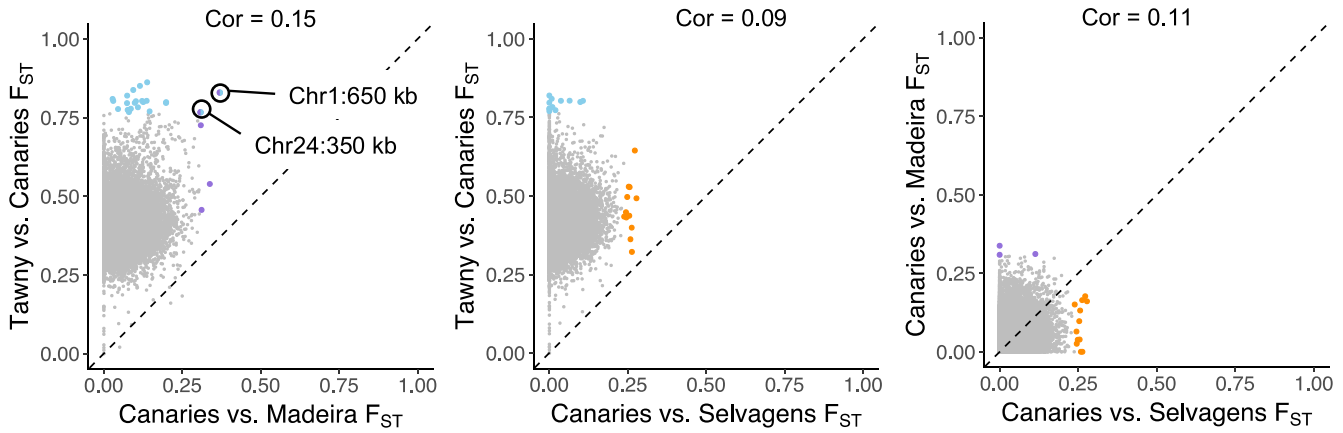


FIGURE 3 Correlated patterns of $F_{ST}$ between Berthelot's pipit population comparisons, and between Berthelot's and tawny pipit populations separated by single colonisation events. $F_{ST}$ was calculated in 50 kb non-overlapping windows, outliers ($_{Z}F_{ST} > 5$) are highlighted; Tawny versus Canary Islands = light blue, Canary Islands versus Madeira = purple, Canary Islands versus Selvagens = orange. The only two outlier windows which occur in multiple population comparisons are circled and the chromosomal positions identified.

the divergence of loci between dyadic comparisons was more strongly correlated when those comparisons involved a common population (because of the characteristics of loci in that common population) and not because of independently reoccurring divergence of the same loci. The highest correlation explains 34.8% of variation in dyadic $F_{ST}$ scores (Pearson's correlation: $r = .59$, $p < 2.2 \times 10^{-6}$, Canary Islands vs. Madeira compared to Selvagens vs. Madeira).

## 3.4 | Identification of peaks and valleys and correlations between population comparisons

The genomic landscape of divergence between the tawny pipit and the Canary Islands Berthelot's pipit population was broadly homogeneous across the genome, with 27 windows >5 s.d. from the genomic autosomal mean ($F_{ST} > 0.41$) (Figure 4, Table 2). The distribution of these areas of elevated divergence was non-random with 12 well defined clusters of high $F_{ST}$, seven of which exceeded the criteria to be considered 'divergence peaks' or 'genomic islands of divergence' (Figure 4, Table S2). Peak size was in the range of 0.05–2 Mb (Table S2) and such divergence peaks covered 0.14% of the genome. Across the Z chromosome, two broad peaks (1–2 Mb) of strong divergence are also observed between the tawny and Canary Island Berthelot's pipit, although we stress that these patterns may be as a consequence of poor contig mapping on this chromosome. The pattern of divergence within the different genomic islands fell into two categories: (i) most commonly, regions of sharp divergence usually include one or two peaks where a "top peak" with the highest associated $F_{ST}$ could be identified or (ii) a broad peak of similar $F_{ST}$ divergence across the island (see Figure 5). Only one such broad peak of divergence was identified approximately 2 Mb in length on chromosome 1A (Figure 5a), with the strongest $F_{ST}$ divergence scores across the genome.

FIGURE 4 Pairwise genomic differentiation, $F_{ST}$, across the genome for Berthelot's and tawny pipit comparisons calculated in non-overlapping 50 kb windows. Genomic islands of divergence or valleys of similarity, where $F_{ST}$ is 5 standard deviations greater than, or less than, the mean window value, are highlighted (indicated by the dashed horizontal line). Pseudochromosomes (derived by comparison with chromosomes of the Zebra finch genome) are shown in alternating light and dark shading. Vertical coloured bars indicate the location of genomic islands within each population comparison. Labelled arrows indicate the location of shared genomic islands and the genomic distance between closely located peak windows across population comparisons. Population comparison highlights: light blue = Tawny versus Canary Islands, Canary Islands versus Madeiran islands = purple, Canary Islands versus Selvagens = orange and Selvagens versus Madeira = navy.


Autosomal divergence between Berthelot's pipit archipelagos showed different patterns. Archipelago comparisons with just one founding step separating them (Canary Islands vs. Madeira and Canary Islands vs. Selvagens) were characterised by low genome-wide divergence with few strongly differentiated 'islands of divergence' (Figures 2 and 4). In contrast, the divergence landscape was

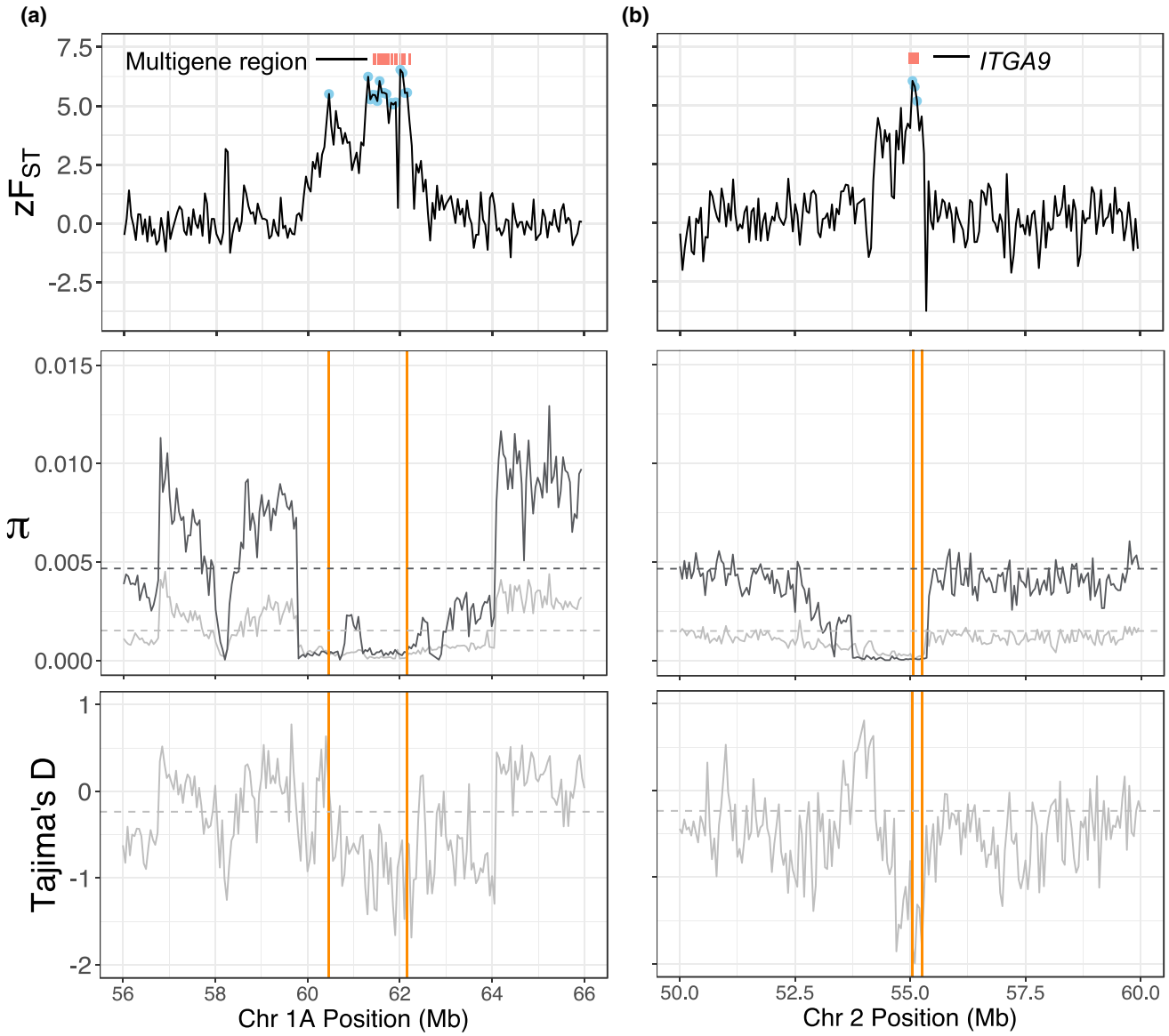


FIGURE 5 Patterns of divergence ($_ZF_{ST}$), genetic diversity ($\pi$) and a measure of the loss of rare alleles (Tajima's *D*) surrounding two genomic islands of divergence between the tawny pipit and Canary Islands Berthelot's pipit comparison. These regions are presented as examples to demonstrate (a) a broad region, or 'plateau' of elevated divergence and (b) a narrow peak of divergence. Values were calculated in 50 kb windows, with $_ZF_{ST}>5$ highlighted in the first panel in light blue, with corresponding genomic locations of the peak start and end indicated by vertical orange lines in panel 2 and 3. Candidate gene locations within peaks are indicated; details in Table S2. Tajima's *D* and $\pi$ are reported for the Canary Islands (light grey) and the corresponding $\pi$ indicated for the tawny pipit (dark grey), with autosomal averages indicated by horizontal dotted lines.

highly heterogenous when the two independently bottlenecked archipelagos, Selvagens versus Madeira were compared. We identified 9–22 genomic islands of divergence in each Berthelot's pipit archipelago comparison (Figure 4, Table 2), which represented 0.05%–0.1% of 50 kb windows. In contrast to the tawny versus Berthelot's pipit comparison, Z chromosome divergence was consistently (but marginally) lower than autosomal divergence between Berthelot's pipit archipelagos comparisons (Table 2), and no strongly divergent genomic windows were identified.

The position of divergent windows varied between population comparisons, with only two shared windows identified between multiple population comparisons (Figure 3). These two windows, mapped to regions of chromosome 1 and 24, and were shared between the tawny pipit versus Canary Island Berthelot's pipit population comparison and between Canary Island Berthelot's pipit versus Madeiran archipelago comparison. No windows of strong divergence were shared between the different Berthelot's pipit comparisons. Between the tawny and Berthelot's pipit, the majority of the genome exhibited strong divergence with the highest window having $F_{ST}=0.87$, and only two windows where $F_{ST}$ was zero (Figures 2 and 4). However, we identified seven genomic regions with low divergence, more than five standard deviations

below the genome-wide mean, which mapped to five distinct chromosome regions (Figure 4). Between Berthelot's pipit archipelagos comparisons many 50 kb regions of the genome were not divergent ($F_{ST}=0$, Figure 4).

## 3.5 | Genes in peaks and valleys and patterns of diversity

The identified regions of elevated divergence between the tawny and Berthelot's pipit consistently exhibit corresponding dips in Tajima's *D* and $\pi$ in the Canary Islands Berthelot's pipit population, indicative of selective sweeps. Patterns of reduced Tajima's *D* and $\pi$, and elevated $F_{ST}$ were consistent across the broad peak on chromosome 1A, suggesting strong linkage disequilibrium in this region, while the other strongly divergent peaks were narrow in width. Across all strongly divergent regions, six of seven peaks harboured protein-coding sequence of which five regions had named candidate genes (Table S2).

A high number of divergence windows were located within the extended genomic island mapped to chromosome 1A (17 of 27 windows identified between the tawny and Berthelot's pipits). This region contained 17 annotated genes, of which at least seven are associated with immune response, three with craniofacial development and two with metabolism (see Table S2). The single most strongly divergent peak in the region included two genes: *CMAS* associated with the innate immune response (O'day et al., 2018; Urbanek et al., 2020) and *ABCC9*, associated with cartilage and bone development (Czeschik et al., 2013) (Table S2).

To further investigate divergence across this broad region on chromosome 1A, we investigated patterns of divergence between the tawny pipit and all Berthelot's pipit populations, and among Berthelot's pipit populations. The results confirm (i) divergence of this region occurs between the tawny pipit and all Berthelot's pipit archipelagos and (ii) this region exhibits low divergence between Berthelot's pipit archipelagos (Figure S4). The other narrow regions (<150 kb) of strong divergence between the tawny pipit and Berthelot's pipit mapped to genomic regions including genes putatively associated with immune response and wound healing (Cui et al., 2017; Sigurðarson, 2020), development of the retina (Xu et al., 2020) and carbohydrate metabolism (Han et al., 1999) (Table S2).

Across the Z chromosome, two broad peaks (1–2 Mb) of high divergence occur between the tawny pipit and Canary Islands Berthelot's pipits. These have corresponding dips in nucleotide diversity in both populations (Figure S2b), which suggests they may be under divergent selection. Identified genes within the top 50 kb divergent window in both peaks, both have strong association with hearing: *ADGRV1* is associated with hearing loss and retina development (Yan et al., 2018); and *PPIP5K2* regulates hearing through growth and maintenance of sensory cells in the inner ear (Yousaf et al., 2018). Nucleotide diversity across the Z chromosome shows a consistent peak across all populations of high diversity at ~45 Mb, likely an artefact of incorrect mapping of W chromosome reads to the Z in female individuals.

Strongly divergent regions between archipelago populations of Berthelot's pipit had varied patterns of $\pi$ and Tajima's *D* across different populations, indicating a range of evolutionary processes occurring within these regions (Table S3). Across the Canary Islands versus Selvagens comparison, several highly divergent regions mapped to genomic locations within previously identified (Martin et al., 2023) long ROH > 1 Mb in the Selvagens individuals (and negative Tajima's *D*). Across the strongly divergent regions for the Canary Islands versus Madeiran archipelago comparison, Tajima's *D* was consistently elevated within the Madeiran archipelago.

Genes in islands of divergence across the Berthelot's pipit range are associated with similar biological functions (Tables S2 and S3). Regions under putative selection within the Selvagens include genes associated with craniofacial shape, apoptosis and inflammation, development of the retina and teeth, metabolism, muscle and growth (Table S3). Within the Madeiran archipelago population regions potentially under selection include genes associated with facial, skin and bone development, immunity (innate immune response and regulation of B and T cells) and eye development.

## 3.6 | Genes with low divergence between species

Between the tawny and Berthelot's pipit, we identify seven low-divergence genomic regions including a total of 11 candidate genes, of which seven were annotated. These likely strongly conserved regions included genes putatively associated with pathogen infection, inflammation and platelet regeneration, growth factor pathways, and muscle and limb development (Table S4). For example, *NFX1* is a transcriptional repressor of major histocompatibility complex (MHC) class II genes (Gewin et al., 2004; Strominger et al., 1994) with biological function in immune and inflammatory response. We also identify a genomic valley with corresponding peaks of nucleotide diversity in Berthelot's pipit (and negative Tajima's *D*) and low diversity in the tawny pipit, putatively associated with recent population growth across the Canary Islands. This loci was within the genomic region containing *RANBP3* which is associated with growth factor pathways including bone morphogenic protein (BMP) signalling (Dai et al., 2009).

## 3.7 | Simulations of divergence

Individual-based modelling captured elevated $F_{ST}$ within genomic regions under both divergent and parallel selection relative to neutrally evolving genomic regions (Figure S5). Over the 100 independent simulations, $F_{ST}$ ranged from 0 to 1. The average level of $F_{ST}$ across the 100 independent simulations indicated a propensity for islands to form at selected loci (as shown by the higher average level of $F_{ST}$ at selected loci in contrast to neutral loci; 125–175 bp locus position) (Figure S5). Genomic divergence was observed most

strongly under divergent selection compared to parallel selection, with loci displaying less variation in $F_{ST}$ scores consistently across the 100 independent simulations.

## 4 | DISCUSSION

We find that divergence accumulates across the genome in association with increasing timeframes since population founding in an island colonising bird, confounded by the cumulative effects of drift though sequential founder events. In this study, we have shown that through population founding across archipelagos, genomic divergence accumulates in few loci and linked regions, but over time drift causes genome-wide loci to also become differentiated across the speciation continuum (from recently divergent Berthelot's pipit populations separated 8–50 kya to the reproductively isolated tawny pipit and Berthelot's pipit separated ca. 2.1 Mya). We find only weakly correlated patterns of genetic divergence and different strongly divergent loci between populations through different founder events (i.e. the same loci rarely show strong divergence across different events). However, we do find that the loci within these genomic islands of divergence are consistently (putatively) associated with the same ecologically important traits, that is, body/head size and immune defence, across the range (see Tables S2 and S3).

### 4.1 | Divergence across the speciation continuum

By comparing $F_{ST}$ frequency distributions between sequentially founded, geographically isolated populations of Berthelot's pipit, we show how genomic differentiation accumulates across timeframes in the absence of gene flow (Figure 2). Berthelot's pipit populations in the early stages of divergence (Canary Islands vs. Selvagens) after a recent colonisation event (<10 kya), have a positively skewed $F_{ST}$ frequency distribution. This includes a handful of strongly divergent genomic regions, which may differ due to differential selection or bottleneck effects on genetic drift. Where populations have been geographically separated for longer timescales (i.e. Canary Islands vs. Madeiran archipelago ca. 50 kya) leading to formation of subspecies, drift has had time to cause overall genomic divergence (Nosil et al., 2009). Over much longer divergence timeframes (i.e. across the speciation event between the tawny and Berthelot's pipit separated >2 Mya), divergence accumulates across the whole genome (mean $F_{ST}=0.41$), approximating a normal distribution with only a few weakly or strongly divergent regions. These patterns are in concordance with speciation models from early- to late-stage divergence with geographical isolation (Seehausen et al., 2014).

Across the Berthelot's pipit system, we can also compare genomic divergence between two independently bottlenecked populations, founded from the same source population (Canary Islands) at different time points (ca. 50 and 8 kya) (Figure 1). Here, the $F_{ST}$ frequency distribution has a greater spread, likely due to the combined consequences of two founder events and subsequent drift. Unlike the comparisons of populations separated by one colonisation event, in this scenario regions that have diverged due to natural selection are more difficult to identify due to high levels of overall genome-wide divergence. Our patterns of genomic divergence among the different Berthelot's pipit archipelago populations concur with comparisons of rapidly speciating island silvereye (*Zosterops lateralis*) populations separated by <200 years where Sendell-Price et al. (2021) only identified a few strongly divergent genomic regions. Between-species comparisons of *Ficedula* flycatchers (Burri et al., 2015) and *Saxicola* stonechats (Van Doren et al., 2017), with divergence times of several millions of years, showed similar normal distribution patterns to that which we observed between the tawny and Berthelot's pipit.

### 4.2 | Independent patterns of divergence through population founding

We identified weakly correlated patterns of $F_{ST}$ across genomes from the different Berthelot's pipit populations, and between Berthelot's pipit and its sister species, the tawny pipit (Figure 3 and Figure S2). That the same loci/regions are not divergent across population comparisons, suggests that long-term linked selection is not a major reason for divergence in the Berthelot's pipit. Instead, such divergent loci may be due to independent evolutionary responses to selection pressures (Munch et al., 2016). Our findings contrast with several studies of genomic landscapes across divergence timescales and through varied gene flow contexts (Burri et al., 2015; Renaut et al., 2013; Stankowski et al., 2019; Van Doren et al., 2017; Vijay et al., 2017), which show parallel patterns of divergence between geographically and morphologically distinct taxa. Instead, we find only weakly correlated $F_{ST}$ scores and different putatively selected genomic regions diverging (see below) through time and speciation events across the range (Figures 3 and 4). Why this is the case in Berthelot's pipit remains unknown, but it may be attributed to the stochastic nature of the founding process, as has been reported in, among others, island colonising silvereyes (Sendell-Price et al., 2021) and laboratory range expansion experiments of red flour beetle (*Tribolium castaneum*; Weiss-Lehman et al., 2019). Largely independent patterns of divergence between population pairs are also reported in a range of other systems, for example through parallel speciation of stick insects (*Timema cristinae*; Soria-Carrasco et al., 2014), sympatric environmental adaptation of flatfish species (Le Moan et al., 2019) and adaptive radiation in Lake Victoria *Pundamilia* cichlid fishes (Meier et al., 2018).

### 4.3 | Genomic islands of divergence and repeated selection for traits

Absence of secondary contact between the tawny and Berthelot's pipit for ca. 2.1 million years has resulted in considerable

genome-wide divergence. Across this speciation event, we identified seven weakly divergent (or conserved) and seven strongly divergent autosomal genomic regions (Figures 2 and 4). The most divergent region formed a broad ~2 Mb peak on chromosome 1A. We also identified two peaks of broad divergence across the Z chromosome (Figure S2), although this may be an artefact of poor-quality variant mapping on this chromosome (Figure S1). All other strongly divergent regions formed narrow sharp peaks. Through subsequent independent founder events from the Canary Islands to the Selvagens and Madeira, we identify 12 and five strongly divergent 50 kb windows, respectively, all mapped to autosomal chromosomes (Table S3).

Individual-based model simulations showed localised genomic islands of divergence forming around selected loci (Figure S5). Our models parameterised for the most recently divergent Berthelot's pipit populations, show that the observed empirical patterns can form under the model. Specifically, genomic islands of divergence can form under selection, as shown by the identified islands and their associated traits under divergent selection (Table S3). However, while simulations show that islands are more likely to form at selected loci, we also show that islands can form under neutral processes, and thus the simulations do not exclusively suggest selection as the explanation for our empirically observed genomic divergence. With an increased divergence timeframe, particularly in the absence of gene flow as simulated here, alternative alleles and genotypic combinations are likely to become more strongly divergent or fix as a consequence of drift.

Signatures of reduced Tajima's D and nucleotide diversity (in the tawny and Berthelot's pipit) were found in all the strongly divergent genomic regions identified across the speciation event between the tawny and Berthelot's pipit (Fay & Wu, 2000), indicative of selective sweeps after the species split. Another explanation for this pattern could be that Berthelot's pipit genomes have accumulated a higher number of slightly deleterious mutations through mainland to island colonisation compared to the tawny pipit (Leroy et al., 2021). Highly divergent genomic regions between the Canary Islands and Selvagens also consistently exhibited a strong decrease in Tajima's D within the Selvagens, commonly associated with large regions of low diversity or ROH. Negative Tajima's *D* can result from either recent selective sweeps or population expansion following a bottleneck (Tajima, 1989). In the case of the Selvagens pipit population we have strong evidence of a very small contemporary $N_e$ with considerable inbreeding (Martin et al., 2023). Consequently, it seems likely that the genomic islands of divergence observed result from selective sweeps, but we cannot rule out the impact of accumulation of deleterious mutations. Such patterns of selection have previously been reported in other wild populations despite small $N_e$ (de Jong et al., 2020) and short divergence timescales (Walsh et al., 2019). This pattern was not observed between other archipelago populations of Berthelot's pipit (Table S3). Genomic islands of divergence between the Canary Islands and Madeiran archipelago population were associated with increased Tajima's *D* values in Madeira which may result from balancing selection or sudden population contractions. Windows with corresponding moderate levels of genetic diversity may indicate balancing selection is driving divergence within these regions (Lindtke et al., 2017; Tetteh et al., 2009), as inbreeding effects due to a population contraction would be expected to result in low diversity regions.

We detected a potential ~2 Mb 'plateau' of divergence between the tawny and Berthelot's pipit, which is conserved across the Berthelot's pipit range (Figure S4). Despite the absence of gene flow between the two sister species it is still surprising to detect such a broad peak of elevated divergence with low genetic diversity in both populations, as this suggests a long-term absence of recombination in this region. Large-scale valleys of recombination commonly coincide with centromeres (Burri et al., 2015; Carneiro et al., 2009), although it is possible that this is as a result of an inversion previous to the speciation event (Kirkpatrick, 2010; Rieseberg, 2001; Sanchez-Donoso et al., 2022). However, lack of recombination maps for Berthelot's pipit or closely related species means we are unable to test this hypothesis here, although long-read sequencing (Shao et al., 2018) in combination with haplotype phasing (Alachiotis et al., 2012; Ferrer-Admetlla et al., 2014) could be utilised to explore this in future studies. The broad 1A divergence peak identified here mapped to a gene dense region with 17 genes, many of which are associated with craniofacial/bone development, migration strategies or the immune response (Table S2). For example, *CMAS*, *TTLL12* and *YBX3* are all associated with viral defence (Carette et al., 2009; Ju et al., 2017; Qin et al., 2020); *TSPO*, *GEC1* and *STYK1* are associated with cellular autophagy or apoptosis (Chakrama et al., 2010; Veenman et al., 2007; Zhou et al., 2020); *KCNJ8* increases wound healing (Zhang & Bei, 2015); *ABCC9* is associated with craniofacial defects in vertebrates (Czeschik et al., 2013; Harakalova et al., 2012) and *SCUBE1* modulates BMP signalling during craniofacial development (Tu et al., 2008; Xavier et al., 2009) and is linked to head morphology adaptation in sticklebacks (Hohenlohe et al., 2010). Inversion haplotypes, and potentially the divergence peak on chromosome 1A, have been found to harbour supergene complexes and may be key facilitators for local adaptation and speciation (Kirkpatrick & Barton, 2006; Taylor & Campagna, 2016; for example, Christmas et al., 2019; Küpper et al., 2016; Porubsky et al., 2020; Roesti et al., 2015; Tuttle et al., 2016).

We identified positively selected candidate genes in Berthelot's pipit within regions exhibiting elevated differentiation that could be associated with transition from migration strategies in the tawny pipit to residency and limited dispersal in Berthelot's pipit (Table S2). For example, two genes within the broad divergence peak on chromosome 1A have previously been associated with migratory to residency transition between continental and island populations of the European blackcap (*Sylvia atricapilla*; Delmore et al., 2020); *GYS2*, which is transcriptionally activated by *CLOCK* which regulates circadian rhythms of hepatic glycogen synthesis (Doi et al., 2010). Circadian rhythms synchronise circannual (seasonal) clocks, which can regulate migratory timing and distance (Gwinner, 1996). The second gene is *TTLL12*, but the function it plays in regulation of these divergent strategies is poorly understood. A further gene in this broad divergence region, *TSPO*, is associated with metabolism through regulating mitochondrial function and glycolysis

(Veenman et al., 2007; Yao et al., 2020). We also detected another gene, *PDE1C*, in a different divergence peak on chromosome 2, associated with metabolic regulation (Lugnier, 2011) that may also be involved in this behavioural transition in the Berthelot's pipit.

Pathogen prevalence varies substantially (and consistently) between different Berthelot's pipit populations, and between those and the tawny pipit, providing a strong environmental selection gradient (Armstrong et al., 2018; Illera et al., 2008; Spurgin et al., 2012). Studies of Berthelot's pipit have previously identified pathogen-mediated selection associated with key immune genes including Toll-like receptor 4 (*TLR4*) (Armstrong et al., 2019; Gonzalez-Quevedo et al., 2015) and MHC class I genes (Gonzalez-Quevedo et al., 2016; Spurgin et al., 2011) within islands and across archipelagos (Armstrong et al., 2018). In the present study, we identified various genes in highly diverged regions that could be involved with host-pathogen evolution including: *ITGA9*, *SENP7* and *NFRKB* which are associated with the innate immune response (Audard et al., 2012; Cui et al., 2017; Sigurðarson, 2020); and *WAC* and *GEC1* which interact in the same pathway to regulate autophagy (Joachim et al., 2015). We did not, however, detect high levels of divergence in regions containing genes associated with pathogen response in comparisons involving the most recently founded Berthelot's pipit archipelago, the Selvagens. This is to be expected given the lack of known pathogens in this very isolated archipelago (Spurgin et al., 2012).

Variation in bill morphology across the species range has been largely attributed to founder effects across the archipelagos (Armstrong et al., 2018; Spurgin et al., 2014). However, our findings suggest that selection may also play a role in shaping craniofacial development through each colonisation event (Tables S2 and S3). Regions of the genome putatively under selection included: *CIT*, *NRXN1*, *PRDM10* and *WAC*, all associated with craniofacial defects in vertebrates (De Santo et al., 2015; Park & Kim, 2010; Shaheen et al., 2016; Zahir et al., 2008); *ABI3BP*, associated with bone development (Zhang et al., 2014); and *SOCS7* which interacts with growth factors (Elliott & Johnston, 2004). These genes may underlie variation in bill morphology that enhances exploitation of differing food resources across the range. Again, studies on foraging behaviour and trophic ecology are now needed to unravel the causes explaining such bill variation across archipelagos.

Our findings provide evidence of repeated divergence – potentially as a result of selection – for genes previously found to be involved in head/bill and body size, eye development, metabolism, wound healing and immune defence, across different temporal scales in Berthelot's pipit. However, as genomic islands of divergence rarely occurred at the same location across inter-population comparisons, it is possible that different genes with similar phenotypic effects may be responding to selection in different locations. Parallel adaptation for traits through different candidate genes has been reported across vertebrates (Langin et al., 2015; Milner et al., 1999; Walsh et al., 2019). Overall, our findings add to growing evidence of immunity and head/bill and body size as ecologically important traits for adaptive divergence and speciation (immune; Davies et al., 2021; Hughes & Yeager, 1998; Jarvi et al., 2001, and bill, Badyaev et al., 2008; Bosse et al., 2017; Dussex et al., 2021; Grant, 1968; Lundregan et al., 2018). Small numbers of individuals sampled per population, as used here, may lead to unrepresentative calculation of population-based statistics, especially between the Tawny pipit and Berthelot's pipit. We therefore caution readers against reliance upon the validity of specific candidate SNPs identified in this study (Tables S2–S4).

### 4.4 | Genomic valleys of divergence through speciation

Between the tawny and Berthelot's pipit, we detected very few low-divergence genomic regions despite speciation in allopatry; similar to observations of diverging *Ficedula* flycatcher species by Burri et al. (2015). Genomic valleys of divergence may be associated with reduced $F_{ST}$ and nucleotide diversity if they are due to parallel selection (Roesti et al., 2014). Alternatively, they may be associated with reduced $F_{ST}$ and moderate nucleotide diversity if they result from balancing selection (Hohenlohe et al., 2010). In our case, we have limited evidence to confirm either of these possibilities. If the genomic regions we have identified are under parallel/balancing selection in the tawny and Berthelot's pipit, this highlights the importance of a further set of genes associated with body and head size and immune response (Table S4). Interestingly, these candidate genes include *NFX1*, a transcriptional repressor of MHC class II genes (Gewin et al., 2004; Strominger et al., 1994), a gene family known to be under balancing selection in many vertebrates (Meyer & Thomson, 2001; Savage et al., 2020), including avian species (Alcaide et al., 2008; Brouwer et al., 2010; Ekblom et al., 2010). Evidence of the long-term retention of MHC alleles is a well-known phenomenon (Klein, 1987), and balancing selection over similar evolutionary timescales has been reported across populations of many taxa (Bryja et al., 2007; Evans et al., 2010; Herdegen-Radwan et al., 2021; Richardson & Westerdahl, 2003). In Berthelot's pipit, island colonisation is initially associated with reduced MHC diversity but there is evidence of rapid in situ generation of diversity via gene conversion (Spurgin et al., 2011).

## 5 | CONCLUSIONS

Island colonisation can dramatically influence genetic diversity within and divergence between populations through a combination of evolutionary neutral (i.e. drift) and selective forces. Disentangling the contributions of such processes is particularly complex in cases where there has been strong and/or sequential founder bottlenecks. Our results offer a population-level view of the divergence history of Berthelot's pipit along the speciation continuum, through differing divergence timeframes across three north Atlantic archipelagos. We observed different sets of strongly divergent loci through colonisation of new islands, potentially due to the stochastic nature of population founding. Despite these unique patterns of divergence across

archipelago populations, strongly divergent loci were putatively associated with the same biologically important traits (i.e. pathogen defence, head/body size), probably reflecting parallel adaptation across islands. Future studies encompassing multiple populations such as ours, including more detailed phenotyping and association mapping for a greater number of individuals, may be able to further clarify the relative contribution of genetic drift and selection in shaping the genomic landscape of divergence in wild populations.

## AUTHOR CONTRIBUTIONS

Claudia A. Martin, David S. Richardson and Lewis G. Spurgin designed the study, with input from Eleanor C. Sheppard and Alex Suh. Fieldwork was undertaken and permit permissions obtained by Lewis G. Spurgin, David S. Richardson and Juan Carlos Illera. Claudia A. Martin conducted the molecular laboratory work, developed the genetic data sets and performed data analysis, with critical input from Lewis G. Spurgin. Hisham A. A. Ali led the individual-based modelling with input from Claudia A. Martin. Claudia A. Martin drafted the manuscript with assistance from David S. Richardson, and all authors read and contributed to the draft and approved submission of this manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

## DATA AVAILABILITY STATEMENT

All data and code used to perform the data analysis within this manuscript are openly available on the Dryad Digital Repository: https://doi.org/10.5061/dryad.1g1jwsv4b (Martin et al., 2024).

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## SUPPORTING INFORMATION

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

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