INTRODUCTION

The establishment of a new population involves phases of founding and recovery leading to long-term persistence. During each stage, the random sampling effect of drift has the potential to affect the degree of genetic diversity and divergence exhibited by a population (Clegg et al., 2002). When populations are founded by few individuals, and especially when combined with longer population recovery times, there is the potential for loss of genetic variation—a "founder effect" (Nei et al., 1975; Wright, 1931). Loss of diversity has important implications on several fronts. For example, it may limit responses to new adaptive landscapes via loss of potentially adaptive
alleles (Lande & Barrowclough, 1987), or in a severe form could result in inbreeding depression (Charlesworth & Charlesworth, 1987) and contribute to population extinction (Frankham, 2005). The stochastic effects of population founding may also result in changes in the frequency of alleles, including loss of rare variants, increased frequency or fixation of rare variants and decreased frequency of previously common alleles, all of which can result in rapid population genetic divergence (Excoffier et al., 2008). The loss and rearrangement of genetic diversity following population founding also forms the basis of “founder-effect speciation” models (Barton & Charlesworth, 1984; Carson, 1968, 1975; Carson & Templeton, 1984; Matute, 2013; Mayr, 1959; Templeton, 1980, 1981, 1999). These models, which seek to explain rapid speciation as catalysed primarily by founding stochasticity, remain controversial, partly because it is unclear whether the founder effects upon which these models rely occur frequently in nature (Coyne & Orr, 2004). When a species experiences a new or substantially altered environment, such as following the colonization of an island, the evolutionary trajectory is also influenced by selection in the new adaptive landscape (Price, 2008; Reznick & Ghalambor, 2001), the strength of which is likely to be strongest in the early stages of divergence (Clegg et al., 2002; Ingley & Johnson, 2016; Reznick et al., 1997). However, what is less well understood is how the stochastic effects of population founding interact with selective processes to influence the evolutionary trajectory of a newly founded population.

It is well established that population bottlenecks, especially those that are severe and sustained, can result in loss of genetic diversity (Hunter et al., 2010; Pastor et al., 2004; Roques & Negro, 2005; Weber et al., 2004). However, there are key differences between in situ population bottlenecks versus population founding, including higher potential for population recovery, and continued gene flow from a source population in the latter (Clegg, Degnan, Kikkawa, et al., 2002). As such, loss of diversity is not an inevitable consequence of population founding, and indeed empirical evidence from founded wild populations is mixed. For example, significant loss of diversity has been reported in house finch (Carpodacus mexicanus) (Hawley et al., 2006), Coqui frogs (Peacock et al., 2009); Lupines (Vásquez et al., 2016) and Steller’s jay (Cyanocitta stelleri) (Burg et al., 2005), but not in South Island saddleback (Philesturnus carunculatus) (Taylor & Jamieson, 2008), Alpine ibex (Capra ibex) (Biebach & Keller, 2012), white-tailed deer (Odocoileus virginianus) (Fuller et al., 2020), New Zealand sea lion (Phocarctos hookeri) (Foote et al., 2020) or humans (Tabbada et al., 2010) to name a few. Empirical evidence for reduced diversity is also mixed following reintroduction/translocation efforts. For example, there was no loss of diversity in American martens (Martes americana) (Williams & Scribner, 2010) or Seychelles warbler (Acrocephalus sechellensis) (Wright et al., 2014) but significant reductions in diversity following translocation in Merriam’s turkey (Meleagris gallopavo merriami) (Mock et al., 2004) and bridled nailtail wallabies (Onychogalea fraenata) (Sigg, 2006).

Several explanations can be proposed for why significant loss of diversity is observed in some studies but not in others. First, the dynamics of a founder event, that is the number of effective founders, the number of generations of small effective population size and the number of founding steps, combine to determine loss of diversity (Nei et al., 1975; Wright, 1931). Where populations are the product of multiple founding steps, for example during a stepwise range expansion, the potential for loss of diversity is expected to be increased and the magnitude of this loss exaggerated (Clegg, Degnan, Kikkawa, et al., 2002). Second, substantial reductions may not be observed in populations that are established from genetically depauperate populations as there is limited diversity to lose (Clegg, Degnan, Kikkawa, et al., 2002; Taylor & Jamieson, 2008). Third, conclusions can vary depending on the form of diversity examined and the type of genetic marker used. For example, allelic richness is more sensitive to diversity loss than heterozygosity (Nei, Maruyama & Chakraborty, 1975) and microsatellites may provide a poorer prediction of genome-wide levels of nucleotide diversity (Roques et al., 2019; Väli et al., 2008) than large numbers of single nucleotide polymorphisms (SNPs) (Kardos et al., 2016).
been previously associated with morphological variation in passerines (Bosse et al., 2017; Lamichhaney et al., 2015, 2016), including within the genus *Zosterops* (Cornetti et al., 2015; Sendell-Price, Ruegg, & Clegg et al., 2020), this system also provides the opportunity to determine whether repeated phenotypic changes following island colonization in the silvereye are produced by a common set of genes or a by suite of alternative genes with similar phenotypic effects (Barghi et al., 2020; Láruson et al., 2020; Zhu et al., 2018).

Here, using restriction-site associated DNA sequencing (RAD-Seq) (Davey & Blaxter, 2010) of silvereye populations representing

### FIGURE 1
(a) The distribution of silvereye forms used in this study. Black circles show sampling locations for the recent colonizer, *Zosterops lateralis lateralis*, and coloured circles indicate sampling locations for the evolutionarily old forms (Australian mainland; Heron Island; and Lord Howe Island). The timing and direction of the recent sequential colonization by *Z. l. lateralis* are indicated by dates and arrows. Numbers within black circles indicate the number of founding steps separating the Tasmanian population from derived populations. (b) Mean body size (as summarized by PC1 of a principal component analysis of morphological traits) for each *Zosterops* form/population used in this study.

### TABLE 1  Sampling information

| Sampling location | Subspecies | Code | Latitude/Longitude | Year sampled | N (sequenced) | N (retained) |
|-------------------|------------|------|---------------------|--------------|---------------|--------------|
| Mainland          | *cornwalli*| ML   | −28.18/153.45       | 2013         | 20            | 18           |
| Tasmania          | *lateralis*| TAS  | −42.88/147.33       | 1997         | 39            | 19           |
| South Island      | *lateralis*| SNZ  | −45.53/170.30       | 1997         | 23            | 21           |
| North Island      | *lateralis*| NNZ  | −40.22/175.57       | 1997         | 17            | 10           |
| Chatham Island    | *lateralis*| CI   | −43.94/176.49       | 1997         | 22            | 18           |
| Norfolk Island    | *lateralis*| NI   | −29.04/167.96       | 1997         | 24            | 12           |
| Tahiti            | *lateralis*| TAH  | −17.58/−149.53      | 2017         | 21            | 9            |
| Heron Island      | *chlorocephalus* | HI  | −23.44/151.91      | 2015         | 20            | 18           |
| Lord Howe Island  | *tephropleurus* | LH  | −31.33/159.05       | 1998         | 20            | 20           |

Note: N (sequenced) = number individuals sequenced; N (retained) = number individuals retained after filtering.
the recent sequentially colonized populations, a mainland population and evolutionarily old island populations for comparison, we address the following questions: (i) How does sequential population founding shape the distribution of divergence across the genome? (ii) What are the consequences of population founding in terms of loss of genetic diversity and the fate of rare alleles? (iii) Are patterns of selection at candidate genes thought to underlie morphological variation consistent across island populations?

2 MATERIALS AND METHOD

2.1 Sample collection

Silvereyes were sampled from nine locations across Australia and islands of the South Pacific (Figure 1a, Table 1). The populations sampled included: (i) Z. l. lateralis from Tasmania—the initial source of the recent colonization sequence; (ii) Z. l. lateralis from the sequentially colonized islands of South Island (New Zealand), Chatham Island, North Island (New Zealand), Norfolk Island and Tahiti; (iii) Zosterops lateralis cornwalli from the east coast of the Australian mainland representing a large, outbreeding continental population; and (iv) Z. l. chlorocephaus (Heron Island) and Z. l. tephropleurus (Lord Howe Island) as representatives of evolutionarily older island colonization events. Unlike other recently founded populations, the Tahitian population is the product of a human-mediated introduction (Thibault & Cibois, 2017). Birds were caught using mist nets or traps and 20–40 µl of blood collected from the brachial wing vein was stored in 1 ml of lysis buffer (Seutin et al., 1991). All sampling was nondestructive, and birds were released at point of capture.

2.2 RAD-sequencing and bioinformatics

DNA extraction and preparation of RAD-Seq libraries was conducted at the University of California, Los Angeles following the protocol outlined by Ali et al., (2016) and using restriction enzyme SbfI-HF (see Supporting methods for a comprehensive description of library preparation). The resulting libraries were sequenced with 150-bp paired-end reads using the Illumina HiSeq4000 platform (Illumina) at the UC Davis Genome Center. Processing of raw sequencing reads was conducted as outlined in the Supporting methods. We aligned cleaned reads of each individual to the Zosterops lateralis melanops genome assembly version 1 (NCBI Assembly GCA_001281735.1) (Cornetti et al., 2015) with bowtie2 version 2.2.6 (Langmead & Salzberg, 2012) using end-to-end alignment and default settings (allowing for a maximum of two mismatches in the seed [–n 2]). SNPs were identified using the gatk (nightly build version 2016-12-05-ga159770) HaplotypeCaller (McKenna et al., 2010) and the outputted VCF file was filtered with vcf-tools version 0.1.13 (Danecek et al., 2011) to remove indels and only include biallelic SNPs where minimum genotype quality = 30, minimum depth = 8, minimum minor allele frequency = 0.01, and where SNPs were called in at least 50% of individuals. Data missingness was then visualized using genoscape-tools (https://github.com/eriqande/genoscapeTools) (Figure S1) and the VCF file was further filtered to retain 89,026 SNPs and 145 individuals (those individuals with <30% of data missing). The number of samples retained per population ranged from nine to 21 (Table 1). As the Z. l. melanops genome is assembled to the scaffold level only (scaffold N50 = 3.5 Mb) (Cornetti et al., 2015), we mapped scaffolds to chromosomes of the Taeniopygia guttata genome assembly version 3.2.4 (NCBI Assembly GCA_000151805.2) (Warren et al., 2010) using satsuma synteny version 3.1.0 (Grabherr et al., 2010). Because chromosomal evolution occurs at an unusually slow rate in birds and synteny between avian genomes is therefore high (Ellegren, 2010), we were able to assign 96.8% of the Zosterops scaffolds to T. guttata chromosomes and determine their order and orientation. Custom scripts were used to reorder the VCF file accordingly and remove SNPs where chromosomal positions could not be determined. This resulted in 87,099 SNPs for downstream analyses, representing ~2.35% of the Z. lateralis genome (based on the number of SNPs previously identified for this species by Cornetti et al., 2015). The final SNP data set had a maximum per-individual missingness of 0.266 and a maximum per-site missingness of 0.06.

2.3 Linkage disequilibrium

We assessed the extent and patterns of linkage disequilibrium within our RAD-Seq data set by calculating the squared correlation coefficient (r^2) between all pairs of SNPs within a physical distance of 1 Mb using poplddecay (Zhang et al. 2019).

2.4 Population structure

We examined patterns of population structure using ADMIXTURE (Alexander et al., 2009). As the authors recommend avoiding SNPs with high linkage disequilibrium (LD), we used plink (Purcell et al., 2007) to remove one of every pair of SNPs with r^2 > 0 within 100-kb sliding windows (LD-pruned data set = 5000 SNPs). We tested K values of 1–9, with 100 independent runs for each value of K, and summarized runs using clumpp (Jakobsson & Rosenberg, 2007).

2.5 Genetic diversity, divergence and the presence of rare alleles

Genetic diversity within populations was measured as average expected heterozygosity (H_e) and average nucleotide diversity (π), both of which were estimated using STACKS version 1.4 (Catchen et al., 2013). Significance of pairwise differences in H_e and π between populations were assessed using Mann-Whitney tests. Confidence intervals were calculated across loci using bootstrap resampling (1000 iterations). For the recently colonized populations, Spearman’s rank correlation was used to test for associations between measures of
genetic diversity and the number of colonization steps proceeding population founding.

Genetic divergence between populations was assessed using Weir and Cockerham’s F_{ST} (Weir & Cockerham, 1984). F_{ST} values were calculated, on an SNP-by-SNP basis, between each population using VCFtools (Danecek et al., 2011), and 95% confidence intervals were estimated using bootstrap resampling (1000 iterations). As the data are in matrix form, Mantel tests were used to test for associations between the number of colonization steps separating any two populations and mean F_{ST} values, and p-values were estimated using 1000 randomizations.

To assess the effects of population founding on rare alleles, for each recently colonized population, we calculated allele frequencies using VCFtools (Danecek et al., 2011). Calculation of allele frequencies was limited to 84,886 SNPs that were present in at least 80% of the individuals within each population. We then identified rare alleles as those SNPs where the minor allele frequency within the original source population of Tasmania was ≤ 0.1 and counted the number of rare alleles that were lost (allele frequency = 0) and the number of rare alleles that become the major allele (allele frequency ≥ 0.5) in subsequently colonized populations.

### 2.6 Distribution and accumulation of genomewide divergence

To determine how divergence accumulates across the genome as populations are established via sequential founding steps, we calculated F_{ST} in nonoverlapping 500-kb windows between the Tasmanian population and each subsequently colonized population, using Python scripts developed by Martin et al., (2013) (https://github.com/simonhmartin/genomics_general). The number of SNPs per 500-kb window ranged from one to 265 (mean = 47.61). Following Sendell-Price, Ruegg, Anderson, et al., (2020), we compared the third moment (skewness) of F_{ST} distributions between population comparisons. Where divergence is limited to few loci, F_{ST} distributions were expected to be highly skewed, whereas if more loci were involved (divergence was more widespread throughout the genome), then distributions were expected to be less skewed. We tested for significant differences in the skewness of empirical distributions using a randomization test. For each pairwise comparison of recently diverging populations (e.g., Tasmania vs. South Island, compared to Tasmania vs. Chatham Island) we first calculated a test statistic (the absolute difference in distributional skew of observed F_{ST} values) and compared this to test statistics calculated for 10,000 null distributions. Null distributions were produced by drawing observed F_{ST} values at random without replacement. The p-values were then calculated as the percentile of the distribution of randomized test statistics on which the observed test statistic lay. We identified highly diverged genomic regions in each comparison as those 500-kb windows with the top 1% of F_{ST} values. For each colonization step, we assessed if highly diverged regions identified in the previous colonization step(s) were maintained.

### 2.7 Identification of outlier loci and candidate genes

We scanned for outlier loci using PCAdapt, a principal components-based method of outlier detection with a low rate of false-positive detection (Luu et al., 2017). PCAdapt requires the choice of K principal components, based on inspection of a scree plot, where K is the number of PCs with eigenvalues that depart from a straight line. PCAdapt then computes a test statistic based on Mahalanobis distance and controls for inflation of test statistics and false discovery rate (FDR). SNPs with high LD were removed using a window size of 200 SNPs and an r^2 threshold of 0.1 and outlier SNPs were then identified using the following settings for all population comparisons: K = 2, MinMAF = 0.05 and FDR = 0.01. Using the Ensembl Biomart database (Yates et al., 2019) we identified known, novel or predicted genes of the T. guttata genome occurring within 50 kb of each outlier SNP. The Biomart database was queried using the R package Biomat (Durinck et al., 2005, 2009). Genes that had previously been associated with body/bill size variation in birds were determined to be candidate genes underlying body size differentiation in silvereyes. Genes known to be involved in craniofacial morphogenesis in nonavian species were also considered candidates. Given the large number of outlier SNPs identified in some comparisons, we performed simulations to assess the probability of candidate genes being identified by chance alone. For each population comparison we selected SNPs at random (without replacement), where the number of randomly drawn SNPs was equal to the number of outlier loci identified using PCAdapt, and counted the number of candidate genes that fell within 50 kb of those SNPs. We performed 10,000 simulations per comparison.

### 2.8 Enrichment analysis

Genes within 50 kb of outlier SNPs were tested for overrepresentation of specific gene ontological terms using the web-based Zebra finch gene ontological (GO) analysis (GOfinch) tool (http://bioinformatics.iah.ac.uk/tools/Gofinch). Enrichment was determined using Fisher and hypergeometric tests and a population gene list consisting of 3596 zebra finch genes that were located within 50 kb of all SNPs contained in our RAD-Seq data set. For GO analyses we used human gene ontologies as zebra finch genes are not as well GO-annotated.

### 2.9 Demographic history

For recently colonized populations, we estimated demographic parameters from the site-frequency spectrum (SFS) using Fastsimcoal2 (Excoffier et al., 2013; Excoffier & Foll, 2011). As Fastsimcoal2 requires the use of unlinked SNPs and is sensitive to missing data, we filtered the LD-pruned data set to include only SNPs present in all individuals (1551 SNPs). VCFs were converted to SFS format using the R package vcf2sfs (https://github.com/shenglin-liu/vcf2sfs) (Liu et al., 2018). As outgroup sequences were unavailable,
demographic inference used the distribution of minor allele frequencies. To avoid problems associated with model overparameterization, we tested two simple demographic models. The first (null model: Figure 2a) represents a population of constant size and is defined by a single parameter, the current population size ($N_{cur}$). The second (bottleneck model: Figure 2b) is characterized by five parameters: the ancestral population size ($N_{anc}$), time of colonization event and start of population bottleneck ($t_{col}$), population size during the population bottleneck ($N_{bot}$), time of population expansion ($t_{exp}$) and the current population size ($N_{cur}$). Parameter search ranges were selected based on historically recorded colonization dates and assumed a generation time of 2.5 years (Kikkawa & Degnan, 1998) (Table S1). For each model, we performed 100 independent model runs per population (40 expectation/conditional maximization [ECM] cycles, 1,000,000 simulations per run) and chose the run with the highest likelihood as the best estimate of demographic parameters under a given model. To determine which of the two models (null or bottleneck) had the highest support we then compared the best maximum-likelihood run under each model using Akaike Information Criterium (AIC).

3 | RESULTS

3.1 | Linkage disequilibrium

Overall mean LD (measured as $r^2$) among SNP pairs was 0.019 and an average LD of 0.050 was estimated for SNPs less than 1 kb apart. LD decayed rapidly as the physical distance increased between SNPs (Figure S2).

**FIGURE 2**  Schematic representation of the demographic scenarios modelled with fastsimcoal2. (a) Null model with a constant effective population size. (b) Bottleneck model incorporating a reduction in population size at time of colonization followed by population recovery. $t_{col}$ = time of colonization event (and start of bottleneck); $t_{exp}$ = time of population expansion (end of bottleneck); $N_{anc}$ = ancestral population size; $N_{cur}$ = current population size; $N_{bot}$ = population size during bottleneck

3.2 | Population structure

Maximum likelihood estimation of individual ancestries using admixture (Alexander et al., 2009) consistently provided the lowest cross-validation error for $K = 5$ (mean cross-validation error across runs $= 0.592$). At $K = 5$, individuals were grouped as follows: (i) Mainland (with some individual exceptions); (ii) Tasmania and sequentially colonized populations excluding Norfolk Island; (iii) Norfolk Island; (iv) Heron Island; and (v) Lord Howe Island (Figure 3).

3.3 | Genomic patterns of divergence in recently colonized populations

The distribution of $F_{ST}$ values was most skewed (divergence was localized to few 500-kb windows) for the single-colonization step comparison (Tasmania vs. the South Island of New Zealand; skew $= 3.964$) and least skewed (divergence was more genome-wide) for the three-colonization step comparison (Tasmania vs. Norfolk Island; skew $= 1.817$). Intermediate levels of distributional skew were observed for populations separated by two founding steps (Table 2). Using randomization tests, we determined that differences in skewness calculated for Tasmania versus South Island and skewness calculated for subsequent colonization steps were significant for all populations (all $p < .001$) with the exception of Tasmania versus North Island (New Zealand) ($p = .246$) (Table 2).

The position of 500-kb windows with the top 1% of $F_{ST}$ values varied between population comparisons, and in many instances highly diverged windows showed reduced divergence in subsequent founding steps (Figure 4). To assess if the differences in the position of highly diverged windows between comparisons reflected differences in selective landscapes between recently founded populations we searched for outlier loci using pcadapt. pcadapt identified between seven and 76 outlier SNPs per population comparison (Figure 5a and Table 2). As outlier SNPs corresponded to the position of the top 1% of $F_{ST}$ windows in only five cases (Table 2), we associate variation in the position of highly diverged genomic windows primarily to drift resulting from the stochastic nature of the founding process.

For recently colonized forms separated by single founding steps, the overall level of divergence (mean genome-wide $F_{ST}$) exhibited was generally low (Table 2 and Figure 6a). However, mean $F_{ST}$ was positively associated with the number of colonization steps separating populations (Mantel test of matrix comparisons, $r = .701, p = .004$), with divergence between populations separated by three founding steps approaching levels seen between the youngest of the evolutionarily old Zosterops forms (Heron Island) and the mainland population (~1000 generations of separation) (Figure 6a).

3.4 | Genetic diversity in recently colonized populations

We found evidence that single founder events often resulted in significant, albeit minor, changes in genome-wide levels of genetic
diversity and differentiation. When each recently colonized population was compared to its immediate source, significant reductions in expected heterozygosity ($H_E$) were observed for all populations (Figure 6b; Wilcoxon $p$-values < .001) with the exception of the South Island of New Zealand which showed a nonsignificant increase in $H_E$ (Figure 6b; Wilcoxon $p$ = .953). Likewise, nucleotide diversity ($\pi$) was significantly reduced in Chatham Island, Norfolk Island and Tahiti (Figure 6b; Wilcoxon $p$ < .001 in all cases), although a significant increase was detected in the North Island of New Zealand (Figure 6c; Wilcoxon $p$ < .001) and no significant change detected in the South Island ($Wilcoxon p$ = .808). Both measures of genetic diversity were negatively associated with the number of founding steps preceding population establishment (Spearman’s rank correlation: $H_E$: $\rho = -0.044$; $p < .001$; $\pi$: $\rho = -0.040$; $p < .001$). Compared to evolutionarily old populations all recently colonized populations had significantly higher $\pi$ and $H_E$ (Figure 6b; Mann–Whitney: $p < .001$ in all cases).

Within the Tasmanian silvereye population 27,950 SNPs were identified as having a rare allele (sites where the minor allele occurred at a frequency ≤ 0.1). Of these, between 8396 and 18,074 were not detected in subsequent colonization steps (Table 2). For the naturally colonized populations (South Island, North Island, Chatham Island and Norfolk Island), the number of undetected rare alleles increased as the number of founding steps from Tasmania increased. The largest loss of rare alleles was detected for Tahiti, a population established via human-mediated introduction. Although the major trend was towards a loss or reduction in the frequency of rare alleles, some rare alleles increased in frequency in subsequent colonization steps (see Figure 6d). In a few cases previously rare alleles became the major allele (allele frequency ≥ 0.5) in the newly founded population (Table 2). Of the 178 rare to major allele transitions, only nine could be attributed to selection, that is transitions occurred at outlier loci identified using pcadapt (Table 2).

| Comparison     | No. founding steps separating populations | Mean $F_{ST}$ | $F_{ST}$ distributional skew | $p$-value | No. of outlier SNPs $^a$ | No. of rare alleles lost | No. of rare alleles that become major allele $^b$ |
|----------------|------------------------------------------|--------------|------------------------------|-----------|-------------------------|-------------------------|------------------------------------|
| TAS versus SNZ | 1                                        | 0.007        | 3.964                        |           | 76 (0)                  | 8396                    | 1 (1)                              |
| TAS versus NNZ | 2                                        | 0.011        | 3.296                        | .2463     | 52 (1)                  | 14,052                  | 5 (1)                              |
| TAS versus CI  | 2                                        | 0.015        | 2.626                        | <.001     | 7 (0)                   | 11,922                  | 9 (1)                              |
| TAS versus NI  | 3                                        | 0.031        | 1.817                        | <.001     | 43 (3)                  | 16,963                  | 95 (5)                             |
| TAS versus TAH | 2                                        | 0.024        | 2.383                        | <.001     | 38 (1)                  | 18,074                  | 68 (1)                             |

For comparisons where populations are separated by two or more founding steps, $p$-values from randomization tests comparing skewness of $F_{ST}$ distributions to Tasmania versus South Island step are also reported.

$^a$Values in parentheses indicate the number of outlier SNPs that corresponded to the top 1% of $F_{ST}$ windows.

$^b$Values in parentheses indicate the number of rare to common allele transitions that corresponded to the position of outlier SNPs.

### 3.5 Candidate genes underlying body size differentiation

Using the Ensembl Biomart database (Yates et al., 2019), we identified 403 known, novel or predicted genes of the zebra finch (T. guttata)
genome within 50kb of `pcadapt` outliers identified across comparisons (Table S2). These genes were not significantly enriched for any GO terms (all Bonferroni-corrected \( p > .05 \)). However, based upon a literature review of gene expression and association studies, 11 genes were previously associated with morphological variation in birds or craniofacial morphogenesis in nonavian species (Figure 5a). These genes were therefore considered candidates underlying the repeated pattern of phenotypic change seen in silvereyes (i.e., larger body/bill size in island populations). The simulations showed that candidate genes were highly unlikely to be identified if SNPs were randomly
drawn. Hence the candidate genes were not identified by chance, leading us to conclude that their association with outlier SNPs is meaningful (Figure 5b). As signatures of selection at candidate genes were inconsistent between population comparisons, this result suggests that a suite of alternative genes may underlie body/bill size variation in the silvereye. However, as we make use of a reduced-representation sequencing technique, an alternative explanation is that our SNP data set missed candidate genes that may underlie repeated morphological change in silvereyes. For example, we are unable to speculate on the role of HMGA2 and ALX1 (both thought to
modulate bill size in Darwin’s finches: Lamichhaney et al., 2015, 2016) as these two genes were not covered by our data set.

3.6 | Demographic history

Statistical comparisons of the two demographic models showed strongest support (lowest AIC values) for the bottleneck model (Figure 2b) for all recently colonized populations (Table 3). Based on this model, point estimates of effective founding population size were in excess of 100 individuals for all naturally colonized populations with the exception of Norfolk Island (Table 3). The lowest estimate of effective founding population size (17 individuals) was estimated for the Tahitian population, which unlike other recently established populations is the product of a human-mediated introduction. Although the exact number of silvereyes introduced to Tahiti is unknown, Guild’s writings (Guild, 1940) suggest the founding population probably consisted of only a handful of individuals.

4 | DISCUSSION

A genome-wide comparison of a historically recent, sequential colonization sequence with more ancient island populations of silvereyes in Australia and the south Pacific has provided both novel insights into the dynamics of population founding at the level of the genome, and valuable empirical validation of some key population genetic principles. We provide empirical evidence that genome-wide divergence accumulates with sequential, natural population founding, and demonstrate that detailed patterns of divergence are highly idiosyncratic in nature, varying across founding steps. Genomic variation is eroded with increasing founder steps, and by tracing the fate of rare alleles across the colonization series, we demonstrate the vagaries of chance, with frequent loss and, much more rarely, increases in frequency of rare alleles. Our results highlight the difference in the potential for founder effects between natural and human-mediated colonizations. In the latter case, a single founder event generated reduced diversity and increased divergence that surpassed the...
cumulative effects of the natural colonization series. Eleven genes previously associated with body size variation in vertebrates were also identified as potential candidates underlying larger body size in insular silvereyes, but patterns of selection at these candidates were highly variable, with no single candidate gene under selection across all populations.

Over the past decade, a major challenge in speciation genomics has been to understand how different evolutionarily processes shape patterns of divergence at the genomic level (Ravinet et al., 2017; Stankowski et al., 2019; Wolf & Ellegren, 2016). However, most research has focused on the role of selection in shaping the genomic landscape, largely ignoring the role of nonselective processes such as genetic drift. By comparing FST frequency distributions between recent, sequentially founded silvereye populations that have been diverging from their original source (Tasmania) for the same amount of time, but with different numbers of prior founding steps, we have shown how sequential population founding amplifies genome-wide divergence. This is seen in the shift from an extreme L-shaped FST frequency distribution in the single-founder step population on South Island (New Zealand), where divergence was limited to few 500-kb windows, compared to the reduced distributional skew (i.e., progression towards more genome-wide divergence) for populations separated from Tasmania by multiple founding steps. Although the process of genetic drift, by comparing FST frequency distributions between populations of divergence has been highlighted previously, our empirical demonstration provides tangible evidence from a natural system.

By tracking the fate of highly diverged genomic regions across different founder events, we show that divergence accumulates idiosyncratically between founding steps. Of the highly diverged regions identified for the single founder step comparison (Tasmania vs. South Island), very few were within the top 1% of windows identified in subsequent colonization steps, and in many instances highly diverged SNPs failed to accumulate under directional selection. Therefore, the position of highly diverged genomic regions varies across populations. However, as illustrated by Dalongeville et al. (2018), all outlier detection methods suffer from false negatives/positives and may provide dramatically different results from false negatives/positives.

The relatively minor impact of single founder events on silvereye genetic diversity and divergence, compared to the obvious number and identity of outlier loci detected, is due to the failure of current methods to identify all selected loci within our data set. However, as illustrated by Dalongeville et al. (2018), all outlier detection methods suffer from false negatives/positives and may provide dramatically different results from false negatives/positives. Further, our empirical demonstration provides tangible evidence from a natural system.

### Table 3: Best parameter estimates from demographic modelling implemented in FASTSIMCOAL2.

| Demographic model | Population | Nanc | Nbot | tcol | tep | Ncur | Recovery time (tcol - tep) | Maximum estimated likelihood | Maximum observed likelihood | AIC |
|-------------------|------------|------|------|------|-----|------|---------------------------|----------------------------|---------------------------|-----|
| Model A           | SNZ        | –    | –    | –    | –   | 64,403| –                         | -1554.951                  | -1529.263                 | 7162.814 |
|                   | CI         | –    | –    | –    | –   | 7679 | –                         | -1114.051                  | -1015.758                 | 5132.395 |
|                   | NNZ        | –    | –    | –    | –   | 44,393| –                         | -1000.642                  | -992.031                  | 4610.127 |
|                   | TAH        | –    | –    | –    | –   | 8327 | –                         | -873.326                   | -844.641                  | 4023.985 |
|                   | NI         | –    | –    | –    | –   | 2120 | –                         | -1040.830                  | -1015.758                 | 4795.199 |
| Model B           | SNZ        | 84,910| 139  | 53   | 67  | 29,513| 14                        | -1536.812                  | -1529.263                 | 7087.405 |
|                   | CI         | 83,492| 357  | 47   | 56  | 957   | 9                         | -1104.225                  | -1015.758                 | 5095.144 |
|                   | NNZ        | 13,002| 100  | 45   | 56  | 84,270| 10                        | -995.022                   | -992.031                  | 4592.388 |
|                   | TAH        | 77,642| 17   | 25   | 32  | 3021 | 7                         | -846.246                   | -844.641                  | 3907.107 |
|                   | NI         | 24,732| 48   | 25   | 37  | 5441 | 12                        | -1019.375                  | -1015.758                 | 4704.395 |

Note: Nanc = ancestral population size; Nbot = population size during population bottleneck; tcol = timing of colonization event; tep = timing of population expansion (end of bottleneck); Ncur = current population size. All timings are in generations (using a 2.5-year generation time). Note: timings are relative to sample collection dates.
cumulative effects of sequential founder events, supports theoretical (Le Corre & Kremer, 1998) and experimental (Bryant & Meffert, 1993) expectations, along with results from a small number of microsatellite studies of sequential colonization in the wild (Lambert et al., 2005; Michaelides et al., 2018; Pruitt & Winker, 2005; Thulin et al., 2011), including a microsatellite study of the silvereye (Clegg, Degnan, Kikkawa, et al., 2002). The greater power and precision provided by the large number of SNPs used here convincingly shows that the erosion of diversity with population founding stems primarily from loss of rare alleles during the colonization process. Following the fate of rare alleles also demonstrated the less common cases of increases in frequencies of rare alleles, as can occur under stochastic or selective processes. Genome-wide \( F_{ST} \) increases with successive founder events showed that average \( F_{ST} \) was approximately five times higher for populations separated by three founding steps compared to one. Despite these marked cumulative effects on both diversity and divergence, multiple founding steps were insufficient to produce reductions of diversity and levels of divergence seen in even the youngest of the evolutionary old forms (Heron Island silvereyes).

If a single founder event is more extreme, then stronger effects on genetic diversity and divergence may be observed (Clegg, Degnan, Kikkawa, et al., 2002). This was the case for Norfolk Island and Tahiti silvereye populations. The effects were most pronounced for the human-mediated introduction to Tahiti which exhibited a more pronounced loss of genetic diversity as measured by both nucleotide diversity and expected heterozygosity. The two populations also exhibited the highest rates of divergence from Tasmania forming highly distinct population clusters despite being the two youngest populations. In the case of Tahiti, these patterns are probably explained by very different demographic dynamics experienced during natural versus human-mediated introductions. Most naturally established silvereye populations are thought to establish via large founding flocks, here estimated as 100+ individuals, undergo rapid population size recovery and have the potential for further gene flow from source populations, all acting to buffer populations from loss of diversity (Clegg, Degnan, Kikkawa, et al., 2002; Estoup & Clegg, 2003). In contrast, the founding size of the introduced silvereye population has been described as “a handful of individuals” (Sendell-Price, Ruegg & Clegg, 2020; Thibault & Cibois, 2017), which is supported by our coalescence based demographic inference with only 17 founding individuals estimated, and the isolated nature of the archipelago precludes further gene flow from the source. However, the estimated number of generations to population recovery was low, as is typical for silvereyes. Consistent with previous inferences from microsatellites (Clegg, Degnan, Kikkawa, et al., 2002; Estoup & Clegg, 2003), the establishment of the Norfolk Island population appears to have involved fewer founders than other natural colonizations, estimated at 48 individuals using our coalescent-based demographic inference. While this could contribute to this population’s high level of divergence, proposed hybridization with an endemic species, Zosterops tenuirostris (Gill, 1970), may also be a factor. Despite continued and long-running interest in the idea that population founding can be a radical short cut to species generation (founder-effect speciation) (Barton & Charlesworth, 1984; Carson, 1968, 1975; Carson & Templeton, 1984; Matute, 2013; Mayr, 1959; Templeton, 1980, 1981, 1999), our results from sequential silvereye founding events, in two cases accompanied by relatively narrow demographic bottlenecks, lend empirical weight to the view that founder effect processes are a relatively uncommon mechanism of speciation (Coyne & Orr, 2004), at least within the silvereye system.

We identified 11 candidate genes potentially underlying body/bill size variation in silvereyes. Candidate genes within 50 kb of outlier SNPs putatively under selection included: BMP7, FOXI1 and TRIM37—all associated with craniofacial defects in vertebrates (Edlund et al., 2014; Graf et al., 2016; Hämäläinen et al., 2004); GSC and NALCN—both of which are associated with bill size variation in Darwin’s finches (Lamichhaney et al., 2015; Lawson & Petren, 2017); ID2—which modulates bone morphogenetic protein (BMP) signalling during craniofacial development (Nimmagadda et al., 2015); LIN28A—which may promote HMGA2 expression (Vignali & Marracci, 2020); NFIA—associated with bill length in the house sparrow (Passer domesticus) (Lundregan et al., 2018) and craniofacial abnormality in humans (Rao et al., 2014); OTX2—which plays a crucial role in craniofacial development across jawed vertebrates (Kimura et al., 1997), and has been associated with bill length in Berthelot’s pipit (Anthus bernotii) (Armstrong et al., 2018); and SOX6—a transcription factor previously associated with bill length in great tits (Parus major) (Bosse et al., 2017). Given that body/bill size follows a highly repeated pattern in the silvereye towards larger size in insular populations (Clegg & Owens, 2002; Leisler & Winkler, 2015) and that several genes of large effect have been associated with morphological variation in passerines, for example ALX1 and HMGA2 in Darwin’s finches (genus: Geospiza) (Lamichhaney et al., 2015, 2016), we had expected some continuity in the position of outlier loci across comparisons and for the position of outlier loci to tightly correspond to candidate genes. However, as outlier loci rarely occurred at the same location across comparisons, the stochastic influence of founder events on the genome noted here raises the possibility that if genes of large effect do explain some variation in bill and body size in silvereyes (as opposed to many genes of small effect), then a suite of alternative genes with similar phenotypic effects may be in operation. However, given the marker density of our RAD-Seq data set (which captures ~2.35% of the Z. lateralis genome) we are not yet able to fully describe the genetic basis of morphological change in this species.

5 | CONCLUSIONS

Our study is the first genome-wide treatment of sequential colonizations in natural populations, and therefore provides an unparalleled empirical demonstration of changes to the landscape of divergence following population founding. Cumulative founding steps generated genome-wide divergence, but this divergence was highly idiosyncratic at the SNP level, emphasizing the role of stochasticity affecting both
neutral and putatively selected loci. We did not identify candidate loci of large effect that would explain repeated phenotypic changes observed, though the possibility remains that these will be revealed by whole genome sequencing. Finally, our study also provides the highest precision yet achieved in quantifying not only the pattern but also the magnitude of cumulative decreases in population-level diversity and increases in divergence. The efficacy of sequential founder events in producing these changes was confirmed, though even multiple founder events are not sufficient to produce values seen in evolutionarily older island silveryeye subspecies. This furthers the view that founder-event speciation may be rare in nature.

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AUTHOR CONTRIBUTIONS
A.T.S.P. and S.M.C. jointly conceived the project, K.C.R. coordinated the genomic data collection, and S.M.C. supervised the project. The manuscript was written by A.T.S.P. and S.M.C., with input from K.C.R. and B.C.R.

DATA AVAILABILITY STATEMENT
Resequencing data produced for this study have been submitted to the European Nucleotide Archive (ENA) under accession no. PRJEB40523, BioSample nos. ER55099157-ER55099397, VCF files and custom scripts used to assign SNPs to Taeniopygia guttata chromosomes are available via Dryad (https://doi.org/10.5061/dryad.4f4qrjbn).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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