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LETTER

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A post-translocation genetic analysis of an endemic wingless grasshopper in urban environments

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Abstract

Translocation is a core conservation strategy, yet the outcomes of invertebrate translocations are often poorly documented due to the small size and limited mobility of invertebrates. Levels of genetic diversity and inbreeding are key indicators of translocation success, reflecting breeding success, adaptive capacity, and long-term viability of translocated populations. This study characterizes the genetic diversity of a recently translocated flightless grasshopper, *Vandiemenella viatica*, two generations after release. Genome-wide sequence data revealed significant reductions in observed heterozygosity (19.07% decrease) and nucleotide diversity (12.69% decrease) in the F_2 generation relative to the source population. Runs of homozygosity were 50.74% more common and 112.98% longer in the F_2 generation relative to the source population. These rapid reductions in diversity underscore the need for genetic monitoring to be integrated into translocation protocols to inform management actions like genetic supplementation for improved conservation outcomes.

KEYWORDS

bottleneck effect, conservation genetics, conservation translocation, genetic diversity, genetic monitoring, matchstick grasshoppers

1 | INTRODUCTION

Translocations play a crucial role in efforts to restore and bolster populations of species threatened by habitat loss, fragmentation, and other anthropogenic pressures (Fischer & Lindenmayer, 2000). By moving individuals, conservation practitioners seek to establish viable populations in suitable habitats, which increases their chances of survival and long-term persistence (Robert et al., 2015; Seddon & Armstrong, 2019). However, for the long-term success of translocations, factors such as population size and genetic diversity need to be considered, given that they are key to maintaining the genetic health and adaptability of a species in the long run (Sgrò et al., 2011).

Translocations typically involve releasing only a fraction of the original population, which reduces the genetic diversity of the new group (founder effect) (Witzenberger & Hochkirch, 2008). The reduced population size increases the likelihood of genetic drift, leading to the loss of rare alleles and reduced heterozygosity (Cardoso et al., 2009). Despite the critical role of these genetic changes, early identification of genetic diversity loss is often overlooked in planning and post-release monitoring (Seddon et al., 2007). Such monitoring is essential to mitigate potential negative

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effects on both short- and long-term population viability (Armstrong & Seddon, 2008). By assessing genetic diversity and divergence from the original population, potential impacts on the translocated population's adaptive capacity can be evaluated (Weeks et al., 2011). Therefore, genetic analyses are useful for practitioners aiming to ensure the sustainability of their translocation programs.

The Larapuna Matchstick grasshopper (*Vandiemenella viatica*) is a pioneering case of conservation translocation for orthoptera in Australia (Yagui et al., 2024). This species comprises several chromosomally distinct races (White et al., 1964), with this study focusing on viatica19 ($2n = 19$). The source population for the translocation had previously been analyzed for genetic variation using single nucleotide polymorphisms (SNPs), revealing slightly lower diversity than in other regions (Hoffmann et al., 2023). Like many morabines, *V. viatica* follows an annual cycle (Kearney et al., 2024). Two years (two generations) post-translocation, multiple populations persisted at release sites, providing an opportunity to test for loss of genetic diversity. Due to the species' flightless nature and the lack of connectivity between sites, movement is restricted. As a result, genetic diversity loss is a likely result of both the initial translocation process and any subsequent population size reductions (Figure S1).

This study compares the genetic diversity of translocated *V. viatica* populations with their source population, analyzing observed autosomal heterozygosity (H_O), nucleotide diversity (π), and runs of homozygosity (ROH) two generations post-translocation. These metrics, derived from DArTseq SNP markers (Hoffmann et al., 2023), help assess genetic variation and inbreeding risks. H_O reflects here genetic variation on non-sex chromosomes within each individual (Schmidt et al., 2021), π measures the average DNA sequence difference among chromosomes within a population (Tajima, 1983), and the number and length of ROH indicate inbreeding and shared ancestry (Broman & Weber, 1999). These analyses provide insights into the genetic consequences of the translocations, opportunities for improving conservation strategies, and support evidence-based decision-making for the conservation of similarly translocated invertebrates.

2 | MATERIALS AND METHODS

2.1 | Study region

The study covered metropolitan Melbourne (Victoria, Australia), which spans a diverse range of remnant vegetation and revegetated areas immersed in an urban-dominated space (Figure 1). A total of 36 translocation sites were established across this region (Yagui et al., 2024). The vegetation at the translocation sites included native forbs and shrubs, with a predominance of Asteraceae from the genera *Chrysocephalum* and *Cassinia*. Sites were small (all <0.2 ha). Each site received 50 individuals, evenly split by sex. Two years post-translocation, numbers varied depending on site modification, with fewer individuals in sites with weed and grass overgrowth. In the most successful sites, early surveys recorded up to 20 individuals, but this declined in later months (Yagui et al., 2024). Note that many individuals in a site were not necessarily detected.

Additional details on the natural history, source habitat, and conservation background of *V. viatica* are provided in Yagui et al. (2024), which describes the species' restricted dispersal ability, dependence on native vegetation, and vulnerability to habitat fragmentation.

2.2 | Sample collection and DArTseq™ processing

The original translocations were conducted in September of 2021. The estimated total source population size before collection was ~3400 (Kearney et al., 2024). F_0 samples (parental generation) were collected during translocations, and F_2 samples (second-generation offspring) were collected in September 2023. The middle legs of 46 translocated F_2 individuals (1–7 per population, Table 1) were collected in the field and preserved in 90% ethanol, with no insects being culled. Leg removal is a standard, non-lethal genetic sampling method in insect studies and has been shown to have minimal impact on survival or reproduction in other taxa (e.g., Holehouse et al., 2003; Koscinski et al., 2011; Vila et al., 2009). The samples were sent to Diversity Arrays Pty Ltd. for DNA extraction and DArTSeq™ assay. Previous work identified the optimal set of restriction enzymes for these samples (Hoffmann et al., 2023). Samples were sequenced on Illumina Hiseq2500/Novaseq6000 platforms (https://www.diversityarrays.com/services/dartseq/). Raw data for the F_0 population was obtained from a previous study by Hoffmann et al. (2023).

2.3 | Data processing and analysis

Raw FASTQ files were processed with Cutadapt v4.7 (Martin, 2011) to remove adapters and anchors. Quality control using FASTQC v0.12.1 (Andrews, 2010) filtered low-quality reads. Cleaned reads were aligned to the *V. viatica* reference genome (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_040549115.1/) with

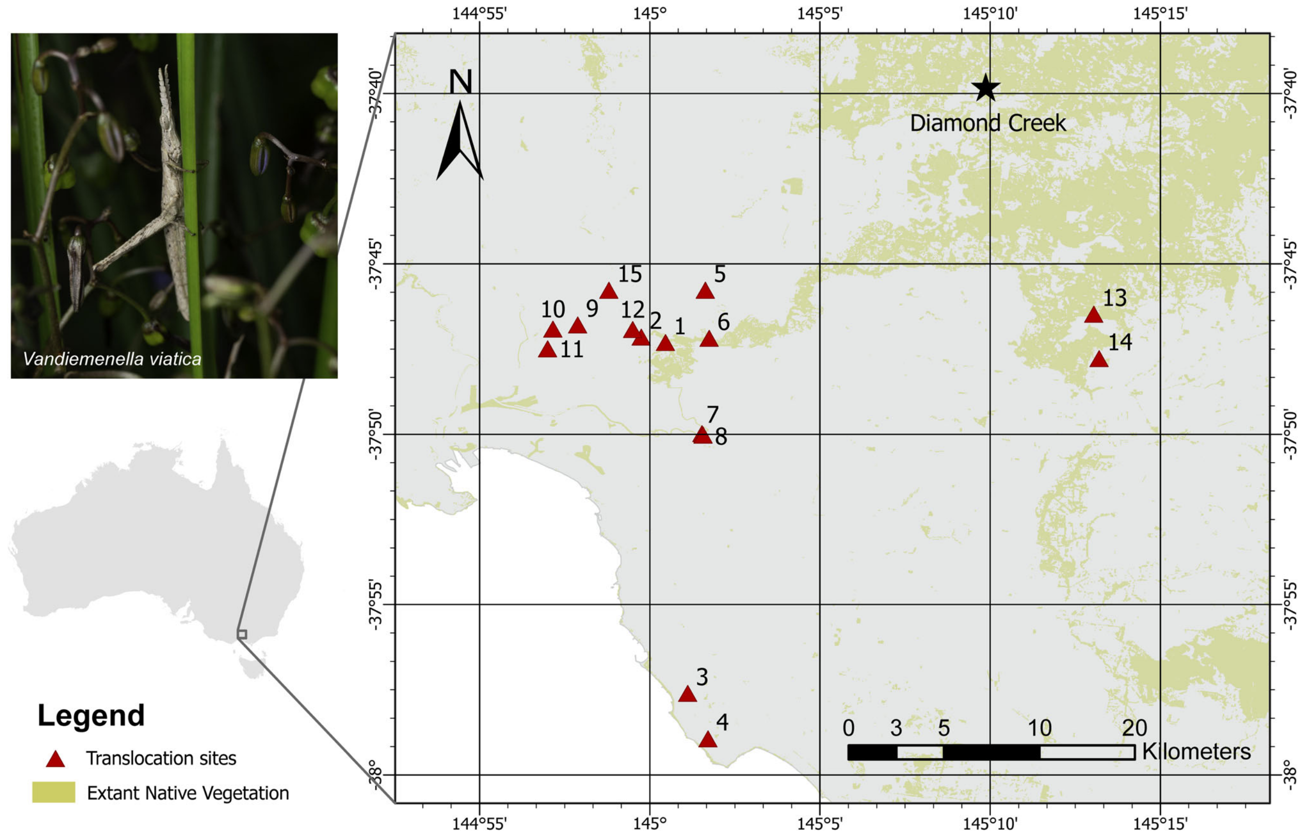

FIGURE 1 Map of *Vandiemenella viatica* collection sites and source population (Diamond Creek). Extant Native Vegetation source: MGV-National Vegetation Information System V6.0 © Australian Government Department of Agriculture, Water and the Environment. https://fed.dcceew.gov.au/maps/e7c56ffd33714b1bbf64893b4f13c34a/about, accessed on August 20, 2024.

Bowtie2 v2.4.5 (Langmead & Salzberg, 2012) using the --very-sensitive preset. Alignments were sorted into BAM format with Samtools v1.16.1 (Li et al., 2009).

Variant calling and genotyping were performed following the pipeline outlined in Schmidt et al. (2024), utilizing GATK (v4.3.0.0) HaplotypeCaller in GVCF mode (McKenna et al., 2010), enabling variant calling across all autosomal chromosomes. Larger chromosomes were split into smaller chunks for processing to prevent computational overload (see Table S1 for chromosome chunk sizes). Following the Schmidt et al. (2024) pipeline, we estimated observed heterozygosity (H_O) using BCFtools (v1.13) (Danecek et al., 2021). Nucleotide diversity (π) was calculated using VCFtools (v0.1.16), and ROH were identified using BCFtools/RoH, which applies a hidden Markov model to detect regions of autozygosity (Narasimhan et al., 2016). As part of a validation process, BWA-MEM (v0.7.17) alignments with default parameters were conducted to compare H_O and ROH estimates, with results provided in Figure S2. In all analyses, we used a minimum depth (DP) threshold of 10 instead of 15 to avoid too much data loss. Following Schmidt et al. (2024), we decomposed complex nucleotide sites with three or four alleles into consecutive SNPs and omitted all sites with any missing genotypes.

To investigate relationships between habitat characteristics and genetic diversity metrics in the F_2 generation, we constructed a correlation matrix using key environmental variables from Yagui et al. (2024) (weed cover, graminoid cover, and plant species richness), including the number of individuals surveyed during offspring sampling. To meet normality assumptions, skewed variables were transformed: the number of surveyed individuals was log-transformed to correct right-skewness, while a cube root transformation was applied to weed cover. To account for the increased risk of false positives when performing multiple comparisons, p-values from the correlation analyses were adjusted using the Bonferroni correction.

3 | RESULTS

The final multi-sample dataset, generated after variant calling and genotyping (pre-depth filter), included both variants and non-variants and contained

TABLE 1 Summary table of samples and collection sites of *Vandiemenella viatica* included in the study (N: number of individuals, H_O: mean observed autosomal heterozygosity, ROH: runs of homozygosity).

Site	Name	Longitude	Latitude	N	Area (m^2)	H_O	π[b]	Total ROH length (kb)	Number of ROH segments	Surveyed in 2023
1	Field Street (FST)	145.0027	−37.7932	1	1200.34	0.00456		392,222.433	102	1
2	Merri C. Trail (MCT)	145.0077	−37.7879	7	1048.76	0.00467	0.00464	258,536.616	71	8
3	Merri C. Bridge (MCB)	144.9958	−37.7853	1	1876.42	0.00545		103,417.622	42	3
4	Bayside R. A. (BRA)	145.0185	−37.9595	1	825.41	0.00440		73,445.359	31	1
5	Bayside D. M. (BDM)	145.0285	−37.9818	3	747.52	0.00435	0.00441	105,215.074	33	3
6	D. Clarendon (DCL)	145.0272	−37.7623	2	404.77	0.00422		141,051.088	40	15
7	Alphington (AP1)	145.029	−37.7858	1	1416.11	0.00395		350,224.290	58	24
8	Burnley 1 (BU1)	145.0255	−37.8324	7	821.94	0.00374	0.00434	303,676.881	63	5
9	Burnley 2 (BU2)	145.0261	−37.8332	3	211.25	0.00432	0.00423	86,546.089	25	8
10	H. Gallagher Lang S. (HLS)	144.9647	−37.7793	1	533.55	0.00413		194,836.297	51	1
11	Royal P. Station (RPS)	144.9526	−37.7813	2	1038.51	0.00438		103,783.763	35	2
12	Royal P. Tree (RPT)	144.9500	−37.7910	7	319.6	0.00455	0.00457	106,040.471	30	43
13	Merry C. Linear (MCL)	144.9916	−37.7816	2	609.11	0.00435		101,046.294	29	5
14	Domeney R. (DOR)	145.2173	−37.7741	5	3366.2	0.00441	0.00459	108,182.404	33	6
15	Loughies B. R. (LBR)	145.2199	−37.7958	1	1979.95	0.00431		159,074.065	40	1
16	Jones Park W. (JPW)	144.9800	−37.7624	2	376.23	0.00399		275,164.303	55	22
[a]	Diamond Creek	145.1599	−37.6720	3		0.00539	0.00511	84,000.743	31	

[a] Source population: Diamond Creek.

[b] π: Nucleotide diversity: Computed only for populations with ≥3 individuals.

2,711,205,677 records. Of these, 2,710,722,450 were non-variant sites, while 414,369 were SNPs and 1653 were insertions or deletions (indels). The dataset exclusively comprised biallelic sites. The dataset exhibited a transitions/transversions (Ts/Tv) ratio of 1.69, with 263,508 transitions and 155,775 transversions. A total of 92,330 singleton SNPs were identified, predominantly transitions (78,199; 84%), resulting in a higher Ts/Tv ratio among singletons. There were 448 singleton indels. The analysis included data from 47 samples, comprising 3 F_0 samples and 44 F_2 samples.

3.1 | Nucleotide diversity

A one-sample t-test was performed to compare the nucleotide diversity (π) of six populations (those with three or more sampled individuals) to that of the F_0 group. Before conducting the t-test, we confirmed that the π data were normally distributed using the Shapiro–Wilk test ($p = .528$). The t-test revealed a significant difference between the F_2 populations and the F_0 group ($t_5 = -9.74$, $p < .001$), indicating that the nucleotide diversity in the F_2 is significantly lower than that in the F_0, with an average reduction of 12.69%.

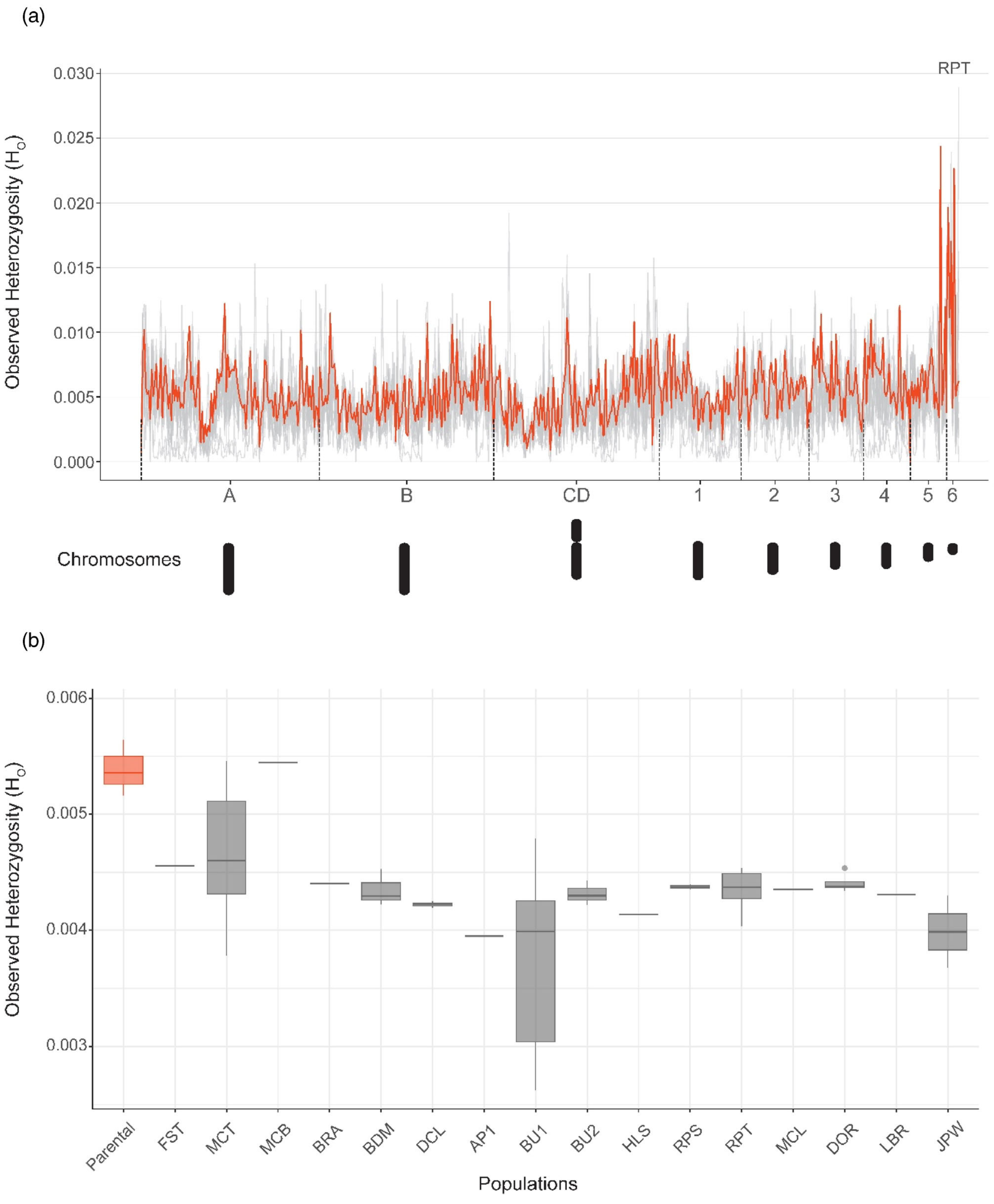

FIGURE 2 (a) Observed autosomal heterozygosity (H_O) for F_0 (red) and F_2 (gray) individuals, averaged across 5 Mbp. The population name displayed corresponds to values over the 0.02 threshold, all belonging to the Royal Park Tree (RPT) population. The schematic chromosomes of *Vandiemenella viatica* chromosomal race 19 (Kawakami et al., 2007; Li et al., 2024a; White et al., 1964) are displayed at the bottom. (b) Boxplot of observed autosomal heterozygosity (H_O) of *V. viatica* populations. Boxes represent population-level distributions; outliers are shown as points. AP1, Alphington; BDM, Bayside D. M.; BRA, Bayside R. A.; BU1, Burnley 1; BU2, Burnley 2; DCL, D. Clarendon; DOR, Domeney R.; FST, Field Street; HLS, H. Gallagher Lang S.; JPW, Jones Park W.; LBR, Loughies B. R.; MCB, Merri C. Bridge; MCT, Merri C. Trail; MCL, Merry C. Linear; RPS, Royal P. Station.

3.2 | Observed heterozygosity

The analysis revealed H_O ranging from 0.00374 in the Burnley 1 (BU1) population to 0.00545 in the Merri C. Bridge (MCB) population, representing the average heterozygosity across individual samples. The highest H_O values were observed in chromosomes 5 and 6 of *V. viatica*, with the Royal Park Tree (RPT) population

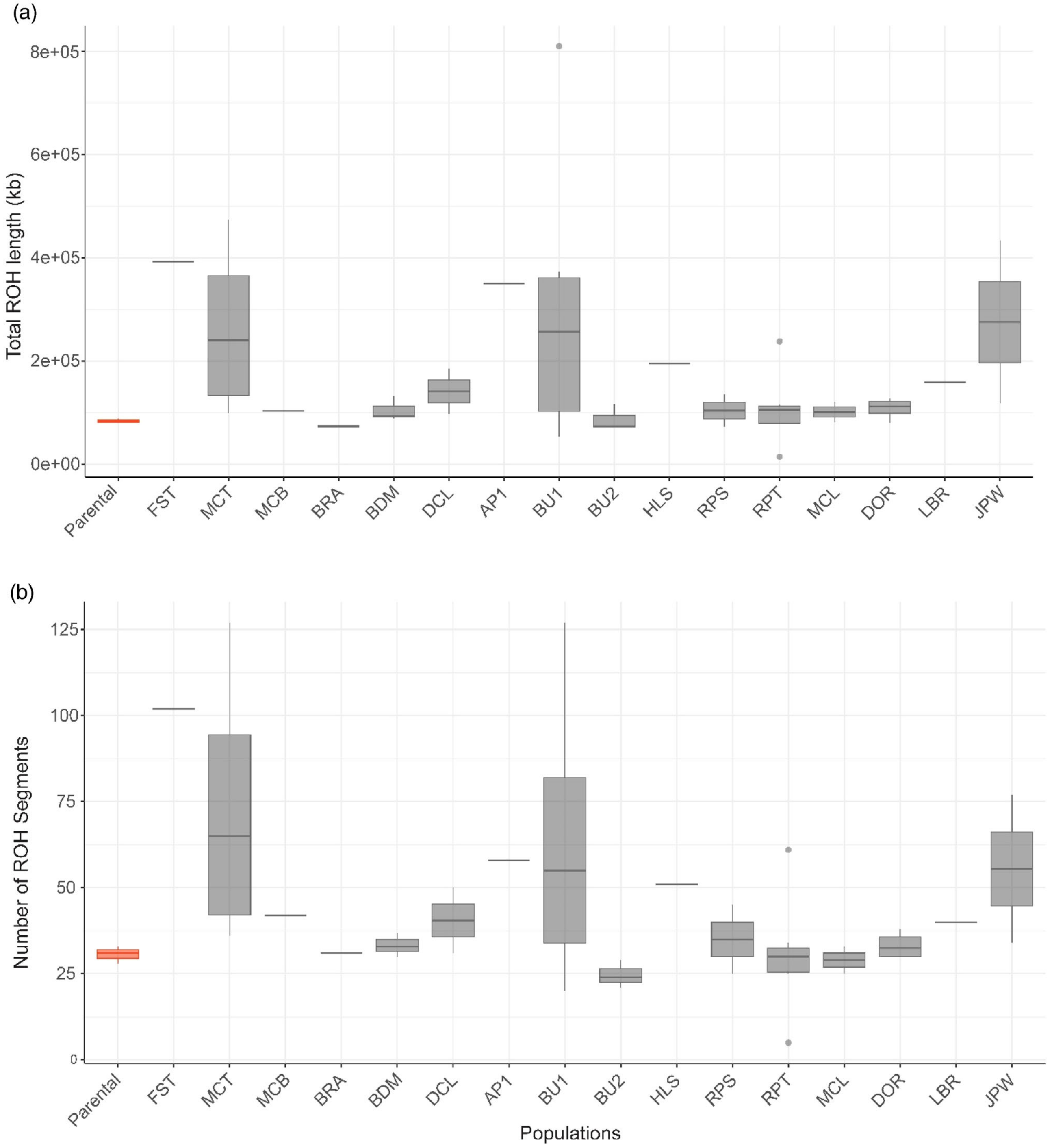

FIGURE 3 (a) Total length of runs of homozygosity (ROH) in kb across populations. (b) Number of ROH segments per individual of *Vandiemenella viatica* across F_0 (red) and F_2 (gray) populations. Boxplots show the distribution of values per population, with outliers represented as points. All analyses included all ROH segments longer than 1 Mb. AP1, Alphington; BDM, Bayside D. M.; BRA, Bayside R. A.; BU1, Burnley 1; BU2, Burnley 2; DCL, D. Clarendon; DOR, Domeney R.; FST, Field Street; HLS, H. Gallagher Lang S.; JPW, Jones Park W.; LBR, Loughies B. R.; MCB, Merri C. Bridge; MCT, Merri C. Trail; MCL, Merry C. Linear; RPS, Royal P. Station; RPT, Royal Park Tree.

exhibiting several outliers on chromosome 6 across different individuals (Figure 2a).

The Shapiro–Wilk test showed that the original heterozygosity data were not normally distributed ($p = .020$). A log transformation improved normality, with the transformed data meeting the assumption of normality ($p = .066$). Levene's test indicated no significant differences in variances between groups ($p = .673$). The mean H_O for the F_2 generation was 0.00436, approximately 19.07% lower than the mean H_O for the F_0 generation ($t_{15} = -10.124$, $p < .001$), indicating a considerable reduction in genetic diversity levels after two generations (Figure 2b). The median H_O for the F_2 generation was lower than that of the parental F_0 population. The interquartile range (IQR) for the F_2 population was broader, reflecting greater variability in H_O compared to the F_0 population. This increased variability was particularly notable in the Merri C. Trail (MCT) and BU1 populations.

3.3 | Runs of homozygosity

ROH varied in length and number across individuals. Several individuals exhibited many large ROH, seen mostly in MCT and BU1 populations, while others displayed fewer, shorter segments. Consistent patterns of ROH were observed in specific genomic regions across individuals. F_0 did not show extensive ROH segments compared to those present in F_2. The distribution of ROH segments exceeding 1 Mb in length is illustrated in Figure S3.

Normality was assessed for total ROH length and ROH segment count using the Shapiro–Wilk test. Total ROH length showed significant deviations from normality in raw data ($p = .005$) and remained non-normal after log ($p = .043$) and square root ($p = .015$) transformations, necessitating non-parametric methods. In contrast, ROH segment count also deviated from normality ($p = .006$), but a log transformation improved its distribution ($p = .306$). A Wilcoxon signed-rank test compared mean ROH length between the F_0 population and other surveyed sites to reveal a significant difference ($V = 134$, $p < .001$), with F_2 showing a 112.98% increase from 84,000.7 to 178,903.9. For ROH segment count, a one-sample t-test on log-transformed values confirmed a significantly higher mean in F_2 than F_0 populations ($t_{15} = 3.138$, $p = .007$), with the F_2 averaging 46.225, a 50.74% increase from F_0 (30.667).

ROH metrics between the F_0 and F_2 populations revealed substantial differences in genetic patterns (Figure 3a,b). In general, F_2 exhibits a higher median ROH length and segment count compared to F_0, indicating a reduction in genetic diversity over two generations, as reflected by an increase in both metrics. Notable exceptions include the Bayside R. A. (BRA) individual for ROH length and the Merry C. Linear (MCL) and Burnley 2 (BU2) individuals for ROH segment count, which deviated from the overall trend. The IQR for F_2 was wider, suggesting greater variability in ROH metrics compared to in the F_0 population.

3.4 | Genetic diversity and other variables

There was a strong positive correlation between ROH length and the number of ROH segments ($r = 0.91$, $p < .001$), which remained significant after correcting for multiple comparisons using the Bonferroni method. A moderate negative correlation was observed between ROH length and plant richness ($r = -0.50$, $p = .046$); however, after Bonferroni correction, this relationship did not reach statistical significance. Similarly, the negative correlation between H_O and ROH length ($r = -0.42$, $p = .094$) was not significant, regardless of correction. All other correlations were weak and not statistically significant (Figure 4, p-values shown in Table S2).

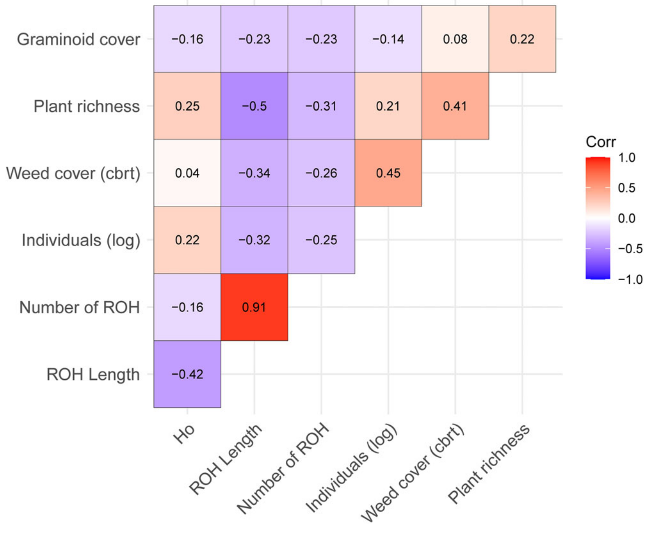

FIGURE 4 Pairwise relationships between genetic diversity metrics of *Vandiemenella viatica* (observed heterozygosity and runs of homozygosity [ROH] values) and habitat variables (Graminoid cover, weed cover, plant species richness, and number of individuals surveyed). Correlations were calculated using Pearson's method. Weed cover was transformed using a cube root transformation (cbrt), while the number of individuals was log-transformed (log) to improve data distribution and reduce skewness. Plot generated with "ggcorrplot" (Kassambara 2023; visualization of a correlation matrix using "ggplot2" R package version 0.1.4.1.).

4 | DISCUSSION

This study underscores the value of monitoring genomic variation when undertaking biodiversity conservation strategies (Hoffmann et al., 2015). Incorporating genetic considerations during the establishment phase can offer valuable early indicators of reintroduction success (Brockett et al., 2022). Early genetic monitoring is essential for detecting changes in genetic diversity after translocation, allowing timely interventions to prevent negative outcomes such as increased risks of inbreeding or genetic drift; the consequences of these have been observed in different reintroduction projects (e.g., Biebach & Keller, 2012; Mueller et al., 2022). By assessing the genetic status of populations within the first few generations, conservation efforts can then be guided to maintain or enhance genetic variation, crucial for long-term adaptability and survival in a new environment (Armstrong & Seddon, 2008).

We released 50 individuals at each site, following the widely recommended $N_e = 50$ guidelines to prevent short-term inbreeding depression (Jamieson & Allendorf, 2012). However, other studies suggest that doubling this number may be necessary to prevent a loss of more than 10% in overall fitness (Frankham et al., 2014). Although our research does not yet provide a complete assessment of population viability or minimum viable population, it lays the groundwork for accurately estimating these critical values essential for future releases.

Using the species-specific reference genome, we estimated observed autosomal heterozygosity (H_O) for the Diamond Creek population (F_0) at 0.00539, compared to 0.00276 reported in a previous study using a de novo assembly and a different analytical pipeline less well-suited for assessing heterozygosity levels (Hoffmann et al., 2023). Across all populations, our H_O estimates ranged from 0.00374 to 0.00545, while the previous study reported 0.00057 to 0.00475 (Hoffmann et al., 2023). The higher mean H_O observed for the F_0 population in this study may reflect methodological differences, including reference genome choice, alignment parameters, or filtering criteria. Despite these differences, the overlapping ranges suggest both approaches provide complementary insights into *V. viatica* population genetic diversity.

Relative to this population, our analysis revealed significant differences in nucleotide diversity (π), observed autosomal heterozygosity (H_O), and total length and number of ROH between the translocated and F_0 populations of *V. viatica* after 2 years since the translocation. These effects appear scattered throughout the genome and likely reflect population contractions, evidenced by a significant increase in the number of ROH (Ceballos et al., 2018), a reduction in π of approximately 13%, and in H_O of nearly 20%. The significant effect on H_O across two generations can be compared to Nei et al. (1975), who found that in a population of the Hawaiian *Drosophila* initiated by a single mated pair, heterozygosity dropped to 65% of its original level after two generations. In the current case, our study on *V. viatica* translocated populations, which began with 50 individuals (25 males and 25 females), showed a retention of nearly 81% of the original heterozygosity after two generations. A population of this introduction size could theoretically retain approximately 98% of the genetic diversity (calculated using $H_t = (1 - 1/(2N_e))^t \; H_0$) (Kimura and Crow (1970), p. 102), and suggests that additional factors such as uneven contributions from the founders and serial bottlenecks have amplified the loss of genetic variation. These findings highlight that genetic outcomes should be assessed separately, as an adequate number of founders does not guarantee the retention of genetic variation.

The comparison of the length and number of ROH between the F_0 and F_2 provides additional insights into the genetic impact of translocation contractions. In the F_2, an increase in both the total length of ROH and the number of ROH segments reflects a reduction in genetic diversity, consistent with the effects of a demographic contraction (Ceballos et al., 2018). In contrast, the F_0 has a larger effective population size and exhibits shorter and fewer ROH segments, indicative of greater genetic variation (e.g., Li et al., 2024a, 2024b). The observed differences between these groups underscore the genetic consequences of translocations above the level of nucleotide variation.

4.1 | Conservation implications and other considerations

π, H_O, and ROH collectively provide a broad view of a population's genetic constitution. Our observations of reduced heterozygosity and π and increased ROH number and length serve to characterize the genetic contractions that may continue to shape the genetic diversity of the *V. viatica* translocations. Understanding the impact of release timing on contractions in translocated populations may be important for informing future conservation strategies. Populations experiencing ongoing genetic erosion may require interventions, such as genetic rescue through the introduction of individuals from other populations (e.g., Weeks et al., 2017), while those showing signs of recovery still warrant monitoring to ensure genetic diversity is maintained.

Despite small sample sizes in some F_2 populations, often due to low recapture success, genetic patterns remained generally consistent across populations. A comparison of populations with only one to two sampled individuals versus those with ≥3 individuals revealed no substantial differences in observed heterozygosity ($t_{12} = -0.505$, $p = .623$), total ROH length ($t_{15} = 0.792$, $p = .441$), or number of ROH segments ($t_{14} = 0.774$, $p = .451$). These findings support the value of opportunistic sampling in post-translocation contexts where individuals are difficult to detect and genomic analyses are possible. While larger sample sizes are ideal for population-level inference, even limited samples can remain informative (Cossu et al., 2022). However, further research would be useful to define optimal sample sizes for robust genetic monitoring in this system.

The observed loss of genetic diversity highlights the need for translocation protocols that prioritize larger founder populations or incorporate individuals from multiple sources (Biebach & Keller, 2012). Regular genetic monitoring should be an integral part of conservation management to detect early signs of inbreeding or further genetic decline. Future research should focus on long-term genetic assessments (Gajdárová et al., 2023) to determine whether descendant populations continue to lose diversity or show signs of genetic recovery, ultimately guiding more effective conservation translocation practices.

AUTHOR CONTRIBUTIONS

Hiromi Yagui: Conceptualization; methodology; investigation; data curation; formal analysis; writing—original draft; writing—review and editing. **Michael R. Kearney**: Conceptualization; funding acquisition; investigation; project administration; writing—review and editing; supervision. **Thomas L. Schmidt**: Conceptualization; methodology; writing—review and editing; supervision. **Ary A. Hoffmann**: Conceptualization; investigation; funding acquisition; methodology; writing—review and editing; project administration; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest regarding the research reported in this manuscript.

DATA AVAILABILITY STATEMENT

Raw sequencing data are deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject accession number (PRJNA1244118). The data can be accessed via the following link: https://www.ncbi.nlm.nih.gov/sra/PRJNA1244118.

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SUPPORTING INFORMATION

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