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# Genomic approaches to mitigating genetic diversity loss in declining populations

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## Abstract

The accelerating pace of global biodiversity loss is exacerbated by habitat fragmentation and subsequent inbreeding in small populations. To address this problem, conservation practitioners often turn to assisted breeding programmes with the aim of enhancing genetic diversity in declining populations. Although genomic information is infrequently included in these efforts, it has the potential to significantly enhance the success of such programmes. In this study, we showcase the value of genomic approaches for increasing genetic diversity in assisted breeding efforts, specifically focusing on a highly inbred population of Western burrowing owls. To maximize genetic diversity in the resulting offspring, we begin by creating an optimal pairing decision tree based on sex, kinship and patterns of homozygosity across the genome. To evaluate the effectiveness of our strategy, we compare genetic diversity, brood size and nestling success rates between optimized and non-optimized pairs. Additionally, we leverage recently discovered correlations between telomere length and fitness across species to investigate whether genomic optimization could have long-term fitness benefits. Our results indicate that pairing individuals with contrasting patterns of homozygosity across the genome is an effective way to increase genetic diversity in offspring. Although short-term field-based metrics of success did not differ significantly between optimized and non-optimized pairs, offspring from optimized pairs had significantly longer telomeres, suggesting that genetic optimization can help reduce the risk of inbreeding depression. These findings underscore the importance of genomic tools for informing efforts to preserve the adaptive potential of small, inbred populations at risk of further decline.

**KEYWORDS**
burrowing owls, captive breeding, conservation breeding, genomics, head-starting, inbreeding, telomere

## 1 | INTRODUCTION

Habitat fragmentation and associated population declines pose a significant threat to biodiversity and ecosystems worldwide (Haddad et al., 2015). Inbreeding in small populations is a major concern, as it can lead to a loss of adaptive potential, a reduction in fitness via inbreeding depression and in extreme cases, an increase in the likelihood of extinction (Keller & Waller, 2002). This cascade effect is often referred to as the "extinction vortex" (Fagan & Holmes, 2006; Frankham, 1995; Gilpin & Soulé, 1986). To address these challenges, managers are increasingly turning to assisted breeding programmes like genetic rescue, captive breeding and head-starting, to prevent extinction and preserve adaptive potential (Frankham, 2015; Whiteley et al., 2015). Despite the potential of genomic data to enhance the success of these programmes, its utilization remains infrequent (but see Galla et al., 2020; Miller et al., 2012). Consequently, there is an urgent need for studies that showcase the value of genomic approaches for augmenting genetic diversity within small and declining populations.

In the past, the integration of genetic information into assisted breeding programmes has varied greatly across different initiatives. Some programmes do not collect genetic data before genetic rescue efforts and instead aim to enhance genetic diversity by translocating individuals from different subspecies (Harrisson et al., 2016). Other genetic rescue programmes focus on translocating individuals from genetically diverse populations of the same species, which is suitable when such populations exist (Pimm et al., 2006; Westemeier et al., 1998). Alternatively, captive breeding programmes commonly utilize genetic information to optimize pairing success (Allendorf et al., 2022; Ballou & Lacy, 1995; Giglio et al., 2016; Ivy & Lacy, 2012), but most of these programmes have yet to explore the power of genomic approaches. In this study, we explore the effectiveness of using genomic approaches to improve the success of a particular type of assisted breeding programme known as head-starting. Head-starting differs from traditional captive breeding in that it involves temporarily removing animals from the wild during their most vulnerable life history stage and then returning them to the wild prior to reproduction. While our focus is on using genomic tools to optimize a head-starting programme, our findings are relevant to all assisted breeding efforts that utilize genomic data.

Robust genetic estimates of relatedness and inbreeding are critical to success of assisted breeding efforts. Prior to genomics, multigenerational pedigrees were the best way to incorporate ancestry information, reduce kinship and estimate inbreeding (Ballou et al., 2010; Ballou & Lacy, 1995; Ivy & Lacy, 2012). With the advent of next-generation sequencing, it has become clear that genome-wide sequencing data have the potential to provide greater precision in estimating co-ancestry (Galla et al., 2020) compared to even the most robust pedigrees. Moreover, several studies have shown that characterizing inbreeding based on long stretches of consecutive homozygous genotypes, also known as runs of homozygosity (ROH), provides a better measure of individual levels of homozygosity than pedigree estimates of inbreeding (Kardos et al., 2015; Purfield et al., 2012). ROH also makes it possible to identify specific regions of homozygosity associated with deleterious variants (Sams & Boyko, 2019; Szpiech et al., 2013; Zhang et al., 2015) or inbreeding depression (Robinson et al., 2019; Stoffel et al., 2021). Thus, if working within a small, inbred population with founders that already exhibit excessively low heterozygosity, even pairing among unrelated individuals, may perpetuate homozygosity if these individuals share ROH (Gómez-Sánchez et al., 2018; Kardos et al., 2018). Despite the advantages of pairing individuals with contrasting region-specific ROH patterns, this information has not yet been incorporated into assisted breeding programmes.

In addition to helping inform optimal pairings, genomic tools can also be used to assess the long-term success of assisted breeding programmes. In the limited number of cases where the follow-up studies have been conducted, researchers have used short-term metrics such as offspring number or genetic diversity to quantify success. However, perhaps the most important metric of success in any breeding programme is improved fitness. While fitness is often difficult to quantify in the field because it requires following offspring for multiple generations, recent advances in telomere research suggest that measuring telomere length may offer a promising, cost-effective method of quantifying long-term fitness. Telomeres are highly conserved, non-coding, repetitive sequences of DNA that form caps at the ends of eukaryotic chromosomes (Blackburn, 2005). Telomeres shorten with each round of cell division and the rate of shortening can be accelerated due to oxidative stress (Chatelain et al., 2020; Monaghan, 2010; Olovnikov, 1996). Progressive shortening has been linked to the aging process as variation in telomere length is correlated with survival in many species (Boonekamp et al., 2014; Sahin & DePinho, 2010; Vedder et al., 2022; Wilbourn et al., 2018). Research has shown that early-life telomere length predicts lifespan in birds (Eastwood et al., 2019; Heidinger et al., 2012), and thus can potentially be used as a proxy for longevity or lifetime reproductive success. Here, we demonstrate the value of measuring telomere length as a method for assessing the success of assisted breeding efforts.

In this study, we used advanced genomic tools to optimize assisted breeding efforts in a highly inbred population of Western burrowing owls (*Athene cunicularia hypugaea*) in northern California. This owl species has been declining in many areas due to various factors, such as habitat loss due to urbanization, secondary poisoning by rodenticides and impacts at wind energy sites (Desmond et al., 2000; Poulin et al., 2020; Smallwood et al., 2009, Trulio & Chromczak, 2007). To address this issue, a head-start breeding programme, the Juvenile Burrowing Owl Overwintering Program, was launched in urban Santa Clara County, CA, with the goal of reducing juvenile mortality and increasing genetic diversity of remaining breeding colonies. To that end, we conducted whole-genome re-sequencing of potential breeders over 3 years and used these data to select optimal breeding pairs for the head-starting programme. Our main aim was to produce the most genetically diverse offspring by analysing levels of inbreeding across the genome of potential parents and pairing individuals with contrasting runs of homozygosity. To evaluate the short-term

success of our strategy, we monitored genetic diversity across multiple generations and collected data on reproductive success, brood size and offspring size. To evaluate long-term success, we measured a telomere length, a genetic tool that is increasingly being used as a proxy for long-term fitness, but has not yet been used in the context of assisted breeding programmes. Our findings have important implications for assisted breeding programmes not only for Western burrowing owls but for other species as well. By providing essential information on the genetic health of individuals and patterns of inbreeding, this study can help inform strategies for enhancing genetic diversity and ensuring the long-term survival of endangered or declining populations.

## 2 | MATERIALS AND METHODS

### 2.1 | Head-start breeding programme

To identify individuals for overwintering in captivity, we monitored burrowing owl nests during the peak breeding season from April 1 through July 31 at four remnant breeding sites in the South San Francisco Bay region. Beginning in early May, we conducted weekly 30-min nest visits and monitored each nest until 14- to 21-day-old chicks were observed or nest failure was determined. Chicks for head-staring ($n=14$ in 2019, $n=13$ in 2020 and $n=17$ in 2021) were collected from successful nests, banded, weighed and measured prior to transporting them to the Peninsula Humane Society (PHS) in Burlingame, California.

Initially, chicks were monitored in incubators and, if necessary, then moved to small indoor cages that contained branches to perch on and logs to hide behind. After acclimation (i.e., eating reliably) and weighing at least 135 g, we collected blood samples from the brachial vein and 5–6 breast feathers from each overwintered owl for genomic analysis. Initial feather analysis was performed by Bird Sexing Solutions. The owls were then relocated to a 10 × 20 × 6 ft outdoor enclosure constructed inside a rooftop aviary, exposed to the sky, where we built 10 aboveground artificial burrow systems (tunnel and nest chamber) secured with river rocks and installed numerous perches. Following relocation outdoors, PHS staff weighed the owls weekly and if their weights were stable, or improving, subsequent weight checks were performed monthly. In December, we separated the owls by sex to prevent premature mating prior to release. Sex was later reconfirmed using genomic estimates of sex prior to release of optimal pairs in early spring (see Methods below).

While the goal of the head-start breeding programme was to release birds back into the wild the subsequent breeding season, in 2020 two breeding pairs were kept in captivity for 2 years in order to initiate a captive breeding programme. While the same methodology for pair optimization was followed, these two captive breeding pairs were excluded from some genomic analyses of reproductive success (see below – brood size, telomere length and body condition measures) due to the different environmental conditions which they experienced during their time in the captive breeding programme.

### 2.2 | DNA extraction and genomic library preparation

Qiagen DNeasy Blood and Tissue Kits (Qiagen Inc.) were used to extract DNA from 44 individuals at the Conservation Genomics Laboratory at Colorado State University. Twenty microliters of blood suspended in Queen's lysis buffer was extracted using the standard blood and tissue extraction protocol. We used a modified version of Illumina's Nextera Library Preparation protocol to prepare whole-genome sequencing libraries and pooled the libraries by equal mass prior to sequencing. In short, the first step in library prep is the tagmentation reaction that fragmented DNA and then tagged the DNA with adapter sequences in a single step. Library amplification is completed using a limited-cycle PCR programme, followed by a reconditioning PCR step and a cleaning step with AMPure XP beads that size selects short library fragments. Libraries were quantified using a Qubit plate reader and normalized to a concentration of 2 ng/μL per individual. Pooled libraries each year were sequenced on an individual Illumina HiSeq4000 (Illumina) lane with a target coverage of 6× per individual.

### 2.3 | Bioinformatic processing

A pipeline adapted from the Genome Analysis Toolkit (GATK) Best Practices Guide (Van der Auwera et al., 2013) was used to process raw reads before genotype calling. Briefly, we processed paired-end raw sequence reads, trimming adapters using TrimGalore (Krueger et al., 2021) and then aligning to the burrowing owl reference genome (Barr et al., in revision) using *bwa-mem* (Li & Durbin, 2009). PCR duplicates were marked with *samtools* version 1.6 (Li et al., 2009), and read groups were added with *picard* 3.0.0 (Broad Institute, 2019). Since we lack a database of known variants, we instead used the bootstrapping method of base quality score recalibration recommended by GATK. We first called raw genotypes with GATK HaplotypeCaller (Van der Auwera et al., 2013) and *samtools* (Li et al., 2009), filtered for missingness and quality scores (minimum base quality Phred score of 20), and then used the intersection of these high-quality variants as input for recalibration with BaseRecalibrator (Van der Auwera et al., 2013).

Joint genotype calling at all sites across the reference genome was performed with GATK HaplotypeCaller for each juvenile set separately using these recalibrated bam files. Genotypes were filtered for quality and depth, leaving only high-quality biallelic SNPs. Only genotypes with at least six supporting reads and high-quality scores (minimum Phred score of 30) were included. Variants failing the recommended GATK hard filters identifying systemic errors were also excluded (QD < 2.0, FS > 60.0, MQ < 40.0, MQRankSum < −12.5, ReadPosRankSum < −8.0, SOR > 3.0, QUAL < 30), as well as excess heterozygosity (>50% of individuals heterozygous).

The burrowing owl reference genome assembly had an average depth of 49× and was highly complete and highly contiguous. 96.8% of the known genes from class Aves are captured by this reference

genome (BUSCO), and the total length of 1.25Gb is spread across 3830 scaffolds at an N50 of 2.6 Mb. To orient the variants across the burrowing owl genome, these scaffolds were mapped to the Zebra finch chromosomal genome assembly (*Taeniopygia guttata*; GCA_000151805.2) using satsuma2 synteny (Grabherr et al., 2010). We used custom R scripts to convert scaffold position of our variants to zebra finch chromosome position, and it was on these chromosome positions that the analyses to detect runs of homozygosity were conducted.

## 2.4 | Genomic analysis of sex, relatedness and inbreeding status

To confirm the sex of individual birds, we used universal PCR primers (Ellegren, 1996), as well as coverage comparison between the first five autosomes and the sex chromosome. In birds, females are the heterogametic sex; therefore, individuals with half the coverage estimated from scaffolds that align to the Z chromosome compared to the autosomes are diagnosed as female, and individuals with comparable coverage levels between autosomes and the Z chromosome are diagnosed as male (Figure S1).

To estimate the average relatedness across the genome of an individual, we used the software program NgsRelate (Hanghøj et al., 2019; Korneliussen & Moltke, 2015). NgsRelate calculates Jaccard coefficients to identify siblings and half siblings in each breeding group based on their probability of sharing at least one allele (K1). We confirmed full siblings identified in NgsRelate with pedigree information and then estimated distantly related individuals based on the distribution of Jaccard values (Figure S2).

To measure inbreeding, we used two methods: (1) an individual measure of inbreeding that estimates the proportion of the genome that is homozygous, $F_{ROH}$; and (2) a region-specific map of homozygosity across the genome. To calculate the proportion of the genome that is homozygous ($F_{ROH}$) and visually assess runs of homozygosity (ROH), we used the R (R Core Team, 2020) program and *detectRuns* program (Biscarini et al., 2019) with the default parameters of the *consecutiveRuns* function, adjusting minSNP = 50, maxGap = $10^6$, minLength = 10 kb, and maxMissRun = 1 parameters. We then used *plink v. 1.9* (Purcell & Chang, n.d.; Chang et al., 2015) with the same parameters, --homozyg-snp 50 and --homozyg-het 3 to calculate runs of homozygosity and created clusters of individuals with a 50% match of overlapping homozygous regions. By mapping homozygous regions (areas with no genetic diversity) interspersed with heterozygous regions (areas with genetic variation) for each individual, we compared specific runs across the genome between individuals and avoided pairing individuals with overlapping homozygous regions in order to maximize genetic diversity.

## 2.5 | Optimal pairing decision tree

Our objective was to create optimal breeding pairs for the headstart programme by combining genomic estimates of kinship and inbreeding with field-based genealogy records. Results from each genomic analysis were summarized into a Decision Tree Matrix (Figure 1) prior to selection of optimal breeding pairs. In order to select genetically optimal pairs, we used pedigree and genetic relatedness estimates to avoid selecting closely related individuals and then compared ROH between individuals to select individuals with non-overlapping regions of homozygosity (Figure 2; Figure S3). Following overwintering at a rehabilitation centre, five genetically optimized pairs were soft released in 2020 and three pairs were soft released in 2021 into different breeding sites from their capture location, whenever possible. In 2021, two genetically optimized pairs were kept in captivity with plans for release in 2023.

## 2.6 | Assessing success of genetic pairings using field and genetic metrics

To compare the success of genetically optimized breeding pairs with natural pairing in the field (i.e.; "non-optimized pairs"), nest success was measured at a total of 14 nests in 2020 (Table 1). Natural pairing included pairings among resident birds or pairings between single overwintered juveniles and a resident bird. Nest success was determined when we observed chicks that were at least 14 days and older. A failed female/pair or nesting attempt was determined by the absence of chicks following food delivery into a nest burrow, the absence of <14-day-old chicks after first sighting, the absence of chick emergence, the death of a female or an evidence of human disturbance to an active nest. Undetermined nest status implied the female/pair vacated the nest and was not observed or the pair did not receive at least three observations in order to determine nest success or failure. In addition, we measured brood size and offspring size (using tarsus length corrected by date measurement as a proxy for size). For discrete field measures, such as reproductive success (success = yes or no), we created a contingency table and tested significance using the Fisher's tests. For continuous measures, significance was established using a *t*-test.

To test whether the nine offspring of six genetically optimized pairs showed a significant increase in genetic diversity when compared to 21 offspring of 11 non-optimized pairs, we compared the proportion of the genome that was homozygous ($F_{ROH}$) between the two groups. Because genetic diversity is not influenced by environmental conditions, offspring from both the four reintroduced pairs and the two captive breeding pairs were included in this analysis.

To assess the potential for increased long-term fitness in genetically optimized pairs relative to non-optimized pairs, we measured telomere length. However, because telomere length is known to be influenced by environmental conditions (Haussmann & Heidinger, 2015; Herborn et al., 2014; Marasco et al., 2021; Nettle et al., 2015), the telomere analysis was restricted to the reintroduced birds only. To measure telomere length, DNA purity and concentration was measured using a NanoDrop 8000 spectrophotometer (Thermo Scientific), and DNA integrity was visually

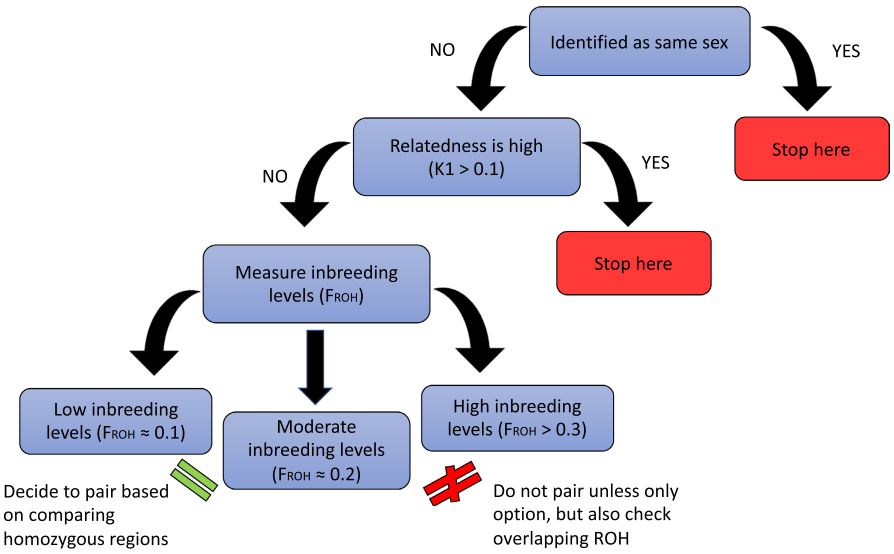


**FIGURE 1** Optimal pairing decision tree that incorporates genomic estimates of sex, relatedness, and genome-wide inbreeding to create optimal burrowing owl breeding pairs.

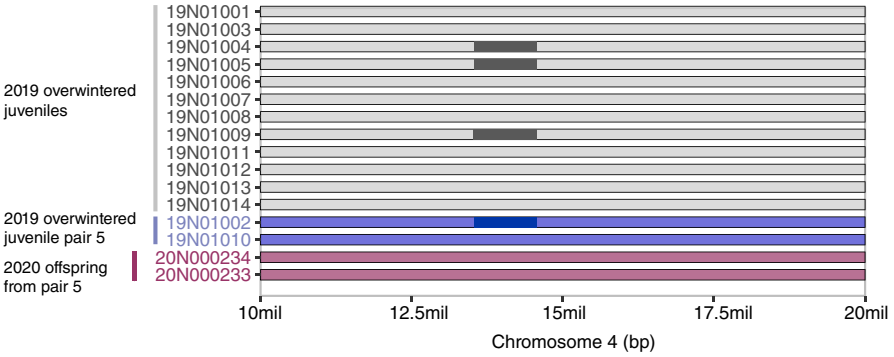


**FIGURE 2** Visualization of runs of homozygosity (ROH, darker solid bar) on chromosome 4 of 2019 overwintered juveniles, the 2019 genetic pair 5 created with 2019 juveniles 19N01002 and 19N01010 and the offspring resulting from pair 5 collected in 2020.

**TABLE 1** Overview of number of pairs, number of offspring and number of collected and sequenced offspring sampled of optimized (OP) and non-optimized (NOP) pairs for the 2020 and 2021 breeding seasons.

| Breeding season | # optimized pairs (OP) | # single owls | # chicks produced by OP | # OP juveniles collected | # failed OP | # non-optimized pairs (NOP) | # chicks produced by NOP | # NOP juveniles collected |
|---|---|---|---|---|---|---|---|---|
| 2020 | 5 pr released | 3 females | 21 | 8 | 0 | 4 pr | 14 | 5 |
| 2021 | 3 pr released; 2 pr captive breeders | 3 males | 4 | 4 | 3 pr released | 10 pr | 18 | 13 |

assessed on an agarose gel (Eastwood et al., 2018). Following Criscuolo et al. (2009), we used qPCR to measure telomere length relative to the glyceraldehyde-3-phosphate dehydrogenase control gene. Samples were run in triplicate in order to estimate intra- and inter-assay repeatability (Eastwood et al., 2018; see Data S1). To test whether telomere length was influenced by field and genetic metrics of success (offspring size, sex, and $F_{ROH}$), we used Akaike information criterion (AIC; Akaike, 1973) model selection (Burnham & Anderson, 2002). Different models had different combinations of success metrics included and the model with the lowest AICc value in each set was considered to be best supported by the data. We also calculated ΔAICc (difference between each model, $w_e$; and the top-ranking model) and Akaike weights ($w_i$, estimates of the probability that it is the best model given the data and the model set).

## 3 | RESULTS

### 3.1 | Genomic analysis of sex, relatedness and inbreeding status

The average depth of sequencing for the 44 sequenced birds was 6.11× (range: 4.55–8.66×). After filtering to remove systematic errors and keeping biallelic loci, we identified 7.55 million, 7.27 million and 6.81 million variants among our juveniles in 2019, 2020 and 2021, respectively. Genetic sexing using coverage comparisons between autosomes and the Z chromosome revealed a slightly skewed sex ratio each year, with nine females and five males in 2019, four females and nine males in 2020, and 10 females and seven males in 2021 (Table S1).

Kinship analysis identified 17 related pairs in 2019, nine in 2020 and 18 in 2021 with a K1 > 0.1; our cut-off for individuals being too related to pair together (Figure S2; within the green circle). Using whole-genome data, inbreeding within an individual was calculated based on the proportion of homozygous chromosomal segments across the genome ($F_{ROH}$), with 1 being complete homozygosity and 0 indicating no stretches of homozygosity and thus no inbreeding (Kardos et al., 2017, 2018). Over all 3 years, $F_{ROH}$ ranged from 0.064 to 0.399 (Table S1). We further compared region-specific homozygous runs between pairs of individuals and oriented the regions along the zebra finch chromosomal assembly. In 2019, we identified long stretches of overlapping homozygosity on 14 chromosomes (1, 2, 3, 4, 5, 7, 8, 9, 12, 13, 17, 21, 23 and the Z chromosome). In 2020, we identified long stretches of overlapping homozygosity on chromosomes 1 and 6 and the Z chromosome. In 2021, we identified long stretches of overlapping homozygosity on six chromosomes (4, 5, 6, 9, 11 and Z).

### 3.2 | Optimal pairing decision tree

We combine all the information to create a step-by-step breeding pair decision tree that was integrated into the Juvenile Burrowing Owl Overwintering Project (Figure 1). The first pairs filtered out were those that were the same sex, greatly reducing potential pairs, especially when the sex ratio was skewed. Given the skewed sex ratio, we retained only 45 viable pairs in 2019, 36 potential pairs in 2020 and 70 potential pairs in 2021. Next, we filtered out pairs if relatedness was too high (K1 > 0.3). Here, we also advised against pairing between individuals with intermediate probability of sharing one allele (K1 > 0.1), which denotes more distantly related individuals. In 2019, 27 pairs passed our kinship filter and remained viable pairs, while 29 remained viable in 2020 and 58 in 2021. Finally, we used genomic inbreeding levels (i.e.; $F_{ROH}$) and region-specific inbreeding maps to pair highly inbred birds with more genetically diverse birds, ultimately removing pairs that had significant ROH overlap. In 2019, we had several clusters of homozygous regions and thus identified pairs with no overlapping runs as "good" pairs ($n = 13$), identified pairs with only 1 region or a short overlapping region as "ok" pairs ($n = 9$), and identified pairs with multiple overlapping regions as "not ideal" pairs ($n = 3$). In 2020, we identified 18 good pairs with no overlapping homozygous regions, and any pairs with overlapping homozygous regions were considered "not ideal" ($n = 12$). In 2021, we identified 40 good pairs with no overlapping homozygous regions, 10 pairs with overlapping regions on the Z chromosome that were considered ok, and 18 pairs that were not considered good due to multiple overlapping homozygous regions.

### 3.3 | Assessing success of genetic pairing

To compare the success of optimized versus non-optimized genetic pairs, we investigated both genomic and field measures of success. Notably, we saw differences in genetic-based metrics of success, such as $F_{ROH}$ and telomere length. In 2020 and 2021, the offspring of optimized pairs had significantly lower levels of inbreeding than the offspring of non-optimized pairs (lower $F_{ROH}$; $p$-value = .0033; Figure 4a). Furthermore, testing whether there was a significant relationship between telomere length, inbreeding and other success metrics using AIC model selection (Table 2), we found that $F_{ROH}$ was the best predictor of telomere length. In addition, linear regression revealed a significant relationship between telomere length and levels of inbreeding, with shorter telomeres being found in birds with higher levels of inbreeding ($p$-value = .0199; Figure 3). AIC model selection also determined the best-fit model underlying telomere length was whether the pair was genetically optimized; in fact, it explained 42% of the variation (Table 3; Figure 4b). Correspondingly, when the telomere length between optimized and non-optimized offspring was compared, we found that juveniles from non-optimized pairs had significantly shorter telomeres ($p$-value = .042; Figure 2b). Alternatively, there was no difference in short-term, field-based metrics of success (brood size or offspring size; Figure 4c,d). We did not see significantly larger brood sizes ($p$-value = .324; Figure 4c) or offspring size as measured by tarsus length corrected by banding age (in days; $p$-value = .463; Figure 4d) in optimized pairs compared to non-optimized pairs.

## 4 | DISCUSSION

Here, we demonstrate how genomic tools can be harnessed to optimize pairing decisions and assess success in head-start and captive breeding programmes. While previous work has used overall estimates of kinship to optimize breeding pairs, here we show that the additional step of pairing males and females with non-overlapping runs of homozygosity results in lower inbreeding in offspring. While the long-term fitness metric, telomere length, was significantly associated with genetic optimization, such that the offspring of optimized pairs have longer telomeres and likely higher lifetime fitness, the short-term metrics of success (i.e., brood size and offspring size) were not different between optimized and non-optimized pairs. Overall, this work shows that genomic methods provide effective tools for maximizing the success and managing the outcomes of assisted breeding programmes in cases where long-term field-based follow-up studies are not feasible.

### 4.1 | Genomic tools to inform optimal pairing

Recent advances in genomics have allowed for greater precision in identifying highly inbred regions in the genome (Kardos et al., 2015). To our knowledge, this work represents that the first-time genome-wide estimates of homozygosity have been used to inform optimal breeding pairs in an assisted breeding programme. By bringing juvenile burrowing owls into captivity for 9–10 months, we were able to keep them alive during a critical period in their life history, map

**TABLE 2** Candidate model set using realistic combinations of variables that best predict telomere length.

| Model | K | AICc | Delta_AICc | AICcWt | Cum.Wt | LL |
|---|---|---|---|---|---|---|
| $F_{ROH}$ | 3 | 29.55 | 0 | 0.22 | 0.22 | −11.25 |
| $F_{ROH}$ + Year | 4 | 29.67 | 0.13 | 0.21 | 0.43 | −9.93 |
| Null model | 2 | 30.54 | 1 | 0.13 | 0.56 | −13.02 |
| $F_{ROH}$ + Size | 4 | 31.61 | 2.07 | 0.08 | 0.64 | −10.85 |
| Year | 3 | 32.21 | 2.67 | 0.06 | 0.7 | −12.58 |
| $F_{ROH}$ + Sex | 4 | 32.29 | 2.74 | 0.06 | 0.75 | −11.23 |
| Size | 3 | 32.47 | 2.92 | 0.05 | 0.81 | −12.69 |
| $F_{ROH}$*Year | 5 | 32.59 | 3.05 | 0.05 | 0.85 | −9.87 |
| Sex | 3 | 32.89 | 3.34 | 0.04 | 0.9 | −12.92 |
| Year + Sex | 4 | 34.32 | 4.78 | 0.02 | 0.92 | −12.25 |
| $F_{ROH}$*Size | 5 | 34.65 | 5.1 | 0.02 | 0.93 | −10.82 |
| $F_{ROH}$*Sex | 5 | 34.78 | 5.23 | 0.02 | 0.95 | −10.96 |
| Year + Size | 4 | 34.95 | 5.41 | 0.01 | 0.96 | −12.52 |
| Year*Size | 5 | 34.99 | 5.44 | 0.01 | 0.98 | −10.99 |
| Sex + Size | 4 | 35.13 | 5.58 | 0.01 | 0.99 | −12.61 |
| Year*Sex | 5 | 37.07 | 7.52 | 0.01 | 1 | −12.11 |
| Sex*Size | 5 | 38.13 | 8.58 | 0 | 1 | −12.57 |

*Note*: Explanatory variables $F_{ROH}$, size (tarsus length corrected for day), breeding season (year) and sex.

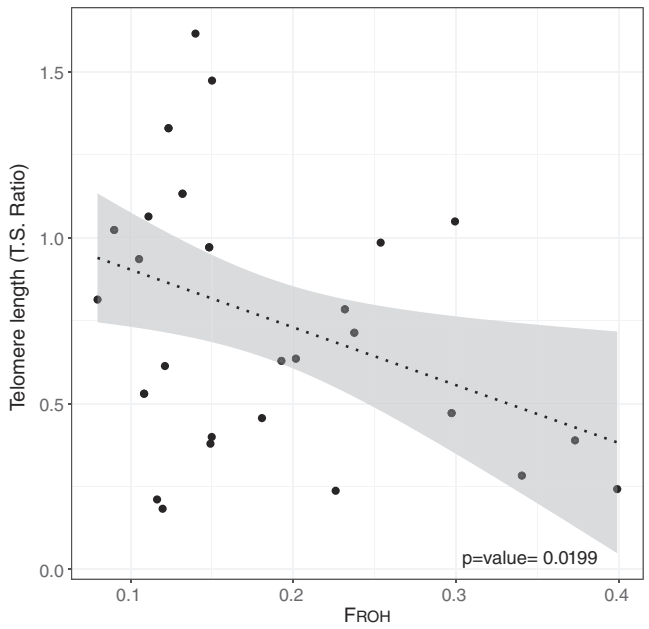


**FIGURE 3** Significant linear relationship between proportion of homozygous regions ($F_{ROH}$) and juvenile telomere length (T.S. ratio).

patterns of inbreeding (ROH) at high resolution, genetically estimate sex and relatedness and use the resulting information to develop an optimal pairing decision tree. Over the course of the study, we released 15 optimized pairs into the field; all five pairs released in the first year of the programme (2020) fledged offspring, while none of the three pairs released the second year (2021) was successful. The difference in nesting success between the years was seen across all wild and optimized pairs and was thought to have resulted from extreme drought and shortages of insects in 2021 (Higgins, personal communication). Across all years, we found that the offspring of optimized pairs had significantly higher genetic diversity and fewer inbred regions than the offspring of non-optimized pairs (Figure 2; Figure S3). In the future, a similar approach could be adapted for reducing the frequency of known deleterious recessive genes in species where the chromosomal positions of such genes are known (Sams & Boyko, 2019; Sumreddee et al., 2020; Szpiech et al., 2013). Overall, our work demonstrates that high-resolution genomic tools provide a powerful method for increasing genetic diversity and maintaining adaptive potential in declining or inbred populations.

## 4.2 | The value of head-starting versus other assisted breeding strategies

Assisted breeding approaches differ in their effectiveness in maintaining genetic diversity over the long-term (Frankham, 2015, 2016). While it is known that translocating individuals from genetically diverse neighbouring populations (i.e., genetic rescue) can benefit genetic diversity in the short-term, such benefits may disappear if not followed up with additional translocations and monitoring. An example is the Isle Royale grey wolf (*Canis lupus*) population, which was supplemented with individuals from a mainland Minnesota population to address the issues of dwindling numbers and high inbreeding. Although translocation initially reduced inbreeding depression, inbreeding levels rapidly increased after the translocation without additional intervention, leading to a sharp decline in population size and imminent extinction (Adams et al., 2011; Hedrick et al., 2014, 2019). In contrast, our findings demonstrate that the repeated use

**TABLE 3** Candidate model set using realistic combinations of variables that best predict telomere length.

| Model | *K* | AICc | △AIC | $w_i$ | −2LL |
|---|---|---|---|---|---|
| Optimized | 3 | 17.21 | 0 | 0.59 | −4.27 |
| Optimized + Size | 4 | 20.74 | 3.53 | 0.1 | −3.51 |
| Null model | 2 | 21.34 | 4.13 | 0.07 | −8.07 |
| Optimized + Sex | 4 | 21.37 | 4.16 | 0.07 | −4.18 |
| Optimized + $F_{ROH}$ | 4 | 21.43 | 4.22 | 0.07 | −4.21 |
| Size | 3 | 23.42 | 6.22 | 0.03 | −7.21 |
| $F_{ROH}$ | 3 | 23.98 | 6.77 | 0.02 | −7.66 |
| Optimized*Sex | 5 | 24.46 | 7.25 | 0.02 | −2.95 |
| Sex | 3 | 24.79 | 7.58 | 0.01 | −8.06 |
| Optimized*Size | 5 | 26.59 | 9.38 | 0.01 | −3.29 |
| Optimized*$F_{ROH}$ | 5 | 26.97 | 9.76 | 0 | −4.2 |
| $F_{ROH}$ + Size | 4 | 27.32 | 10.11 | 0 | −6.8 |
| Size + Sex | 4 | 27.67 | 10.46 | 0 | −6.98 |
| Size*Sex | 5 | 31.57 | 14.37 | 0 | −5.79 |
| $F_{ROH}$*Size | 5 | 32.05 | 14.84 | 0 | −6.02 |
| Optimized*Size*Sex | 8 | 68.1 | 50.89 | 0 | −2.05 |
| Optimized*Size*$F_{ROH}$ | 9 | 101.06 | 83.85 | 0 | 3.47 |

*Note*: Explanatory variables include genetic pair optimizations (yes or no), $F_{ROH}$; size (tarsus length corrected for day) and sex.

of head-starting in combination with genomic tools can increase genetic diversity over generations. For example, we show that the levels of inbreeding in the wild-caught parents in the first year of our study ranged from 9% to 39.9%, whereas levels in the offspring of optimized pairs ranged from 8% to 20.2%. Our work suggests that a genomically informed head-starting programme spanning multiple years may be a promising alternative to genetic rescue efforts for improving the genetic health of declining populations. Future work monitoring the levels of inbreeding across all optimized and non-optimized pairs for more generations will reveal the extent to which our findings continue to improve the genetic health of burrowing owls in this region.

### 4.3 | Measuring the success of assisted breeding programmes

To assess the effectiveness of assisted breeding programmes, reproductive output metrics such as annual variation in clutch size, offspring size and offspring survival can be measured (Ostermann et al., 2001). When inbreeding becomes extreme, as in the case of inbreeding depression, these reproductive metrics can decline due to the accumulation of deleterious recessive alleles in offspring from inbred parents. A study of the helmeted honeyeater, for example, found that extremely high levels of inbreeding (75%–95% homozygosity across the genome) led to a reduction in reproductive output (Harrisson et al., 2019). In our study, the highest percentage of homozygosity across the genomes of wild-caught burrowing owls we observed was 39%, which is below the level seen in the honeyeater population. Furthermore, unlike the case of the helmeted honeyeater, we did not detect any annual difference in reproductive output between optimized and non-optimized pairs, suggesting that non-optimized pairs did not suffer from inbreeding depression relative to optimized pairs. There was also no correlation between nest success and genetic diversity of offspring, although this may be due to environmental factors during the study period overshadowing any minor differences in reproductive output due to genetic diversity. While these findings suggest that inbreeding depression may not be a major concern in our population at the present time, it is important to note that an individual's true fitness is determined over their lifespan. Therefore, a more comprehensive evaluation of the success of our programme would be to investigate whether there are any differences in long-term fitness.

Typically, measuring the impact of inbreeding on lifetime fitness in small populations requires long-term field data, which can be expensive and difficult to obtain (Fox & Reed, 2011; Losdat et al., 2016). To circumvent this challenge, we employed the use of telomere length as a proxy for lifetime fitness, as this relationship has been previously validated across species (Eastwood et al., 2019; Haussmann et al., 2005; Heidinger et al., 2012; Wilbourn et al., 2018). Here, we find a significant correlation between telomere length and inbreeding, with birds that exhibit lower levels of homozygosity throughout their genomes having longer telomeres. A similar relationship between inbreeding and telomere length was recently documented in two small island populations of wild house sparrows (*Passer domesticus*) known to be affected by inbreeding depression (Pepke et al., 2022). Furthermore, we demonstrated that optimized breeding pairs showed a significant association with telomere length, with offspring of optimized pairs exhibiting relatively longer telomeres. If telomere length is truly

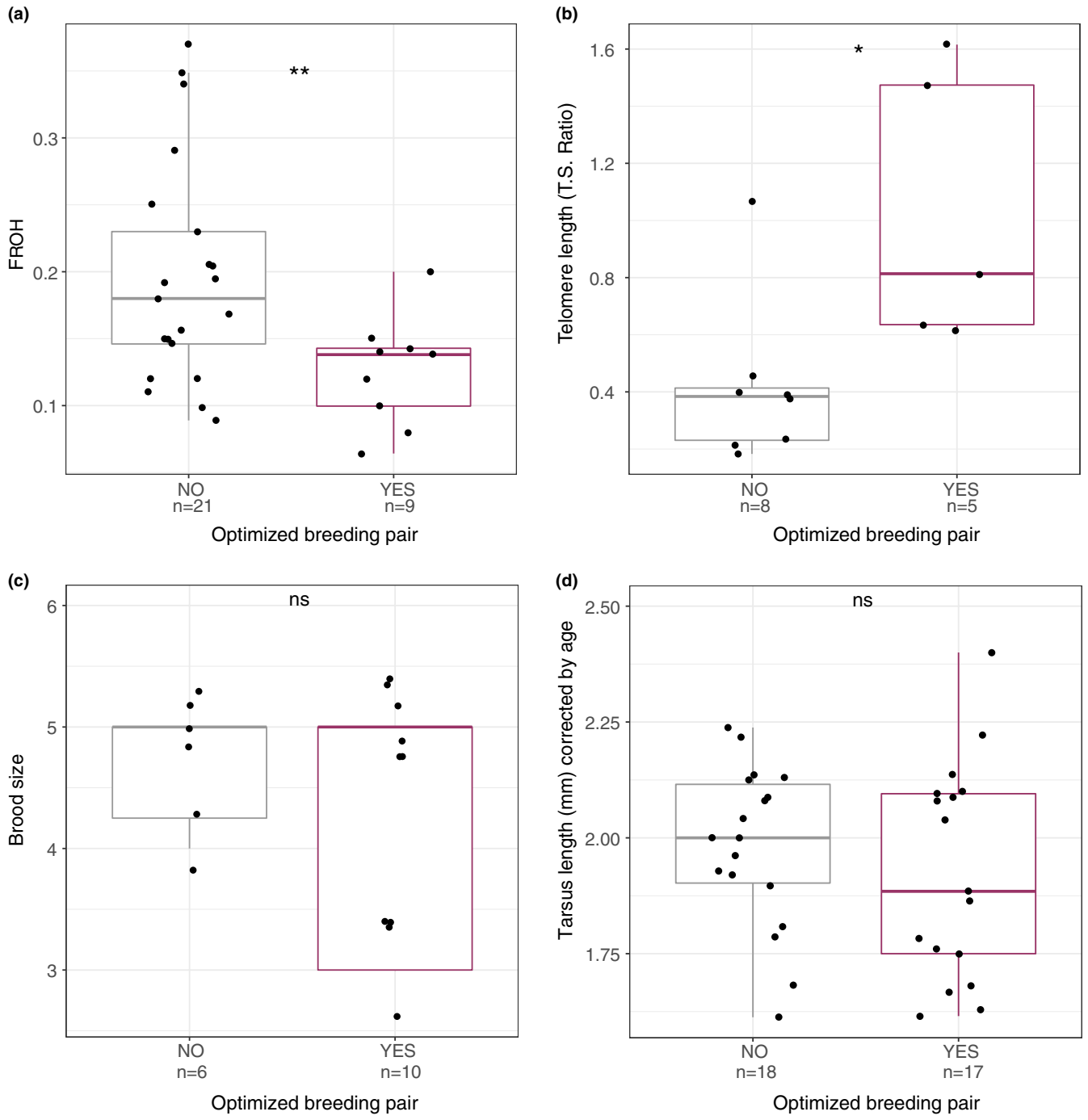


**FIGURE 4** Success of optimized (purple) genetic pairs compared to non-optimized (grey) resident breeders as measured by (a) proportion of homozygosity in offspring, $F_{ROH}$, (b) offspring telomere length in 2020, (c) nest brood size and (d) offspring tarsus length (mm) corrected for day of banding. Offspring of optimized breeding pairs had significantly lower levels of homozygosity (a) and longer telomeres (b), indicating greater long-term fitness than non-optimized resident breeding pairs; however, there was no significant differences in short-term field-based metrics of fitness (c and d).

a proxy for lifetime fitness in the burrowing owl, our results suggest that optimizing breeding pairs using genomic tools helps reduce the chance of inbreeding depression over the lifespan of an individual. To have confidence in our results, follow-up work will include long-term field-based research analysing the association between optimization, telomere dynamics and lifelong reproductive success. This work represents a first step in utilizing telomeres as a biomarker of success in assisted breeding programmes without the need for expensive long-term studies.

While we demonstrate a clear link between telomere length and inbreeding in burrowing owls, understanding the potential mechanistic basis behind this correlation is less clear. Individual differences in telomere length are established early in life (Entringer et al., 2018) and reflect costs associated with environmental stressors (Bauch

et al., 2022; Dupoué et al., 2022; Kärkkäinen et al., 2019) and somatic functioning (Metcalfe & Olsson, 2022). Since all burrowing owls in our telomere analysis were exposed to similar environmental conditions, one possible explanation for our findings is that the decrease in genetic diversity in the offspring of non-optimized pairs is linked to a reduction in cellular functioning stemming from inbreeding (Bebbington et al., 2016). Previous studies have shown that inbreeding depression can result in a weaker immune response (Charpentier et al., 2008; Reid et al., 2003) and lower metabolic efficiency (Ketola & Kotiaho, 2009; Kristensen et al., 2005), which can both elevate oxidative stress (de Boer et al., 2018). Consequently, inbred individuals might have higher levels of oxidative damage that could lead to shorter telomeres (von Zglinicki, 2002). To further test these ideas, future research should focus on comparing levels of oxidative stress and immune response in relation to inbreeding.

## 5 | CONCLUSIONS

The results of this study indicate that optimizing breeding pairs based on genomic estimates of homozygosity can effectively increase genetic diversity in assisted breeding programmes. Although short-term reproductive outcomes were similar for optimized and non-optimized pairs, long-term fitness estimates, as measured by telomere length, showed that genetic optimization can help reduce the risk of inbreeding depression. While more research is needed to confirm telomere length as a fitness indicator and understand the underlying mechanisms, our findings suggest that it could be a cost-effective biomarker for assessing the success of assisted breeding programmes. These results highlight the potential of genomic tools for preserving adaptive potential in populations that are at risk of further decline in a changing world.

### AUTHOR CONTRIBUTIONS

C.M.B., L.A.T. and K.C.R. conceived the study; D.A.C. and P.G.H. collected juvenile samples, overwintered juveniles, released pairs, conducted field surveys, and monitored nests; C.R. performed lab work and Fluidigm genotyping; C.M.B. performed the population genetic and inbreeding genetic analyses with contributions from K.C.R.; M.R. performed telomere analyses with contributions from C.M.B.; and C.M.B. and K.C.R. wrote the paper with contributions from all authors.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

### OPEN RESEARCH BADGES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://doi.org/10.5068/D11974.

### DATA AVAILABILITY STATEMENT

Individual genotype data, metadata linking juvenile telomere length, genetic estimates of inbreeding, FROH, sex and unique sample identifier tags, metadata related to field-based metrics of fitness (e.g., clutch size, offspring size and nest success) and custom scripts will be available on Dryad (Bossu et al., 2023; https://doi.org/10.5068/D11974).

### BENEFIT-SHARING STATEMENT

Benefits generated: a research collaboration was developed with scientists from the Santa Clara Valley Habitat Agency (SCVHA), which provided the genetic samples, conducted the field surveys and overwintering of juvenile burrowing owls that were soft released each spring. All collaborators are included as co-authors.

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## SUPPORTING INFORMATION

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