American Genetic Association

Journal of Heredity, 2020, 628–639
doi:10.1093/jhered/esaa056
Original Article
Advance Access publication December 4, 2020

OXFORD

Original Article

Heterozygosity-Fitness Correlations in a Continental Island Population of Thorn-Tailed Rayadito

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Received September 7, 2020; First decision November 24, 2020; Accepted December 4, 2020.

Corresponding Editor: Robert C. Fleischer

Abstract

Heterozygosity-fitness correlations (HFCs) have been used to monitor the effects of inbreeding in threatened populations. HFCs can also be useful to investigate the potential effects of inbreeding in isolated relict populations of long-term persistence and to better understand the role of inbreeding and outbreeding as drivers of changes in genetic diversity. We studied a continental island population of thorn-tailed rayadito (*Aphrastura spinicauda*) inhabiting the relict forest of Fray Jorge National Park, north-central Chile. This population has experienced a long-term, gradual process of isolation since the end of the Tertiary. Using 10 years of field data in combination with molecular techniques, we tested for HFCs to assess the importance of inbreeding depression. If inbreeding depression is important, we predict a positive relationship between individual heterozygosity and fitness-related traits. We genotyped 183 individuals at 12 polymorphic microsatellite loci and used 7 measures of reproductive success and estimates of apparent survival to calculate HFCs. We found weak to moderate statistical support (*P*-values between 0.05 and 0.01) for a linear effect of female multi-locus heterozygosity (MLH) on clutch size and nonlinear effects on laying date and fledging success. While more heterozygous females laid smaller clutches, nonlinear effects indicated that females with intermediate values of MLH started laying earlier and had higher fledging success. We found no evidence for effects of MLH on annual fecundity or on apparent survival. Our results along with the long-term demographic stability of the study population contradict the hypothesis that inbreeding depression occurs in this population.

Subject Area: Conservation genomics and biodiversity
Key words: apparent survival, demographic history, inbreeding depression, outbreeding depression, relict population, reproductive success

The loss of genetic diversity is a major conservation concern in small and isolated populations where genetic drift and inbreeding often increase the risk of extinction (Soulé 1987; Höglund 2009). Isolated island populations are more likely affected by inbreeding depression than mainland populations due to founder effects and smaller population sizes (Frankham 1995, 1998). In addition, isolated populations can experience local adaptation, increasing their susceptibility to outbreeding depression (Edmans 2007). For instance, breeding between local individuals and immigrants from a distant population (i.e., admixture) can lead to the dilution of locally adapted genomes or the disruption of co-adapted gene complexes (Lynch and Walsh 1998; Verhoeven et al. 2010).

Inbreeding and outbreeding have been central topics in evolutionary biology due to their profound consequences on the viability of populations (Frankham 2005). However, monitoring the effects of inbreeding and outbreeding in natural populations is logistically difficult, and thus evolutionary biologists have relied on an indirect approach by estimating heterozygosity-fitness correlations (hereafter HFCs; reviewed in Coltman and Slate 2003; Chapman et al. 2009; Szulkin et al. 2010; Miller and Coltman 2014). This approach relates a measure of within-individual single-locus or multi-locus heterozygosity (SLH and MLH, respectively) to variation in fitness. There is ample evidence that individual genetic diversity affects fitness in a variety of taxa (Kempenaers 2007), and that measures of heterozygosity can be useful to estimate inbreeding (Balloux et al. 2004). Consequently, HFCs are a popular and convenient way to investigate the impact of inbreeding and outbreeding in natural populations (Szulkin et al. 2010).

Two mechanisms are hypothesized to explain the correlation of heterozygosity and fitness. The first possibility is that HFCs reflect the effect of heterozygosity at functional loci, either because the loci themselves have an effect (direct effect hypothesis), or because the genetic markers are in linkage disequilibrium (LD) with functional regions in the genome (local effect hypothesis; Kempenaers 2007). The alternative is that HFCs result from a genome-wide association between a group of markers and fitness loci (general effect hypothesis). This requires heterozygosity to covary across loci (i.e., identity disequilibrium ID; David 1998), so that a multi-locus measure of heterozygosity (MLH) will adequately represent genome-wide inbreeding/outbreeding (Kempenaers 2007; Szulkin et al. 2010). These hypotheses are not mutually exclusive, and HFCs in natural populations lie in a continuum between purely single-locus and genome-wide effects (Balloux et al. 2004).

In a conservation genetics context, HFCs can be viewed as a "warning signal" of genetic erosion in wild populations (Szulkin et al. 2010). Nevertheless, most HFC studies have been conducted in large and relatively outbred populations (Grueber et al. 2008; Velando et al. 2015). Studying large populations is valuable to gain a better insight into the balance of inbreeding and outbreeding depression in nature (Szulkin and David 2011), but the relevance of HFCs in small, threatened populations requires further assessment (Velando et al. 2015). Studies performed on threatened populations have only focused on those having undergone recent bottlenecks or those founded by few individuals (e.g., Brouwer et al. 2007; Grueber et al. 2010, 2011; Gibbs and Chiucchi 2012; Ruíz-López et al. 2012; Brommer et al. 2015; Brambilla et al. 2017; Moss et al. 2019). So far, no consideration has been given to relict populations that have remained demographically stable for prolonged periods. Although reduced genetic diversity can be found in such cases (Westemeier et al. 1998; Keller and Waller 2002), there are also examples of relict populations retaining moderate to relatively high levels of genetic variation (e.g., Grant et al. 2000; Brekke et al. 2011; Väli et al. 2019). This can be observed when population size has remained large over time, or when historic levels of genetic diversity were high (Brekke et al. 2011). Alternatively, associative overdominance (AO; Ohta and Kimura 1969) or heterozygote advantage may result in high levels of genetic variability. The latter possibility can be investigated by assessing HFCs.

Here we report on a study of a geographically isolated, relict population of thorn-tailed rayadito (*Aphrastura spinicauda*). The thorn-tailed rayadito is a socially monogamous, secondary-cavity nesting bird that breeds in the temperate forests of southern South America (Remsen 2003). We studied the population in Fray Jorge National Park, north-central Chile, in the northern margin of the species' distribution (Figure 1). Genetic evidence indicates limited connectivity between this and other populations of rayaditos (González and Wink 2010), and estimates of contemporary gene flow suggest that immigration rates in Fray Jorge are low (~0.1; Botero-Delgadillo et al. 2020a). Despite its long-term isolation (see *Study area and species*), the population still harbors a moderately high level of genetic diversity (observed heterozygosity based on multi-locus genotypes, HO = 0.67), and shows no evidence of recent bottlenecks or demographic expansions (Botero-Delgadillo et al. 2020a). In this study, we calculated HFCs based on measures of reproductive success and estimates of apparent survival to investigate the occurrence of inbreeding depression. If that would be the case, we predict a positive relationship between individual heterozygosity and fitness-related traits, assuming that AO or heterozygote advantage are causal factors for the level of genetic diversity in this population.

Materials and Methods

Study Area and Species

This research is part of a long-term study of the breeding biology of thorn-tailed rayadito carried out since 2008 in Fray Jorge National Park (30°38′ S, 71°40′ W), Coquimbo Region, north-central Chile (Figure 1). This is a semi-arid region dominated by xerophytic vegetation in which relict islands of Valdivian temperate forest persist on coastal hilltops (500–700 m asl) due to oceanic fog-water inputs (del-Val et al. 2006). Fray Jorge is a remnant of an ancient forest community that underwent a gradual process of isolation caused by post-glacial aridisation in north-central Chile, such that forest patches became restricted to the humid coastal mountain range during the Quaternary (Villagrán et al. 2004). The forest relicts in Fray Jorge and Cerro Talinay (13 km south; see Supplementary File 1) are in the northern margin of the distribution of thorn-tailed rayadito, located 170 km north from the nearest known population at Cerro Santa Inés (Figure 1). Our study site in Fray Jorge is ~5.3 km^2, in which several fragments of variable size (0.5–22.5 ha) sum up to approximately 2.4 km^2 of suitable habitat. During the study period, 101–157 nestboxes were available for rayaditos within the forest fragments (Figure 1). Nestboxes were regularly distributed taking into consideration previous estimates of breeding bird density (8.2 pairs/ha; Vergara and Marquet 2007).

Thorn-tailed rayaditos are socially monogamous and exhibit biparental care (Moreno et al. 2007). Only one clutch is laid per season during the austral spring, between September and December (Moreno et al. 2005), although replacement clutches after failure of the first clutch are not uncommon. Eggs are laid on alternate days and incubation only starts after clutch completion

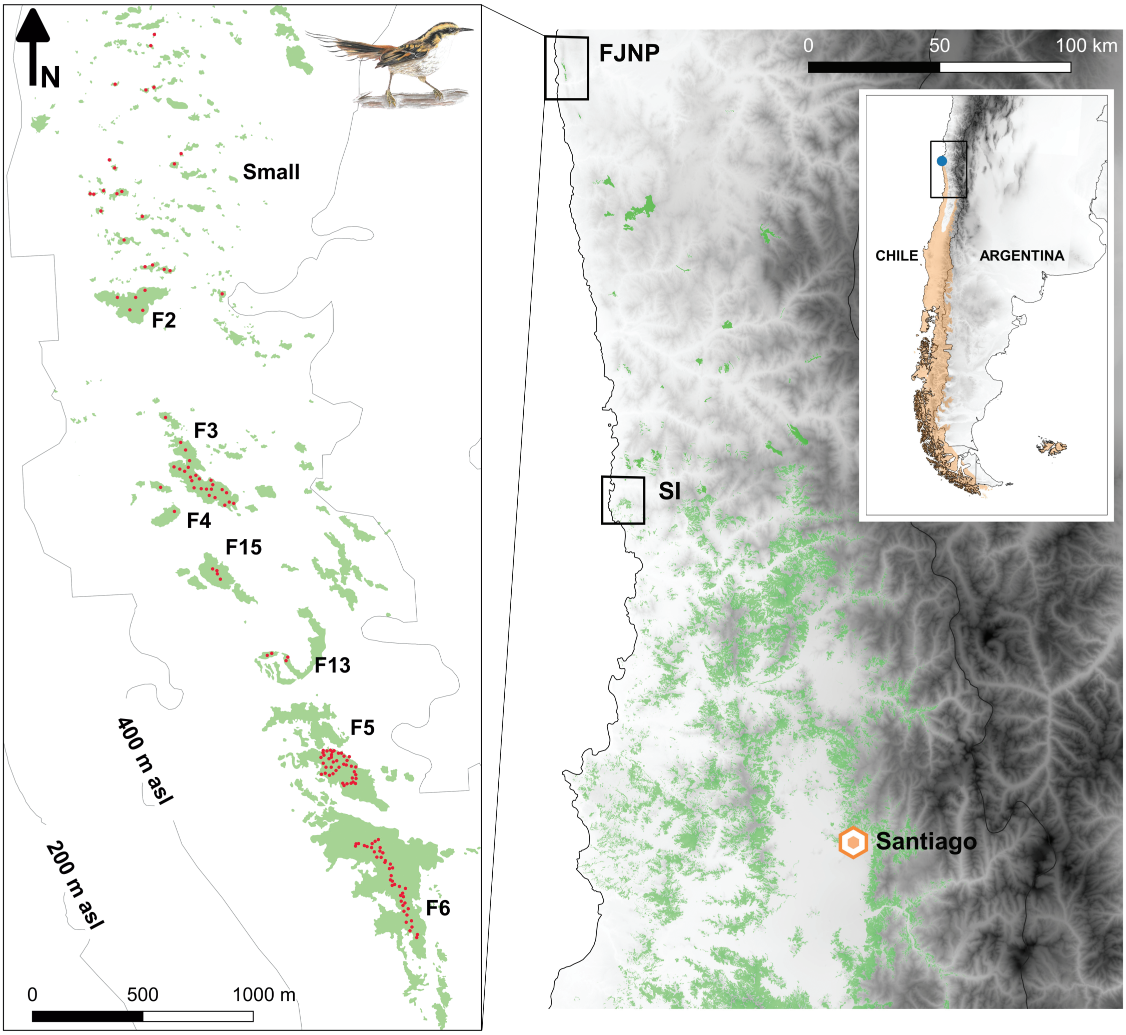

Figure 1. Location of Fray Jorge National Park (FJNP) in the northern margin of the distribution of thorn-tailed rayadito (light brown on the small map). Native forest (light green) is naturally fragmented in north-central Chile, north of Santiago, and the nearest known population of rayadito occurs in the forest relict at Cerro Santa Inés (SI). Nestboxes were mounted in 2008 among fragments of different size (0.5–22.5 ha) on the coastal hilltop landscape of FJNP (left panel). The studied fragments are shown with their corresponding names (small fragments were grouped together for data analysis). Illustration of thorn-tailed rayadito by Priscila Escobar Gimpel.

(Moreno et al. 2005). Clutch size in Fray Jorge is 1–4 eggs, and females start breeding between 1 to 3 years of age (Botero-Delgadillo et al. 2017). Extra-pair paternity in this population is not rare; the yearly proportion of broods with at least 1 extra-pair young (EPY) during 2012–2017 was 13–25%, and the percentage of EPY was 8–18% (Botero-Delgadillo et al. unpublished manuscript). The estimated apparent survival for adult females and males is about 68% (Botero-Delgadillo et al. 2017). Movements of rayaditos are affected by landscape structure both at the local and regional scales (Vergara and Marquet 2007; Vergara et al. 2010). Locally, daily movements through the scrub matrix seldom involve crossing open spaces larger than 100 m (Vergara and Marquet 2007). Natal dispersal is restricted and female-biased (median distance: females = 780 m; males = 85 m), which generates fine-scale genetic structuring for males (Botero-Delgadillo et al. 2017, 2019). Breeding dispersal occurs (~30% of individuals) and mostly involves movements within forest fragments by both members of a breeding pair (Botero-Delgadillo et al. 2020b). Connectivity between Fray Jorge and southerly populations appears limited, as forested vegetation is reduced in the northernmost part of the species' range (Botero-Delgadillo et al. 2020a).

Field Procedures and Genetic Analysis

Data for this study were collected during 10 consecutive breeding seasons in 2008–2017. Details on the field protocol are given elsewhere (Quirici et al. 2014; Botero-Delgadillo et al. 2017). Briefly, we visited all nestboxes every 3–5 days to detect nest building by rayaditos. We increased the frequency of visits for occupied nestboxes to record data on laying and hatching dates, clutch size, and the number of hatchlings and fledglings produced. We caught adults inside the nestbox while they were feeding 12–14-day-old nestlings and marked them as well as their nestlings with a uniquely numbered metal band. In total, we marked 248 breeding adults (132 females, 116 males) and 730 nestlings. We determined the exact age for 77 breeding individuals that were banded as nestlings (33 females and 37 males), and a minimum age for the remaining birds (Botero-Delgadillo et al. 2017). Combining these data, we assigned birds to 2 categories: yearling or adult. We took a ~15 µl blood sample by brachial venipuncture from all adults (from 2010 onwards) and nestlings (from 2012 onwards), and stored samples on filter paper (FTA™ Classic Cards, Whatman™) for later genetic analysis.

We extracted DNA from blood samples for genotyping and molecular sexing following the protocol described in Botero-Delgadillo et al. (2017). We genotyped a total of 183 adults and 412 nestlings at 12 autosomal, polymorphic microsatellite loci, using 7 species-specific markers and 5 cross-species amplifying markers (Supplementary Table S1). Sex was determined by amplifying a sex-specific length polymorphism in the CHD gene using the primers P2/P8 (see details in Griffiths et al. 1998). As outlined in Botero-Delgadillo et al. (2019), we used the multi-locus genotypes for parentage analysis.

We estimated the frequency of null alleles and tested for deviations from Hardy-Weinberg Equilibrium (HWE) at each locus and for linkage disequilibrium (LD) between all pairs of loci, using the adegenet (Jombart 2008) and poppr (Kamvar et al. 2014) packages in R3.5.2 (R Core Team 2018). The frequency of null alleles was < 0.03 for all loci, and no locus deviated from HWE (Supplementary Table 1). The value for the standardized index of association ($\bar{r}_d = 0.0042$) suggested that covariation among loci was low (Supplementary Figure 1). Although this already indicated that there was no linkage among loci, we also mapped the microsatellite sequences onto the annotated genome of the blue tit (*Cyanistes caeruleus*) to determine their location. We first entered the GenBank accession number of each microsatellite-containing sequence into the Nucleotide database of the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov), and then performed a BLAT search of the corresponding FASTA sequence against the v2.0 blue tit genome, using the C. caeruleus Genome Browser at the Sequencing Core Facility of the Max Planck Institute for Molecular Genetics (http://public-genomes-ngs.molgen.mpg.de). Only half of the markers were assigned a position, and 5 out of 6 were located on different chromosomes (Supplementary Table 1).

Measures of Fitness-Related Traits of Breeding Adults

We considered 8 variables as proxies of fitness-related traits. 1) Clutch size and 2) laying date (defined as the date of the first egg) as female-specific traits. 3) Male siring success, defined as the total number of offspring sired (including both within- and extra-pair young) and 4) male paternity loss, defined as a binary variable describing the presence of extra-pair young (EPY) in the male's nest (yes/no). We also included 5) hatching and 6) fledging success, calculated as the proportion of eggs in a clutch that hatched, and the proportion of nestlings that fledged, respectively. 7) Annual fecundity (the number of fledglings produced per season) was considered for both sexes as well. Lastly, we estimated 8) apparent survival for males and females based on capture-mark-recapture analysis (see below).

Population Genetic Structure

We explored genetic diversity and assessed evidence for a cryptic genetic structure in the study population using multivariate analyses in the adegenet package. This is relevant given that assuming distinct genetic groups with varying degrees of inbreeding as a panmictic unit can increase the strength of HFCs due to elevated levels of ID in those populations (Chapman et al. 2009). We used a Principal Component Analysis (PCA) to explore genetic substructure (Patterson et al. 2006), performing analyses for all birds combined as well as for each sex separately. We used a Discriminant Analysis of Principal Components (DAPC; Jombart et al. 2010) to determine whether individuals captured in the same forest fragment (Figure 1) formed identifiable genetic clusters. Therefore, the DAPC was used to calculate probabilistic assignments of individuals to *a priori* defined groups. We selected the number of retained PCs for the DAPC so as to optimize the *a*-score, using the optim.a.score function in adegenet. The number of retained PCs (optimal number: 15) allowed us to obtain an *a*-score ~1, indicating that the DAPC solution was strongly discriminating and stable (Jombart et al. 2010).

Heterozygosity Measures

We used 2 measures of MLH to test for general effects: homozygosity by locus (HL; Aparicio et al. 2006) and the mean-squared distance between alleles (md^2; Coulson et al. 1998). We implemented the GENHET function in R (Coulon 2010) to compute HL and we used a custom routine in R based on the formula described in Coulson et al. (1998) to estimate md^2. We log-transformed the values of md^2 for subsequent statistical analyses as recommended by Coltman et al. (1998). The HL index was selected over other measures because it shows a higher correlation with the inbreeding coefficient (Aparicio et al. 2006). The md^2 index was used as a complementary measure, given that some authors recommend its use for measuring outbreeding (see Marshall and Spalton 2000; Coltman and Slate 2003). To test local effects, we calculated single-locus heterozygosity (SLH). Each locus was coded as a binary variable representing either a homozygous or a heterozygous state.

Estimating Inbreeding and ID

The population mean inbreeding coefficient F was estimated using the inbreeding function in the adegenet package. For each genotyped individual, we computed a likelihood function of F, from which we obtained 10 000 samples to calculate a mean value. Individual mean values were then used to estimate the population mean and the 95% confidence interval (CI) around F.

To assess ID (i.e., heterozygosity correlations across loci), we calculated the g_2 statistic (David et al. 2007) and tested whether the empirical value differed from the value expected under random association of loci using the R package inbreedR (Stoffel et al. 2016). A g_2 value different from zero potentially indicates that MLH and inbreeding are correlated and that the observed HFCs are likely caused by a genome-wide effect (Szulkin et al. 2010). We obtained a 95% CI around the empirical g_2 value by bootstrapping over individuals ($n = 1000$), and a *P*-value by simulating a frequency distribution of g_2 estimates randomly generated using permutations ($n = 1000$).

We plotted the observed distribution of MLH to visualize the variation in inbreeding in our study population. We also compared the observed pattern against simulated distributions under different scenarios, which were generated from our dataset by permuting alleles over individuals using the RMES software (David et al. 2007). This allowed us to assess variation in inbreeding under random association of loci ($g_2 = 0$) and under different degrees of ID caused by inbreeding ($g_2 = 0.1$; $g_2 = 0.2$).

Effects of Heterozygosity on Reproductive Success

We assessed the effects of individual heterozygosity by fitting mixed-effects models using the package lme4 (Bates et al. 2015) in R, with each measure of reproductive success as the response variable. As several individuals bred in the study area in multiple years, we entered individual identity as a random intercept in all models (female id and/or male id, depending on the trait). Following Colquhoun (2014), *P*-values between 0.05 and 0.01 were interpreted as weak to moderate evidence of HFCs, and values ≤ 0.001 were taken as strong evidence of a real effect.

We fit linear regressions for clutch size, laying date, siring success and annual fecundity, and binomial models (with logit-link function) for paternity loss and hatching and fledging success. For the first 4 variables, we controlled for between-season effects by calculating *Z*-scores using the mean value and standard deviation for each year. All models included age (yearling or adult) as a non-genetic cofactor and a measure of MLH (either HL or md^2) and its squared term as predictors (Olano-Marín et al. 2011, Botero-Delgadillo et al. 2020c). For hatching and fledging success, we included age of both parents as predictors in the models, and female and male identity as random effects.

To assess the potential local effects of heterozygosity, we fit similar models as described above, with the only difference that SLH terms were entered instead of the MLH measures (Supplementary Tables 2–3). We compared the MLH and SLH models using likelihood ratio tests (Küpper et al. 2010, Bichet et al. 2019). As the SLH and MLH models were not strictly nested, we used Vuong tests for non-nested models (Vuong 1989). First, we performed a variance test to assess the models' distinguishability, and when the hypothesis of indistinguishability was rejected, we used a non-nested likelihood ratio test to compare the fit of the 2 models (Schneider et al. 2020). Local effects were interpreted and analyzed in detail only when a SLH model fit the data better than the MLH model. We ran the Vuong's test using the nonnest2 package (Merkle and You 2018) in R.

Effects of Heterozygosity on Apparent Survival

Although we had a 10-year capture-mark-recapture (CMR) dataset to estimate apparent survival (φ) and recapture rates (p), we excluded data from the 2008 and 2009 breeding seasons because sampling effort was lower compared to the 2010–2017 period. Excluding these data ensured that recapture probabilities of all marked adults occupying nestboxes were comparable. We used the Cormack-Jolly-Seber model in MARK 5.1 (White and Burnham 1999), entering the recapture history data for 77 females and 72 males as input files. Given that 58% of all adults were captured as remated breeding pairs, recapture probabilities of members of a social pair were not independent as they often dispersed or remained in the study plot together (Botero-Delgadillo et al. 2020b). Therefore, we modeled each sex separately.

We generated different candidate models with varying restrictions on the parameters. For example, either φ or p could be held constant between capture intervals, or vary across time (Supplementary Table 4). We first fit a "full" model that included time-dependent (7 intervals) and age-specific (yearling vs. adult) variation in survival and recapture probability. Then, we fit simplified versions of the full model, and selected the most parsimonious model using the small-sample corrected Akaike Information Criterion (AICC). This model was then used as the "basic" model structure to test the effects of heterozygosity on apparent survival, introducing a measure of MLH (HL or md^2) and its squared term as individual co-variates. Given that introducing the 12 SLH terms into a model was not possible given sample size limitations, we only tested general effects. Co-variates were analyzed using the logit-link function.

Results

Population Genetic Structure

We found no evidence of genetic substructure in the population. The PCA based on all sampled individuals and the sex-specific analyses showed no signs of genetic clustering (Supplementary Figures 2–3). Likewise, individuals did not cluster according to the forest fragment where they were first captured (Supplementary Figure 4). The latter result was supported by the DAPC, which showed considerable admixture between individuals captured in different forest fragments (Supplementary Figure 5).

Patterns of Heterozygosity

The average expected heterozygosity (HE) in the population was 0.68 (SD = 0.05; n = 183), and the number of alleles per locus (Na) ranged from 3 to 13, with a mean of 7.4 (SD = 0.9). Measures of MLH were qualitatively similar for females (HL = 0.28 ± 0.12, md^2 = 1.14 ± 0.23) and males (HL = 0.29 ± 0.11, md^2 = 1.12 ± 0.22; see also Supplementary Figure 6). The correlation between HL and md^2 was relatively low ($r = -0.42$, $P < 0.0001$).

Inbreeding and ID

The mean value for the individual inbreeding coefficient ranged from 0.09 to 0.40 (Supplementary Figure 7). The population mean inbreeding coefficient was 0.18 (95% CI = 0.17–0.19; Supplementary Figure 8). We found no evidence for a correlation of heterozygosity across loci in this population. The g_2 estimated for the population (n = 183) was small and statistically not distinguishable from zero ($g_2 = -0.007$, 95% CI = −0.013–0.0015, $P = 0.98$). The distribution of MLH in the population followed the expected pattern under random association of loci (i.e., when $g_2 = 0$; see Supplementary Figure 9).

Effects of Heterozygosity on Reproductive Success

Analyses using HL and md^2 showed relatively small effects on measures of reproductive success and inconsistent results (Tables 1 and 2, Figure 2). We found no clear statistical support for an effect of MLH—either measured as HL or md^2—on male siring success, paternity loss, hatching success, and annual fecundity (Figure 2). However, there was moderate statistical evidence for an effect of female MLH on clutch size, laying date, and fledging success (Tables 1 and 2, Figure 2). Less heterozygous females laid larger clutches (Figure 3A) and females with intermediate md^2 values started laying earlier (Figure 3B) and had a higher proportion of nestlings that fledged (Figure 3C).

Vuong tests showed that MLH models were not distinguishable from the SLH models (Supplementary Table 2). An additional comparison between models without the quadratic term of MLH indicated that multi-locus and single-locus models were indistinguishable (Supplementary Table 3).

Effects of Heterozygosity on Apparent Survival

Based on the AICC, the "basic" model structure that best fit recapture history data for both sexes included a constant value for survival and recapture probability through time (Supplementary Table 4). Apparent survival and recapture probability were φ = 0.75 ±

Table 1. Effects of heterozygosity (estimated as HL) and age (yearling or adult) on measures of male and female reproductive success in a population of thorn-tailed rayadito in Fray Jorge National Park, north-central Chile

Clutch size	Parameter	Estimate ± SE	*t* / Z	*P*
Females (n = 93; n_{obs} = 210)	Intercept	0.01 ± 0.07		
	HL	0.10 ± 0.04	2.31	0.026
	HL^2	−0.0006 ± 0.03	−0.02	0.99
	Age (adult)	−0.05 ± 0.07	−0.71	0.48
Laying date				
Females (n = 93; n_{obs} = 194)	Intercept	2.34 ± 1.67		
	HL	0.38 ± 1.05	0.36	0.72
	HL^2	−0.16 ± 0.85	−0.18	0.85
	Age (adult)	−3.26 ± 1.63	−2.01	0.047
Siring success				
Males (n = 75; n_{obs} = 164)	Intercept	−0.19 ± 0.19		
	HL	0.08 ± 0.10	0.80	0.43
	HL^2	0.08 ± 0.08	1.05	0.30
	Age (adult)	0.12 ± 0.21	0.57	0.57
Paternity loss				
Males (n = 75; n_{obs} = 164)	Intercept	−1.41 ± 0.56		
	HL	0.06 ± 0.30	0.21	0.83
	HL^2	−0.27 ± 0.25	−1.05	0.29
	Age (adult)	−0.31 ± 0.54	−0.57	0.57
Hatching success				
Breeding pairs (n_F = 86; n_M = 78)	Intercept	1.89 ± 0.55		
	$HL_{FEMALES}$	0.10 ± 0.21	0.49	0.63
	$HL^2_{FEMALES}$	−0.31 ± 0.18	−1.76	0.078
	HL_{MALES}	0.30 ± 0.23	1.31	0.19
	HL^2_{MALES}	−0.05 ± 0.19	−0.27	0.79
	$Age_{FEMALES}$ (adult)	−0.20 ± 0.48	−0.41	0.68
	Age_{MALES} (adult)	0.48 ± 0.50	0.96	0.34
Fledging success				
Breeding pairs (n_F = 86; n_M = 78)	Intercept	1.89 ± 0.54		
	$HL_{FEMALES}$	−0.24 ± 0.22	−1.11	0.27
	$HL^2_{FEMALES}$	−0.08 ± 0.17	−0.46	0.65
	HL_{MALES}	0.07 ± 0.22	0.33	0.74
	HL^2_{MALES}	0.06 ± 0.18	0.33	0.73
	$Age_{FEMALES}$ (adult)	−0.26 ± 0.44	−0.58	0.56
	Age_{MALES} (adult)	−0.27 ± 0.47	−0.57	0.57
Annual fecundity				
Females (n = 93; n_{obs} = 210)	Intercept	0.02 ± 0.11		
	HL	0.09 ± 0.07	1.38	0.17
	HL^2	−0.02 ± 0.05	−0.38	0.71
	Age (adult)	−0.01 ± 0.11	−0.08	0.94
Annual fecundity				
Males (n = 75; n_{obs} = 164)	Intercept	−0.08 ± 0.19		
	HL	0.04 ± 0.09	0.41	0.68
	HL^2	0.05 ± 0.07	0.64	0.52
	Age (adult)	0.05 ± 0.20	−0.23	0.82

Linear regressions were fitted for clutch size, laying date, male siring success, and annual fecundity. Binomial models were used for male paternity loss, hatching success, and fledging success. All models included individual identity as a random effect. Clutch size, laying date, male siring success, and annual fecundity were transformed to *Z*-scores using the mean value and standard deviation for each year. Parameter estimates and SE were estimated relative to "yearling" level in variable "Age." HL was scaled for interpretability of effect sizes–coefficients represent the estimated effect of a 1-unit change (1 SD) in HL. n_F = number of females, n_M = number of males.

0.04 and p = 0.66 ± 0.05 for females and φ = 0.78 ± 0.03 and p = 0.77 ± 0.04 for males. The models including the effects of MLH showed no indication that individual heterozygosity affected apparent survival in either sex (Table 3, Figure 2). Because models with age-specific survival rates also had acceptable support (Supplementary Table 4), we also used these models to test the effects of MLH. We found no evidence for an effect of MLH (Supplementary Table 5), or for an interaction between MLH and age (Supplementary Table 6).

Discussion

We investigated the association between individual heterozygosity and fitness-related traits in a relict population of thorn-tailed rayadito that has undergone a slow process of isolation. Our results suggest some potential effects of MLH on measures of female reproductive success, including clutch size, laying date and fledging success. However, we found no evidence for effects of MLH on male-specific reproductive variables or on annual

Table 2. Effects of heterozygosity (estimated as m*d*^{2}) and age (yearling or adult) on measures of male and female reproductive success in a population of thorn-tailed rayadito in Fray Jorge National Park, north-central Chile

Clutch size	Parameter	Estimate ± SE	t / Z	P
Females (n = 93; n_{obs} = 210)	Intercept	−0.02 ± 0.07		
	md^2	−0.03 ± 0.04	−0.57	0.57
	(md^2)^2	0.04 ± 0.03	1.25	0.22
	Age (adult)	−0.06 ± 0.07	−0.86	0.39
Laying date				
Females (n = 93; n_{obs} = 194)	Intercept	0.24 ± 1.60		
	md^2	0.93 ± 0.99	0.94	0.35
	(md^2)^2	1.84 ± 0.74	2.49	0.015
	Age (adult)	−3.03 ± 1.61	−1.88	0.06
Siring success				
Males (n = 75; n_{obs} = 164)	Intercept	−0.21 ± 0.19		
	md^2	0.09 ± 0.10	0.96	0.34
	(md^2)^2	0.09 ± 0.07	1.22	0.23
	Age (adult)	0.14 ± 0.21	0.69	0.49
Paternity loss				
Males (n = 75; n_{obs} = 164)	Intercept	−1.80 ± 0.60		
	md^2	−0.37 ± 0.28	−1.32	0.19
	(md^2)^2	0.21 ± 0.21	1.00	0.32
	Age (adult)	−0.38 ± 0.54	−0.70	0.48
Hatching success				
Breeding pairs (n_F = 86; n_M = 78)	Intercept	1.19 ± 0.44		
	md$^2_{\mathrm{FEMALES}}$	0.05 ± 0.22	0.21	0.83
	(md^2)$^2_{\mathrm{FEMALES}}$	−0.11 ± 0.13	−0.85	0.40
	md$^2_{\mathrm{MALES}}$	−0.21 ± 0.28	−0.74	0.46
	(md^2)$^2_{\mathrm{MALES}}$	0.40 ± 0.23	1.75	0.079
	Age$_{\mathrm{FEMALES}}$ (adult)	−0.03 ± 0.44	−0.08	0.94
	Age$_{\mathrm{MALES}}$ (adult)	0.32 ± 0.45	0.72	0.47
Fledging success				
Breeding pairs (n_F = 86; n_M = 78)	Intercept	1.97 ± 0.52		
	md$^2_{\mathrm{FEMALES}}$	0.18 ± 0.15	1.22	0.22
	(md^2)$^2_{\mathrm{FEMALES}}$	−0.4 ± 0.23	−2.10	0.036
	md$^2_{\mathrm{MALES}}$	−0.08 ± 0.26	−0.32	0.75
	(md^2)$^2_{\mathrm{MALES}}$	0.10 ± 0.16	0.62	0.54
	Age$_{\mathrm{FEMALES}}$ (adult)	−0.23 ± 0.44	−0.52	0.61
	Age$_{\mathrm{MALES}}$ (adult)	−0.33 ± 0.47	−0.69	0.49
Annual fecundity				
Females (n = 93; n_{obs} = 210)	Intercept	0.01 ± 0.11		
	md^2	−0.03 ± 0.07	−0.47	0.64
	(md^2)^2	−0.001 ± 0.05	−0.02	0.98
	Age (adult)	−0.02 ± 0.12	−0.16	0.87
Annual fecundity				
Males (n = 75; n_{obs} = 164)	Intercept	−0.15 ± 0.18		
	md^2	−0.08 ± 0.09	−0.82	0.42
	(md^2)^2	0.12 ± 0.07	1.78	0.08
	Age (adult)	0.05 ± 0.19	0.27	0.79

Linear regressions were fitted for clutch size, laying date, male siring success, and annual fecundity. Binomial models were used for male paternity loss, hatching success, and fledging success. All models included individual identity as a random effect. Clutch size, laying date, male siring success, and annual fecundity were transformed to Z-scores using the mean value and standard deviation for each year. Parameter estimates and SE were estimated relative to "yearling" level in variable "Age." m*d*^{2} was scaled for interpretability of effect sizes–coefficients represent the estimated effect of a 1-unit change (1 SD) in md^2. n_F = number of females, n_M = number of males.

fecundity and apparent survival. HFCs may vary with environmental fluctuations, and hence may remain undetected during some years (Coulson et al. 1998; Ferrer et al. 2016). Including effects on longer-term fitness-related traits such as apparent survival is therefore critical to understand the longer-term implications of individual heterozygosity (e.g., Brouwer et al. 2007; Bichet et al. 2019).

The population studied here along with the relict population at Cerro Santa Inés (Figure 1) constitute a demographically independent unit that experiences reduced gene flow with other localities (Botero-Delgadillo et al. 2020a). Our results can help defining conservation priorities for the isolated and restricted population in Fray Jorge National Park, but should be interpreted with caution as the sample size and the number of genetic markers used were relatively low (see *Limitations and future work*). The near absence of HFCs in this study suggests that neither associative overdominance (AO) nor heterozygote advantage are the main explanation for the levels of heterozygosity previously

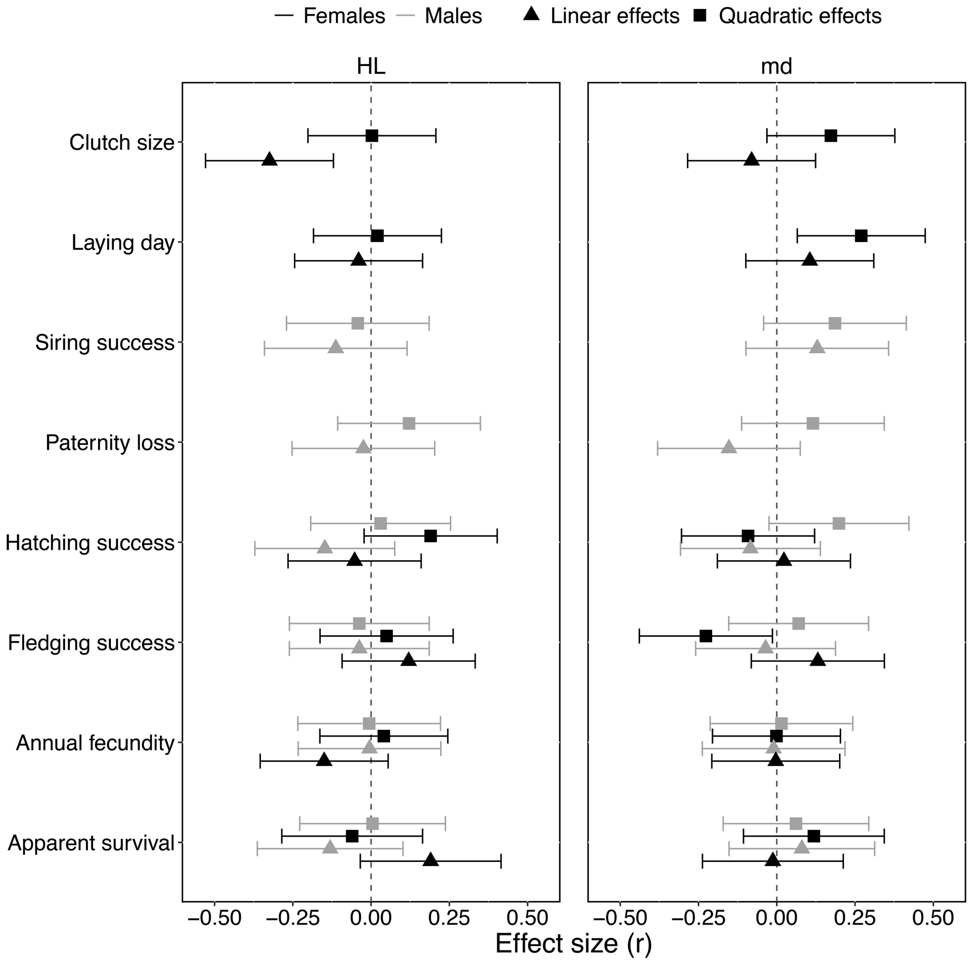

Figure 2. Effect sizes of individual heterozygosity at multiple loci (MLH) on fitness-related traits in a population of thorn-tailed rayadito in Fray Jorge National Park. Shown are the effects of MLH measured as **(A)** HL and **(B)** md^2. Standardized effect sizes were calculated by transforming model coefficients to *r*, the equivalent of the Pearson product-moment correlation coefficient (Coltman and Slate 2003). Bars denote the 95% confidence interval around *r*. The sign of the effects for HL was changed such that positive values indicate positive effects of heterozygosity. For sample sizes, see Tables 1–3.

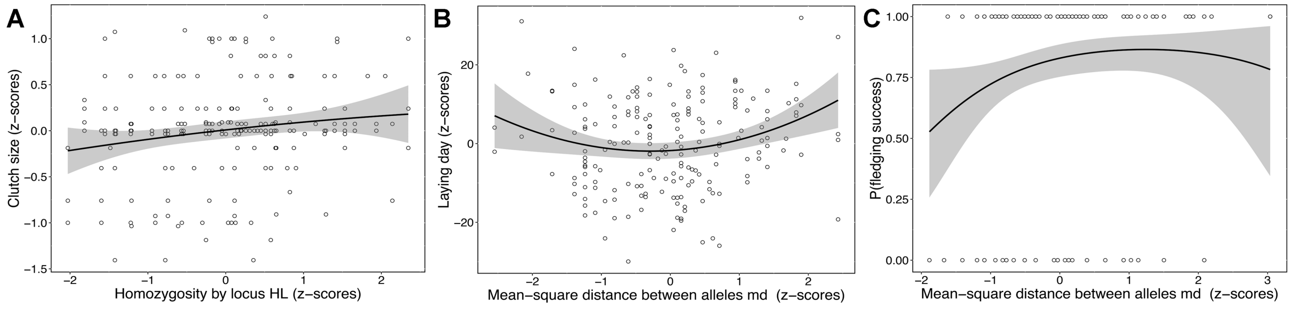

Figure 3. Relationship between individual multi-locus heterozygosity (MLH) and measures of female reproductive success in a population of thorn-tailed rayadito in Fray Jorge National Park. MLH (measured either as HL or md^2) and (**A**) clutch size, **(B)** laying date, and (**C**) fledging success. Shown are the raw data (points) and model predictions with 95% confidence intervals (lines and shaded area).

reported. Below we discuss some alternative explanations by considering the historical and ecological context of the Fray Jorge population.

Effects of MLH on Female Reproductive Success

There was moderate statistical support for both negative and positive effects of MLH on measures of reproductive success in females. A negative, linear relationship between heterozygosity and clutch size was detected using HL as a measure of MLH. The *r* value observed for this association was the highest obtained ($r = -0.32$), and the unstandardized parameter estimate from the corresponding model (Table 1) indicates that an increase in 1 SD of HL (SD = 0.12) corresponds to an increase in 0.1 SD in clutch size (equals 0.6 eggs). This implies that the most homozygous female in our population

Table 3. Effects of heterozygosity on adult apparent survival in a population of thorn-tailed rayadito in Fray Jorge National Park, north-central Chile. Results are from binomial models with logit-link function

	Parameter	Estimate ± SE	95% CI
Females (n = 77; n_e = 164)			
HL	Intercept (φ)	1.04 ± 0.24	0.57–1.5
	HL	−0.31 ± 0.19	−0.67–0.05
	HL^2	0.07 ± 0.14	−0.20–0.35
	P	0.64 ± 0.22	0.21–1.07
md2	Intercept (φ)	0.90 ± 0.25	0.42–1.38
	md^2	−0.02 ± 0.22	−0.46–0.41
	(md^2)^2	0.26 ± 0.25	−0.23–0.75
	P	0.60 ± 0.23	0.14–1.05
Males (n = 72; n_e = 169)			
HL	Intercept (φ)	1.28 ± 0.23	0.83–1.74
	HL	0.20 ± 0.18	−0.15–0.56
	HL^2	−0.01 ± 0.14	−0.28–0.27
	P	1.20 ± 0.23	0.74–1.66
md2	Intercept (φ)	1.23 ± 0.20	0.85–1.63
	md^2	0.12 ± 0.18	−0.23–0.47
	(md^2)^2	0.01 ± 0.03	−0.40–0.07
	P	1.20 ± 0.23	0.75–1.66

Coefficients below are from the most parsimonious models that included apparent survival (φ) and recapture probabilities (P) as fixed parameters through time. n_e: effective sample size (i.e., total number of encounters).

(HL = 0.55) would lay 1 more egg per breeding season compared to the most heterozygous female (HL = 0.03).

The md^2 index showed a non-linear relationship with laying date (r = 0.27) and fledging success (r = −0.23). The effect on fledging success could arise by several mechanisms, including direct maternal effects such as differential investment in eggs (e.g., Heeb et al. 1998; Potti 1999; Eising et al. 2001), or differences in the quality of incubation or maternal care during the nestling period (Brouwer et al. 2007). The non-linear effects detected with md^2 imply that eggs from females with intermediate MLH are laid earlier and survive better until fledging (Figure 3). We speculate that intermediate levels of MLH reflect an optimal degree of inbreeding/outbreeding in the female population (Bateson 1983). As most females in Fray Jorge have intermediate levels of MLH–measured as md^2–, it is possible that selection on heterozygosity may be relatively weak or transitory (e.g., Brouwer et al. 2007; Ferrer et al. 2016), only affecting individuals with extreme values. Rayaditos with very low levels of MLH might descent from local lineages with higher rates of consanguineous matings. On the other hand, individuals with relatively high MLH might be second-generation migrants (i.e., the offspring from a locally born individual and a long-distant immigrant) whose admixed genetic architecture may be disadvantageous compared to locally adapted individuals (Marr et al. 2002).

It is important to highlight that we found no evidence for an effect of MLH on annual fecundity, suggesting that neither the negative effect on clutch size nor the non-linear effects on laying date and fledging success might ultimately affect female reproductive output.

The reasons for the different results obtained with each MLH index are unclear and can be manifold, but one could argue that md^2 is a more meaningful index for this relict population. While HL quantifies the proportion of homozygote loci carried by an individual weighed by variability at each locus (Aparicio et al. 2006), md^2 focuses on events deeper in the population history and may therefore reflect both mutations accumulated since isolation of a population and allelic differences resulting from immigration of individuals from a genetically distinct population (Coulson et al. 1998). Since Fray Jorge has a prolonged history of gradual isolation with limited–but not zero–long-distance immigration events (Botero-Delgadillo et al. 2020a), md^2 may measure the effects of moderate levels of inbreeding and infrequent admixture (see Marshall and Spalton 2000).

HFCs Despite Low Variance in Inbreeding?

This study reports low to moderate correlations between fitness traits and measures of MLH. The reported correlation coefficients (r) in HFC studies are often small, usually < 0.1 (reviewed in Chapman et al. 2009). Here, absolute values of r ranged from 0.005 to 0.32, but the majority of effects were < 0.1 and were statistically inconclusive.

We found no evidence that certain loci were contributing more heavily to observed HFCs than others (i.e., no local effects). This suggests the occurrence of general effects, at least for traits such as laying date and female fledging success. Such effects are unexpected given that inbreeding in the population is not high and there is no evidence of ID. High variation in inbreeding and ID are likely to arise after bottlenecks or admixture (Szulkin et al. 2010), but previous studies have not detected signs of recent demographic changes in the Fray Jorge population (Yáñez 2013; Botero-Delgadillo et al. 2020a). It has been shown, however, that the absence of statistical evidence for ID does not preclude the observation of HFCs (Chapman et al. 2009; Szulkin et al. 2010; Kardos et al. 2014).

No Strong Evidence for Inbreeding Depression: Alternative Explanations

HFCs are likely detected in small populations where the influence of genetic drift is strong (David 1998), in populations that expanded in numbers after a bottleneck or a founder event (Wall et al. 2002), or after recent admixture (Briscoe et al. 1994). Although relict populations are often expected to exhibit low genetic diversity and signs of inbreeding depression (Frankham 1998; Höglund 2009), some have unexpectedly high levels of heterozygosity despite being isolated for several generations (e.g., HO = 0.68 in a remnant island population of hihi *Notiomystis cincta*; Brekke et al. 2011). In the absence of inbreeding depression, several hypotheses have been invoked to explain the relatively high genetic diversity, including large periods of demographic stability and large population sizes, or high historical levels of genetic diversity (see Brekke et al. 2011; Väli et al. 2019). Below we discuss some of these alternatives.

The demographic history of the study population can help explain the lack of strong evidence for HFCs. As stated earlier, there is no genetic evidence of recent population expansions or bottlenecks in the population of Fray Jorge. Although this population might have experienced an isolation process starting in the Plio-Plesitocene period (Villagrán et al. 2004), genetic diversity is relatively high (Botero-Delgadillo et al. 2020a). Furthermore, the estimated effective population size (N_e) is comparable to other populations that maintain recurrent gene flow (Botero-Delgadillo et al. 2020a). It is thus possible that genetic diversity has been reduced slowly, with limited influence of genetic drift, hence explaining the moderate levels of inbreeding and the absence of ID. The occasional immigrants into Fray Jorge might also slow down the depletion of genetic diversity, but the effect might be limited given the low frequency of such events and the fitness costs of outbreeding when an immigrant pairs with a locally born individual (see above). Coalescent-based analyses will be useful to assess whether intense inbreeding and reductions in population size likely occurred earlier in the history of this population. If this was the case, purging of deleterious alleles as a result of historical inbreeding might have reduced the population mutational load (Charlesworth and Charlesworth 1987; Crnokrak and Barrett 2002), hence decreasing variance in fitness with respect to inbreeding (Grueber et al. 2008). This could lead, however, to an increase of the drift load and a decrease in mean fitness in the population due to the fixation of recessive, partially deleterious mutations (van Oosterhout 2020).

The ecological context of Fray Jorge is also relevant for interpreting our results. Natal dispersal in this population is female-biased. While males remain relatively close to their natal territories, females typically disperse over larger distances, usually between forest fragments (Botero-Delgadillo et al. 2017, 2019). Several studies have shown that sex-biased dispersal along with demographic processes can decrease the levels of inbreeding in wild populations (Arcese 1989; Gibbs and Grant 1989; Lebigre et al. 2010). Mating strategies are also relevant for the avoidance of inbreeding (Sugg and Cheeser 1994; Pearse and Anderson 2009). In Fray Jorge, the socially induced population structure consists of genetically related males and unrelated females occurring in close proximity, but pairing between genetically related individuals occasionally occur in this population (12% of the breeding pairs captured during 2010–2015 (n = 107) had a coefficient of relatedness > 0.125; Botero-Delgadillo 2017). Extra-pair mating is thus another behavior that could reduce the potential effects of inbreeding or increase offspring genetic compatibility (Kempenaers 2007), but this requires further study.

Limitations and Future Work

As is typical in studies on endangered populations, obtaining large sample sizes is difficult, and thus HFCs may show large fluctuations around the estimated “true” effect size (Chapman et al. 2009). We cannot dismiss the possibility that the observed results were influenced by the number of markers used. In addition, a limited number of genetic markers (e.g., ~10 microsatellite loci) might not be representative of genome-wide heterozygosity (Grueber et al. 2011), and could reduce statistical power to detect an association between MLH and fitness (Coltman and Slate 2003). It has been argued that 10–25 microsatellites may suffice to reveal an effect, provided there is variation in inbreeding in a population (Szulkin et al. 2010; Kardos et al. 2014). The distribution of MLH in our study system indicated low variation in inbreeding. Nevertheless, a review by Miller and Coltman (2014) suggested that the magnitude of g_2 or HFCs in 50 HFC studies was not associated with the number of markers used or the sample size. Moreover, the number of loci we used is close to the average used in other HFC studies (Chapman et al. 2009; Miller and Coltman 2014), and the fact that we found some statistically supported HFCs indeed suggests that there was sufficient power in our dataset. Whether or not our marker panel is informative of genome-wide heterozygosity in thorn-tailed rayadito needs further investigation.

Because we only assessed HFCs in the adult population, it is possible that the effects of inbreeding might be stronger at an earlier stage. The fact that age-specific models showed no differential effects of MLH on apparent survival (Supplementary Table 6) suggests that viability selection might be weak or absent in Fray Jorge. Yet, further study is needed to assess whether individual heterozygosity affects, for example, juvenile survival or the probability of local recruitment and whether breeding adults on average show higher individual genetic diversity than juveniles (Doyle et al. 2019).

Overall, this study provides weak to moderate support for linear and nonlinear effects of heterozygosity on female reproductive traits, and no evidence for effects on annual fecundity and apparent survival. Our results along with the historical and ecological context of Fray Jorge contradict the hypothesis that inbreeding depression occurs in this population. However, there are 3 key issues that should be addressed in future work to better assess the population status and its conservation priorities.

1. Confirmation of the observed HFCs using a larger panel of genetic markers. We recommend the use of other markers to estimate heterozygosity. Preferably, these markers should be mapped to an annotated genome of the thorn-tailed rayadito, which is being assembled (Quirici et al. unpublished manuscript). This would allow inference of the genetic architecture of confirmed HFCs and better tests for local effects.
2. Examining HFCs in functional loci. This approach will reveal if inbreeding has negative effects on functional areas of the genome that are unlikely detected by the set of markers used in our study, such as the MHC complex (Kempenaers 2007).
3. A comparative study to determine whether the mean population fitness in Fray Jorge is lower than that in other, less isolated populations. Our study suggests no effects of inbreeding, but the drift load due to a distant bottleneck could be depressing fitness of all individuals of the population without being detected (van Oosterhout 2020). Quantifying the drift load using a genomic approach or comparing mean fitness across populations will permit a better assessment of the extinction risk of this relict population.

More studies on populations that have experienced long-term demographic stability despite their isolation would be helpful to increase our understanding of how the demographic history of populations relates to the occurrence of inbreeding and outbreeding depression.

Supplementary Material

Supplementary material is available at *Journal of Heredity* online.

Funding

Funding was provided by the Max Planck Society to B.K.; grants from Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) Nos. 1100359 and 1140548, grant ICM-P05-002, PFB-23-CONICYT-Chile, PAIFAC 2019 (Sciences Faculty, Universidad de Chile), and AFB-170008-CONICYT-Chile to R.A.V.; grants from FONDECYT Nos. 3110059 and 11130245 to V.Q.; graduate fellowships CONICYT-Chile 63130100 and ICM-P05-002 to E.B-D.

Acknowledgments

We thank Pamela Espíndola, Cristóbal Venegas, Matías Acevedo, Javier Bustos, Sandra Escudero, and Isidora Núñez for their collaboration during fieldwork, and especially Juan Monárdez for his assistance at Fray Jorge National Park. Fieldwork in protected areas was possible thanks to people from Parque Nacional Bosque Fray Jorge. Sylvia Kuhn and Alexander Girg provided valuable help with genotyping. Special thanks to Priscila Escobar Gimpel for her illustrations of thorn-tailed rayadito. This work was approved by the Ethics

Committee of the Sciences Faculty, Universidad de Chile following guidelines from Biosecurity Manual from CONICYT (version 2008) and Chilean law 20380 about animal protection. Full permission for sampling and animal ethics approval were granted by Servicio Agrícola y Ganadero (SAG; permits Nos. 5193/2005, 6295/2011, 1101/2013, 7542/2015, 5158/2016, 8185/2016, 404/2017, 4209/2017, 2667/2018) and Corporación Nacional Forestal (CONAF), Chile.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability

Data has been deposited on Dryad, doi:10.5061/dryad.c866t1g5f, including individual identity, sex and age, measures of reproductive success, recapture histories, and microsatellite genotypes.

References

Aparicio JM, Ortego J, Cordero PJ. 2006. What should we weigh to estimate heterozygosity, alleles or loci? *Mol Ecol*. 15:4659–4665.

Arcese P. 1989. Intrasexual competition, mating system and natal dispersal in song sparrows. *Anim Behav*. 38:958–979.

Balloux F, Amos W, Coulson T. 2004. Does heterozygosity estimate inbreeding in real populations? *Mol Ecol*. 13:3021–3031.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Soft*. 67:1–48.

Bateson P. 1983. Optimal outbreeding. In: Bateson P, editor. *Mate choice*. Cambridge (UK): Cambridge University Press. Pp. 257–268.

Bichet C, Vedder O, Sauer-Gürth H, Becker PH, Wink M, Bouwhuis S. 2019. Contrasting heterozygosity-fitness correlations across life in a long-lived seabird. *Mol Ecol*. 28:671–685.

Botero-Delgadillo E. 2017. *Patterns, strategies and dispersal syndromes of the Thorn-tailed Rayadito Aphrastura spinicauda (Furnariidae) in two contrasting environments in central and southern Chile*. Ph.D. dissertation. Santiago (Chile): Universidad de Chile.

Botero-Delgadillo E, Gilsenan C, Mueller JC, Kempenaers B. 2020a. Negative effects of individual heterozygosity on reproductive success in a wild bird population. *Mol Ecol*. 29:3196–3216.

Botero-Delgadillo E, Quirici V, Poblete Y, Cuevas E, Kuhn S, Girg A, Poulin E, Kempenaers B, Vásquez RA. 2019. Ecological and social correlates of natal dispersal in female and male Thorn-tailed Rayadito (*Aphrastura spinicauda*) in a naturally isolated and fragmented habitat. *Auk*. 136:ukz016.

Botero-Delgadillo E, Quirici V, Poblete Y, Acevedo M, Cuevas É, Bravo C, Cragnolini M, Rozzi R, Poulin E, Mueller JC, *et al*. 2020. Range-wide genetic structure in the thorn-tailed rayadito suggests limited gene flow towards peripheral populations. *Sci Rep*. 10:9409.

Botero-Delgadillo E, Quirici V, Poblete Y, Cuevas É, Kuhn S, Girg A, Teltscher K, Poulin E, Kempenaers B, Vásquez RA. 2017. Variation in fine-scale genetic structure and local dispersal patterns between peripheral populations of a South American passerine bird. *Ecol Evol*. 7:8363–8378.

Botero-Delgadillo E, Quirici V, Poblete Y, Poulin E, Kempenaers B, Vásquez RA. 2020b. Variation in fine-scale genetic structure and local dispersal patterns between peripheral populations of a South American passerine bird. *J Avian Biol*. 51:e02262.

Brambilla A, Keller L, Bassano B, Grossen C. 2017. Heterozygosity-fitness correlations at the major histocompatibility complex despite low variation in Alpine ibex (*Capra ibex*). *Evol App*. 11:631–644.

Brekke P, Bennett PM, Santure AW, Ewen JG. 2011. High genetic diversity in the remnant island population of hihi and the genetic consequences of re-introduction. *Mol Ecol*. 20:29–45.

Briscoe D, Stephens JC, O'Brien SJ. 1994. Linkage disequilibrium in admixed populations: applications in gene mapping. *J Hered*. 85:59–63.

Brommer JE, Kekkonen J, Wikström M. 2015. Using heterozygosity-fitness correlations to study inbreeding depression in an isolated population of white-tailed deer founded by few individuals. *Ecol Evol*. 5:357–367.

Brouwer L, Komdeur J, Richardson JS. 2007. Heterozygosity-fitness correlations in a bottlenecked island species: a case study of the Seychelles warbler. *Mol Ecol*. 6:3134–3144.

Chapman JR, Nakagawa S, Coltman DW, Slate J, Sheldon BC. 2009. A quantitative review of heterozygosity-fitness correlations in animal populations. *Mol Ecol*. 18:2746–2765.

Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. *Ann Rev Ecol Syst*. 18:237–268.

Coltman DW, Bowen WD, Wright JM. 1998. Birth weight and neonatal survival of harbor seal pups are positively correlated with genetic variation measured by microsatellites. *Proc Roy Soc B*. 265:803–809.

Colquhoun D. 2014. An investigation of the false discovery rate and the misinterpretation of p-values. *R Soc Open Sci*. 1:140216.

Coltman DW, Slate J. 2003. Microsatellite measures of inbreeding: a meta-analysis. *Evolution*. 57:971–983.

Coulon A. 2010. genhet: an easy-to-use R function to estimate individual heterozygosity. *Mol Ecol Resour*. 10:167–169.

Coulson TN, Pemberton JM, Albon SD, Beaumont M, Marshall TC, Slate J, Guinness FE, Clutton-Brock TH. 1998. Microsatellites reveal heterosis in red deer. *Proc Biol Sci*. 265:489–495.

Crnokrak P, Barrett SC. 2002. Perspective: purging the genetic load: a review of the experimental evidence. *Evolution*. 56:2347–2358.

David P. 1998. Heterozygosity-fitness correlations: new perspectives on old problems. *Heredity (Edinb)*. 80 (Pt 5):531–537.

David P, Pujol B, Viard F, Castella V, Goudet J. 2007. Reliable selfing rate estimates from imperfect population genetic data. *Mol Ecol*. 16:2474–2487.

del-Val E, Armesto JJ, Barbosa O, Christie DA, Gutiérrez AG, Jones CG, Marquet PA, Weathers KC. 2006. Rain forest islands in the Chilean semiarid region: fog-dependency, ecosystem persistence and tree regeneration. *Ecosystems*. 9:598–608.

Doyle JM, Willoughby JR, Bell DA, Bloom PH, Bragin EA, Fernandez NB, Katzner TE, Leonard K, DeWoody JA. 2019. Elevated heterozygosity in adults relative to juveniles provides evidence of viability selection on eagles and falcons. *J Hered*. 110: 696–706.

Edmans S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol. Ecol*. 16:463–475.

Eising CM, Eikenaar C, Schwabl H, Groothuis TG. 2001. Maternal androgens in black-headed gull (Larus ridibundus) eggs: consequences for chick development. *Proc Biol Sci*. 268:839–846.

Ferrer ES, García-Navas V, Sanz JJ, Ortego J. 2016. The strength of the association between heterozygosity and probability of interannual local recruitment increases with environmental harshness in blue tits. *Ecol Evol*. 6:8857–8869.

Frankham R. 1995. Inbreeding and extinction: a threshold effect. *Conserv Biol*. 9:792–799.

Frankham R. 1998. Inbreeding and extinction: island populations. *Conserv Biol*. 12:665–675.

Frankham R. 2005. Genetics and extinction. *Biol. Conserv*. 126:4908–4918.

Gibbs HL, Chiucchi JE. 2012. Inbreeding, body condition, and heterozygosity-fitness correlations in isolated populations of the endangered eastern massasauga rattlesnake (*Sistrurus c. catenatus*). *Conserv Genet*. 13:1133–1143.

Gibbs HL, Grant PR. 1989. Inbreeding in darwin's medium ground finches (geospiza fortis). *Evolution*. 43:1273–1284.

González J, Wink M. 2010. Genetic differentiation of the Thorn-tailed Rayadito *Aphrastura spinicauda* (Furnariidae: Passeriformes) revealed by ISSR profiles suggests multiple paleorefugia and high recurrent gene flow. *Ibis*. 152:761–774.

Grant PR, Curry RL, Grant BR. 2000. A remnant population of the Floreana mockingbird on Champion island, Galápagos. *Biol Conserv*. 92:285–290.

Griffiths R, Double MC, Orr K, Dawson RJ. 1998. A DNA test to sex most birds. *Mol Ecol*. 7:1071–1075.

Grueber CE, Laws RJ, Nakagawa S, Jamieson IG. 2010. Inbreeding depression accumulation across life-history stages of the endangered Takahe. *Conserv Biol*. 24:1617–1625.

Grueber CE, Wallis GP, Jamieson IG. 2008. Heterozygosity-fitness correlations and their relevance to studies on inbreeding depression in threatened species. *Mol Ecol*. 17:3978–3984.

Grueber CE, Waters JM, Jamieson IG. 2011. The imprecision of heterozygosity-fitness correlations hinders the detection of inbreeding and inbreeding depression in a threatened species. *Mol Ecol*. 20:67–79.

Heeb P, Werner I, Kolliker M, Richner H. 1998. Benefits of induced host responses against an ectoparasite. *Proc. Roy. Soc. B* 265:51–56.

Höglund J. 2009. *Evolutionary conservation genetics*. Oxford (UK): Oxford University Press.

Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*. 24:1403–1405.

Jombart T, Devillard S, Balloux F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet*. 11:94.

Kamvar ZN, Tabima JF, Grünwald NJ. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*. 2:e281.

Kardos M, Allendorf FW, Luikart G. 2014. Evaluating the role of inbreeding depression in heterozygosity-fitness correlations: how useful are tests for identity disequilibrium? *Mol Ecol Resour*. 14:519–530.

Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. *Trends Ecol Evol*. 17:230–241.

Kempenaers B. 2007. Mate choice and genetic quality: a review of the heterozygosity theory. *Adv Stud Behav*. 37:189–278.

Küpper C, Kosztolányi A, Augustin J, Dawson DA, Burke T, Székely T. 2010. Heterozygosity-fitness correlations of conserved microsatellite markers in Kentish plovers *Charadrius alexandrinus*. *Mol Ecol*. 19:5172–5185.

Lebigre C, Alatalo RV, Siitari H. 2010. Female-biased dispersal alone can reduce the occurrence of inbreeding in black grouse (*Tetrao tetrix*). *Mol Ecol*. 19:1929–1939.

Lynch M, Walsh B. 1998. *Genetic analysis of quantitative traits*. Sunderland (MA): Sinauer Associates Inc.

Marr AB, Keller LF, Arcese P. 2002. Heterosis and outbreeding depression in descendants of natural immigrants to an inbred population of song sparrows (*Melospiza melodia*). *Evolution*. 56:131–142.

Marshall TC, Spalton JA. 2000. Simultaneous inbreeding and outbreeding depression in reintroduced Arabian oryx. *Anim Conserv*. 3:241–248.

Merkle EC, You D. 2018. nonnest2: Tests of Non-Nested Models. R package version 0.5–2. https://CRAN.R-project.org/package=nonnest2.

Miller JM, Coltman DW. 2014. Assessment of identity disequilibrium and its relation to empirical heterozygosity fitness correlations: a meta-analysis. *Mol Ecol*. 23:1899–1909.

Moreno J, Merino S, Lobato E, Rodríguez-Gironés MA, Vásquez RA. 2007. Sexual dimorphism and parental roles in the thorn-tailed rayadito (*Furnariidae*). *Condor*. 109:312–320.

Moreno J, Merino S, Vásquez RA, Armesto JJ. 2005. Breeding biology of the thorn-tailed rayadito (Furnariidae) in south-temperate rainforests of Chile. *Condor*. 107:69–77.

Moss JB, Gerber GP, Welch ME. 2019. Heterozygosity-fitness correlations reveal inbreeding depression in neonatal body size in a critically endangered rock iguana. *J Hered*. 110:818–829.

Ohta T, Kimura M. 1969. Linkage disequilibrium at steady state determined by random genetic drift and recurrent mutation. *Genetics*. 63:229–238.

Olano-Marin J, Mueller JC, Kempenaers B. 2011. Correlations between heterozygosity and reproductive success in the blue tit (*Cyanistes caeruleus*): an analysis of inbreeding and single locus effects. *Evolution*. 65:3175–3194.

Patterson N, Price AL, Reich D. 2006. Population structure and eigenanalysis. *PLoS Genet*. 2:e190.

Pearse DE, Anderson EC. 2009. Multiple paternity increases effective population size. *Mol Ecol*. 18:3124–3127.

Potti J. 1999. maternal effects and the pervasive impact of nestling history on egg size in a passerine birD. *Evolution*. 53:279–285.

Quirici V, Venegas CI, González-Gómez PL, Castaño-Villa GJ, Wingfield JC, Vásquez RA. 2014. Baseline corticosterone and stress response in the Thorn-tailed Rayadito (*Aphrastura spinicauda*) along a latitudinal gradient. *Gen Comp Endocrinol*. 198:39–46.

R Core Team. 2018. *R: a language and environment for statistical computing, version 3.5.2*. Vienna (Austria): R Foundation for Statistical Computing.

Remsen JV. 2003. Family Furnariidae (Ovenbirds). In: del Hoyo J, Elliot A, Christie D, editors. *Handbook of the birds of the world*. Vol. 8. Barcelona (Spain): Lynx Edicions. p. 162–357.

Ruíz-López MJ, Gañan N, Godoy JA, Del Olmo A, Garde J, Espeso G, Vargas A, Martinez F, Roldán ER, Gomendio M. 2012. Heterozygosity-fitness correlations and inbreeding depression in two critically endangered mammals. *Conserv Biol*. 26:1121–1129.

Schneider L, Chalmers RP, Debelak R, Merkle EC. 2020. Model selection of nested and non-nested item response models using Vuong tests. *Multivariate Behav Res*. 55:664–684.

Soulé ME. 1987. *Viable populations for conservation*. Cambridge (UK): Cambridge University Press.

Stoffel MA, Esser M, Kardos M, Humble E, Nichols H, David P, Hoffman JI. 2016. inbreedR: an R package for the analysis of inbreeding based on genetic markers. *Methods Ecol Evol*. 7:1331–1339.

Sugg DW, Chesser RK. 1994. Effective population sizes with multiple paternity. *Genetics*. 137:1147–1155.

Szulkin M, Bierne N, David P. 2010. Heterozygosity-fitness correlations: a time for reappraisal. *Evolution*. 64:1202–1217.

Szulkin M, David P. 2011. Negative heterozygosity-fitness correlations observed with microsatellites located in functional areas of the genome. *Mol Ecol*. 20:3949–3952.

Väli Ü, Dombrovski V, Dzmitranok M, Maciorowski G, Meyburg BU. 2019. High genetic diversity and low differentiation retained in the European fragmented and declining Greater Spotted Eagle (*Clanga clanga*) population. *Sci Rep*. 9:3064.

Van Oosterhout C. 2020. Mutation load is the spectre of species conservation. *Nat Ecol Evol*. 4:1004–1006.

Velando A, Barros A, Moran P. 2015. Heterozygosity-fitness correlations in a declining sea bird population. *Mol Ecol*. 24:1007–1018.

Vergara PM, Hahn IJ, Zevallos H, Armesto JJ. 2010. The importance of forest patch networks for the conservation of the Thorn-tailed Rayadito in central Chile. *Ecol Res*. 25:683–690.

Vergara PM, Marquet PA. 2007. On the seasonal effect of landscape structure on a bird species: the thorn-tailed rayadito in a relict forest in northern Chile. *Landsc Ecol*. 22:1059–1071.

Verhoeven KJF, Macel M, Wolfe LM, Biere A. 2010. Population admixture, biological invasions and the balance between local adaptations and inbreeding depression. *Proc Roy Soc B*. 278:2–8.

Villagrán C, Armesto JJ, Hinojosa LF, Cuvertino J, Pérez C, Medina C. 2004. El enigmático origen del bosque relicto de Fray Jorge. In: Scheo FA, Gutiérrez JR, Hernández IR, editors. *Historia natural del Parque Nacional Bosque Fray Jorge*. La Serena (Chile): Ediciones Universidad de La Serena. pp. 3–43.

Vuong QH. 1989. Likelihood ratio test for model selection and non-nested hypotheses. *Econometrica*. 57:307–333.

Wall JD, Andolfatto P, Przeworski M. 2002. Testing models of selection and demography in *Drosophila simulans*. *Genetics*. 162:203–216.

Westemeier RL, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, Paige KN. 1998. Tracking the long-term decline and recovery of an isolated population. *Science*. 282: 1695–1698.

White GC, Burnham KP. 1999. Program MARK: survival estimation from populations of marked animals. *Bird Study*. 46(Supp):120–138.

Yáñez DI. 2013. *Diversidad y estructura genética de tres poblaciones contrastantes de Aphrastura spinicauda*. Undergraduate thesis. Santiago (Chile): Universidad de Chile.