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Contemporary evolution of the innate immune receptor gene *TLR3* in an isolated vertebrate population

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Abstract

Understanding where genetic variation exists, and how it influences fitness within populations is important from an evolutionary and conservation perspective. Signatures of past selection suggest that pathogen-mediated balancing selection is a key driver of immunogenetic variation, but studies tracking contemporary evolution are needed to help resolve the evolutionary forces and mechanism at play. Previous work in a bottlenecked population of Seychelles warblers (*Acrocephalus sechellensis*) show that functional variation has been maintained at the viral-sensing Toll-like receptor 3 (*TLR3*) gene, including one nonsynonymous SNP, resulting in two alleles. Here, we characterise evolution at this *TLR3* locus over a 25-year period within the original remnant population of the Seychelles warbler, and in four other derived, populations. Results show a significant and consistent temporal decline in the frequency of the $TLR3^{C}$ allele in the original population, and that similar declines in the $TLR3^{C}$ allele frequency occurred in all the derived populations. Individuals (of both sexes) with the $TLR3^{CC}$ genotype had lower survival, and males - but not females - that carry the $TLR3^{C}$ allele had significantly lower lifetime reproductive success than those with only the $TLR3^{A}$ allele. These results indicate that positive selection on the $TLR3^{A}$ allele, caused by an as yet unknown agent, is driving *TLR3* evolution in the Seychelles warbler. No evidence of heterozygote advantage was detected. However, whether the positive selection observed is part of a longer-term pattern of balancing selection (through fluctuating selection or rare-allele advantage) cannot be resolved without tracking the $TLR3^{C}$ allele over an extended time period.

KEYWORDS
genetic variation, reproductive success, selection, Seychelles warbler, survival, TLR

1 | INTRODUCTION

Genetic variation is key to both the fitness of individuals and the persistence of populations (Reed & Frankham, 2003). Loss of genetic variation can result in inbreeding depression and a reduction in the adaptive potential of the population, which may be especially detrimental in small or bottlenecked populations (Lande, 1995). Therefore, understanding the factors and mechanisms that shape genetic variation within such populations is important from

both an evolutionary and conservation perspective (Frankham, 1996).

Various interacting evolutionary forces act to shape genetic variation within populations, either through 'neutral' processes such as genetic drift, or 'adaptive' processes such as selection (Lande, 1976; Wright, 1931). Determining the relative importance of these forces in shaping genetic diversity is key to understanding the adaptive potential of populations (Lacy, 1987; Sutton et al., 2011). In small populations genetic drift is usually predominant, resulting in a decrease in genetic variation across the genome (Robinson et al., 2016). Nevertheless, selection can also act on functional genes, either counteracting or reinforcing the effect of drift. Directional or purifying selection can push alleles to fixation, resulting in a reduction in genetic variation and reinforcing drift (Mukherjee et al., 2009). In contrast, balancing selection (caused by a suite of potential mechanisms) may maintain genetic variation and counteract the effect of drift (Hedrick, 1998).

Pathogens can have considerable negative impact on the survival and reproductive success of individuals (Daszak et al., 2000), and are strong drivers of evolutionary change in natural populations (Haldane, 1992). Consequently, immunogenetic loci - i.e., those involved in the detection and combating of pathogens – are excellent candidates in which to investigate the evolutionary forces underlying the maintenance of genetic variation (Croze et al., 2016; Sommer, 2005). Indeed, pathogen-mediated selection is thought to be a key driver of balancing selection (Spurgin & Richardson, 2010). Three non-mutually exclusive mechanisms driving pathogen-mediated selection have been proposed: heterozygote advantage (Doherty & Zinkernagel, 1975), rare allele advantage (Slade & McCallum, 1992), and fluctuating selection (Hill et al., 1991). These three mechanisms – along with other forces such as sexual selection – can act independently, in concert, or in trade-off with one other (Apanius et al., 1997; Ejsmond et al., 2014; Spurgin & Richardson, 2010).

Immunogenetic research on wild populations has focused mainly on receptor genes of the acquired immune system: in particular on the exceptionally polymorphic major histocompatibility complex (MHC) (reviewed in Piertney & Oliver, 2005). However, high levels of diversity (Hedrick, 1994), gene duplication (Bollmer et al., 2010), conversion, recombination (Miller and Lambert, 2004a), and epistasis (van Oosterhout, 2009) makes it hard to tease apart the evolutionary forces driving MHC variation (Spurgin & Richardson, 2010). In contrast, the genes involved in the innate immune response, while still often polymorphic, exhibit relatively lower complexity. Furthermore, the innate immune system is the host's first line of response to pathogens, enabling a broad defence against an assortment of organisms (Aderem & Ulevitch, 2000). Consequently, innate immune genes can be more tractable candidates with which to study the evolutionary forces shaping immunogenetic variation in wild populations (Acevedo-Whitehouse & Cunningham, 2006).

Toll-Like Receptor (TLR) genes encode receptor molecules which bind to pathogen-associated molecular patterns - evolutionary conserved molecular motifs that are integral to the pathogen's survival (Medzhitov, 2001). Once bound, the TLR molecule triggers a cascade of processes associated with the innate and adaptive immune responses (Akira et al., 2006). Vertebrate TLRs can be divided into six families, depending on the pathogen-associated molecular patterns they detect (Roach et al., 2005). For example, *TLR3* binds to viral dsRNA (Barton, 2007), while *TLR5* binds to bacterial flagellin (Brownlie & Allan, 2011). While the majority of the TLR structure is structurally conserved (Roach et al., 2005), there is variation in the leucine-rich repeat domain of TLR genes, resulting in functional variation at the binding site. Such TLR polymorphisms have been associated with resistance (Antonides et al., 2019), or susceptibility to specific pathogens (Kloch et al., 2018), or associated with increased survival (Bateson et al., 2016; Grueber et al., 2013). TLRs can evolve rapidly as a result of pathogen-mediated selection (Downing et al., 2010) and evidence of balancing selection at TLR genes has been reported for various taxa (e.g., Areal et al., 2011; Velová et al., 2018). Nevertheless, most of these studies only inferred past selection from sequence variation and could not determine if selection was still acting, or determine the specific mechanisms involved. Moreover, in various bottlenecked populations, genetic drift may override selection as the dominant evolutionary force shaping TLR variation (Gonzalez-Quevedo et al., 2015; Grueber et al., 2013).

Here, we investigate the contemporary evolution of TLR variation in a natural population of Seychelles warblers (*Acrocephalus sechellensis*). The last remaining population of this species on Cousin island underwent a bottleneck in the 1900s resulting in decreased genome-wide genetic variation (Spurgin et al., 2014). Extensive longitudinal monitoring and a lack of dispersal (Komdeur et al., 2004) means that virtually all individual warblers on Cousin Island are sampled, marked and tracked throughout their entire lives (Hammers et al., 2015; Komdeur, 1992). This allows for accurate measures of survival and reproductive success (Hammers et al., 2019). As part of a conservation programme, individuals have been translocated from Cousin to establish populations on four additional islands (Komdeur, 1994; Richardson et al., 2006; Wright et al., 2014), allowing spatial TLR variation to be investigated. A previous study found that five of seven TLR loci examined in the contemporary population (2000–2008) of Seychelles warbler on Cousin Island were polymorphic and detected a signature of past positive selection at two loci, one of these being *TLR3* - a viral sensing TLR (Gilroy et al., 2017). One of the three SNPs at this *TLR3* loci was singled out for investigation because it is nonsynonymous, found within the functionally important leucine-rich repeat domain region, and had a relatively high minor allele frequency (32%, n = 28). However, if and how balancing selection maintains variation at this locus has yet to be investigated.

We first assessed how the frequency of this *TLR3* SNP has changed over 25-years in the Seychelles warbler on Cousin Island. We then tested the role of selection in shaping *TLR3* variation in this population; specifically, if survival and reproductive success are associated with individual *TLR3* genotypes. Lastly, we compared patterns of *TLR3* evolution over time in, and between, the Cousin population and the newly established (translocated) populations. These analyses allow us

to better understand which evolutionary forces shape immunogenetic variation in small populations of conservation concern.

2 | MATERIALS AND METHODS

2.1 | Study species and system

The Seychelles warbler is a small (ca 15 g) insectivorous passerine endemic to the Seychelles. The species was distributed across the archipelago prior to human colonisation (Spurgin et al., 2014), but underwent a severe population reduction in the 1900s due to anthropogenic effects, with just ca 29 individuals remaining on Cousin Island (4°20′S, 55°40′E; 0.29 km^2) by the 1960s (Crook, 1960). After intensive conservation, the population recovered to carrying capacity on Cousin (ca 320 adults present in ca 110 territories) by the 1980s (Brouwer et al., 2009; Komdeur, 1992). Additional populations were established by translocations to four nearby islands (Table S1): Aride (29 birds in 1988), Cousine (29 birds in 1990), Denis (58 birds in 2004), and Frégate (59 birds in 2011) (Komdeur, 1994; Richardson et al., 2006; Wright, Spurgin, et al., 2014). Founder individuals (all from Cousin) were selected based on sex, age, body condition, and breeding experience but without reference to genetic characteristics (Wright, Spurgin, et al., 2014). Translocations to Aride and Cousine were undertaken before blood sampling became routine, whereas sampling of all the founders of the Denis and Frégate populations was undertaken (Wright, Spurgin, et al., 2014). Of the translocated populations, two are now at carrying capacity (Aride: ca 1850 individuals; Cousine: ca 210 individuals (Wright, Spurgin, et al., 2014)), while the populations on the other islands are still increasing (Denis: ca 424 birds in 2015 (Doblas & McClelland, 2015); Frégate: ca 141 birds in 2016 (Johnson et al., 2018)).

The Seychelles warbler population on Cousin Island has been monitored since 1986 (Hammers et al., 2019; Komdeur, 1992). A comprehensive population census has taken place every year during the major breeding season (June–September), and since 1997 also during the minor breeding season (November–March), except in 2000–2002 and in 2006 (Brouwer et al., 2010). Individuals were recorded as present if caught, or observed, during the field season. The other populations have not been censused regularly, and only sporadic census data are available.

The rate of annual resighting of individuals on Cousin is high (0.98, Brouwer et al., 2010) and there is virtually no inter-island dispersal (0.1%, Komdeur et al., 2004), thus enabling accurate survival estimates (Brouwer et al., 2006). Individuals can be confidently presumed dead if not seen for two consecutive breeding seasons; the date of death is assigned as the end of the last season in which a bird was observed (Hammers et al., 2013). Ages were rounded to the nearest 0.5 years. Adult annual survival is high (84%), with mortality being greatest in first-year birds (Brouwer et al., 2006). Median lifespan is 5.5 years post-fledging, and maximum lifespan is 19 years (Hammers & Brouwer, 2017).

Females typically lay single-egg clutches (Richardson et al. 2001) and only occasionally two or three eggs (Komdeur, 1991). They are facultatively cooperative breeders, with a socially monogamous dominant breeder pair defending strict territories year-round (Komdeur, 1992). Some adult birds delay independent breeding and become subordinates (Kingma et al., 2016), and may help raise offspring (Hammers et al., 2019; Komdeur, 1992). Although 44% of female subordinates gain reproductive success by cobreeding, male subordinates rarely gain paternity (Raj Pant et al., 2019; Richardson et al., 2002). Extra-pair paternity is frequent in this species (Richardson et al., 2001), with 41% of offspring fathered outside the natal territory (Raj Pant et al., 2019).

Individuals are caught either by mist-net, or as nestlings, and are aged based on hatch date, behaviour, and eye colour at first catch (for details see Komdeur, 1992; Wright, 2014). Each bird is given a metal British Trust for Ornithology (BTO) ring and a unique combination of three colour rings (Richardson et al., 2001). Routine blood sampling began in 1993. Since 1997, >96% of the Cousin population has been ringed and blood sampled (Raj Pant et al., 2019). Samples (ca 25 µl) are collected by brachial venipuncture and stored in 0.8 ml of absolute ethanol at 4°C.

2.2 | Molecular methods

Genomic DNA was extracted from blood using either a salt extraction technique (Richardson et al., 2001) or, since 2013, the DNeasy blood and tissue kit (Qiagen). Sex was determined via PCR (Griffiths et al.,1998). Individuals were genotyped at 30 polymorphic microsatellite loci (Richardson et al., 2001). Parentage assignment was carried out using MasterBayes 2.52 (Hadfield et al., 2006); for full details see Sparks et al. (2021). Parentage assignment was conducted for 1966 offspring that hatched between 1993–2018, with 89% of fathers and 86% of mothers assigned at ≥80% accuracy. Standardised individual and maternal microsatellite heterozygosity (H_s) was calculated using the R package GENHET 3.1 (Coulon, 2010). Two of the microsatellite loci were excluded from this heterozygosity analysis due to pooled alleles (see Sparks et al., 2021). Variation at exon 3 of the MHC class I loci (MHC-I) had previously been screened in individuals from Cousin (1148 individuals hatched between 1992–2009) (Richardson & Westerdahl, 2003; Wright, Spurgin, et al., 2014).

Variation within the leucine-rich repeat domain of *TLR3* exon 4 had previously been characterised; of the three SNPs found, only one SNP was nonsynonymous and had a minor allele frequency of >0.05 (Gilroy et al., 2017). This focal SNP is found at 198 bp in the Seychelles warbler *TLR3* reference sequence (NCBI accession number: KM657704.2), where the presence of an A or C nucleotide caused a change of amino acid from lysine (+ charge), to asparagine (polar). Variation at KM657704.2:g.198A>C (hereafter referred to as *TLR3* SNP) was genotyped in 1647 individuals using the KASP genotyping technology by LGC Genomics, Hertfordshire.

2.3 | Analyses

Unless otherwise stated, all analyses were conducted in R 3.6.1.

2.4 | Temporal patterns of *TLR3* variation on Cousin

In total, 1190 birds hatched on Cousin from four cohorts 1992–1994, 1997–1999, 2005–2010, and 2016–2018, were sequenced at the *TLR3* SNP. The earliest and latest of the sampled cohorts were used to assess temporal changes. In addition, the years 1997–1999 and 2005–2010 were selected; (a) to avoid hatch years in which translocations happened (2004, 2011), as the subsequent reduction in population density may have a positive effect on juvenile (<1 year) survival in that year (Brouwer et al., 2006), and, (b) to focus on individuals with the most complete MHC and life-history data. Temporal allelic variation was analysed using a linear model (LM) and significance was assessed using the F-statistic. Frequency of the *TLR3* allele in the sampled adult or juvenile population was the response variable, while year was the fixed factor.

2.5 | Contemporary selection on *TLR3* variation on Cousin

2.5.1 | Survival

A mixed-effects Cox proportional hazards model in the package COXME 2.2–14 (Therneau, 2019) was used to determine whether *TLR3* genotypes differed in survival. Model diagnostics using Schoenfeld's residuals confirmed that proportional hazards assumptions were met (Grambsch & Therneau, 1994). Age at death was standardised to bi-annual levels corresponding to the major and minor seasons. Fieldwork was not conducted for four minor breeding seasons (2000–2002, 2006), so accurate bi-annual survival estimates could not be calculated for 77 individuals. Instead, the minimum date of death was assigned (i.e., the last season an individual was observed). Excluding these individuals did not qualitatively alter the results, so they were retained in the model. Birds first caught as an adult (>1 year, n = 21) were excluded to prevent any survivorship bias by including individuals that have already survived the first year of life, and because Seychelles warblers cannot be reliably aged past one year of age (Wright, 2014). Individuals that were translocated to other islands (n = 39), and those still alive after the major 2018 breeding season (n = 42) were right-censored. Previous work has found that in low-quality seasons maternal heterozygosity affected offspring survival (Brouwer et al., 2007), and MHC diversity positively affected survival in juveniles, while individuals with the MHC-I allele (*Ase-ua4*) have a greater life expectancy (Brouwer et al., 2010). Due to these fitness component differences, and the fact that MHC-I has similar properties to *TLR3* in that it primarily binds intracellular peptides, we also include MHC-I characteristics in subsequent analysis. *TLR3* genotype ($TLR3^{AA}$/$TLR3^{AC}$/$TLR3^{CC}$), MHC diversity (2–8 different alleles), presence of the *Ase-ua4* allele (Yes/No), individual heterozygosity (H_s), maternal heterozygosity (Maternal H_s), sex (Male/Female), and season hatched (Minor/Major) were included as fixed factors in the model, with hatch year included as a random factor. Individuals hatched on Cousin between 1997–1999 or 2005–2010, for which these data were available, were included (n = 517). Cox proportional hazards models in the package SURVIVAL 2.44-1.1 (Therneau & Lumley, 2015), without the random effects, were used to plot Kaplan–Meier survival curves.

2.5.2 | Reproductive success

A zero-inflated generalised linear mixed model (GLMM) with a Poisson error structure was run using the package GLMMTMB 0.2.3 (Brooks et al., 2017) to test whether lifetime reproductive success (LRS) was associated with *TLR3* variation. LRS was measured as the number of offspring that survived to independence (3 months) produced throughout an individual's lifespan. Both social and extra-pair offspring were included. Individuals that were translocated, or still alive after the minor 2018 season, were excluded due to incomplete data. Individuals first caught over one year of age, for which we did not have accurate age and longevity data, were also excluded. All other birds hatched on Cousin between 1997–1999 and 2005–2010 were included (n = 487). *TLR3* genotype, MHC diversity, presence of the *Ase-ua4* allele, and individual H_s were fixed factors in the model, with year of hatch as a random factor to control for cohort effects. The sexes were modelled separately as it is likely that different factors and constraints act upon males and females.

As LRS is strongly correlated with longevity (GLMM, p < .001, Table 2), and survival was strongly correlated with *TLR3* genotype (COXME, p = .026, Figure 2, Table 1), we tested if lifetime reproductive rate (defined as reproduction controlling for longevity) was associated with *TLR3* genotype. The model and data set used was the same as used for LRS, except for two key differences: (a) Individuals which died before reaching adulthood (i.e., one year of age) were excluded from this analysis (resulting in n = 323), (b) Age at death (i.e., longevity and longevity^2) were included as fixed factors. The inclusion of longevity, and the exclusion of non-adult individuals, allows reproductive success to be isolated from survival; thus gaining a measure of the rate of reproduction during the individual's adult life.

For both LRS and rate of reproduction models all continuous factors were standardised (scaled and centred) using the package ARM 1.10-1 (Gelman et al., 2018). Collinearity between fixed effects was tested using variance inflation factors. We used the package DHARMA 0.2.4 (Hartig, 2017) to confirm that there was no over or under dispersion, residual spatial or temporal autocorrelation in the GLMM models. We used model averaging using the dredge function in the MUMIN package 1.43.6 (Barton & Barton, 2019) to select plausible models. All models within seven AICc of the top model were included in the averaged model to get the final conditional model.

2.5.3 | Selection coefficient

Mean values of LRS were calculated for each genotype from the raw data, relative fitness per *TLR3* genotype was calculated by dividing

TABLE 1 Time-dependent Cox Regression modelling to test the effects of *TLR3* genotype on bi-annual survival in the Seychelles warbler population (n = 517) on Cousin

Factor	coef	SE (coef)	HR	z	p
TLR3: ^{AC}	−0.01	0.10	0.99	−0.08	.940
***TLR3*:^{CC}**	**0.32**	**0.14**	**1.37**	**2.25**	**.024**
Individual H_s	−0.12	0.23	0.89	−0.52	.600
Ase-ua4	**−0.29**	**0.13**	**0.75**	**−2.20**	**.028**
MHC Diversity	−0.02	0.03	0.98	−0.77	.440
Maternal H_s	−0.08	0.22	0.92	−0.37	.710
Season hatched	−0.22	0.12	0.80	−1.86	.062
Sex	−0.02	0.10	0.98	−0.19	.850
Random effects	Variance	517 individuals			
Hatch year	0.015	9 hatch years			

Note: An HR >1 indicates increased hazard of mortality, and <1 indicates decreased hazard of mortality.

Coefficient estimates are in reference to *TLR3* = ^{AA}, *Ase-ua4* = Present, Season hatched = Major, Sex = Female.

Significant terms are in bold and underlined.

Abbreviations: Coef, hazard rate; HR, hazard ratio; SE (coef), standard error of the hazard rate.

the mean for all three genotypes by the mean from the genotype with the greatest fitness. The data set used was the same as that used for LRS – except that mean LRS was measured as the total number of offspring produced by an individual that survived to recruitment (>1 year) as this is a more accurate measure of genotype contribution to the next generation.

2.5.4 | Hardy-Weinberg Equilibrium in young birds on Cousin

Deviation from Hardy-Weinberg Equilibrium (HWE) was tested using exact tests (Guo & Thompson, 1992) based on allelic frequencies in GENEPOP 4.2 (Rousset, 2008). p-values were estimated with Markov chain algorithms (1000 dememorisations, 100 batches, 1000 iterations), and F_{IS} values are presented using Robertson and Hill estimates (Robertson & Hill, 1984). First, all birds from Cousin first caught before three months of age (before independence) were tested (n = 591). Second, to determine if early-life mortality changed HWE proportions, this test was repeated including only individuals that survived until adulthood (n = 361). To determine if any deviation from HWE was caused by a temporal Wahlund-like effect (as in Pusack et al., 2014) we also re-ran the analysis separately for each hatch year.

2.6 | Spatial and temporal *TLR3* variation across islands

The earliest available samples from the source population Cousin (120 birds caught in 1993 and 1994), were used to provide a proxy estimate of the initial *TLR3* diversity on Aride and Cousine (which were established in 1988 and 1990 before sampling took place). Samples from 56 of the 58 birds translocated to Denis, and all 59 birds translocated to Frégate were used to determine initial *TLR3* diversity on these islands. The most recent population samples were of 58 individuals caught in 2018 on Frégate, 158 individuals caught in 2015 on Denis, 54 individuals caught in 2012 and 2016 on Aride, 72 individuals caught in 2019 on Cousine, and 196 individuals caught in 2018 on Cousin (Table S1).

GENEPOP 4.2 (Rousset, 2008) was used to test if the different island populations conformed to HWE (as above). We tested for temporal and spatial divergence in *TLR3* frequencies among populations using genic differentiation tests (Raymond & Rousset, 1995) in GENEPOP 4.2 (Rousset, 2008). Fisher's exact test and the Markov chain algorithm parameters were as above. First, we tested for differentiation between the initial (translocated or 1993–1994 samples) and most recent samples from each population. Second, we tested for differentiation among populations using the most recent samples.

2.7 | Ethics statement

Fieldwork was carried out in accordance with local ethical regulations and agreements. The Seychelles Department of Environment and the Seychelles Bureau of Standards approved the fieldwork.

3 | RESULTS

In total, 1608 out of 1647 (0.98) samples were genotyped successfully at one *TLR3* SNP: 756/1608 (0.47) of these individuals had genotype $TLR3^{AA}$, 659/1608 (0.41) had $TLR3^{AC}$, and 193/1608 (0.12) had $TLR3^{CC}$.

3.1 | Temporal patterns of *TLR3* variation on Cousin

In the adult population on Cousin, the frequency of the minor $TLR3^{C}$ allele decreased significantly over time from 0.40 in 1993 to 0.29 in 2018, with a corresponding increase in the $TLR3^{A}$ allele (LM: R^2 = 0.85, $F_{1,24}$ = 140, p < .001, Figure 1). Likewise, the minor $TLR3^{C}$ allele also significantly decreased over time in the juvenile population from 0.44 in 1993 to 0.23 in 2018 (LM: R^2 = 0.68, $F_{1,12}$ = 28.7, p < .001, Figure 1).

3.2 | Testing for contemporary selection on *TLR3* variation on Cousin

There were significant differences in lifetime survival probabilities between *TLR3* genotypes. Individuals (first caught as juveniles) with the $TLR3^{CC}$ genotype had a 37% increased mortality risk compared

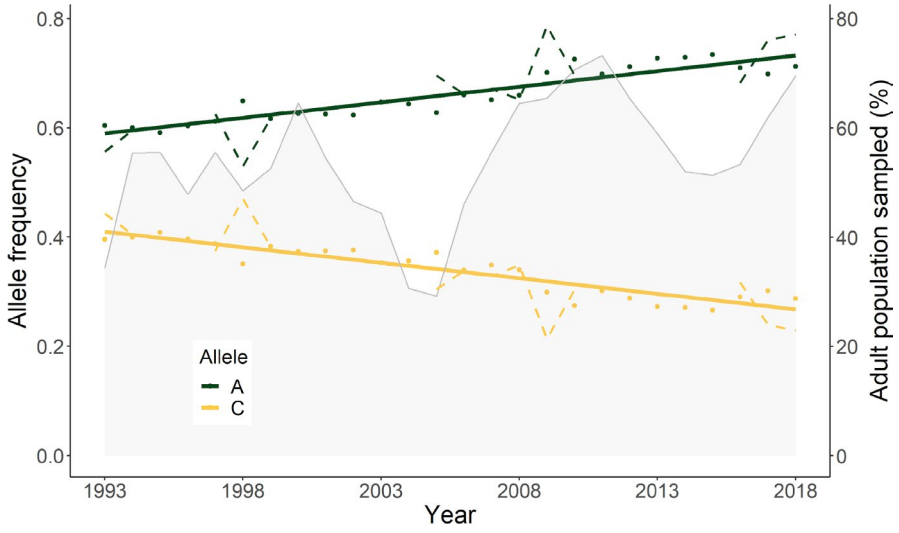

FIGURE 1 Allele frequency change at a nonsynonymous *TLR3* SNP in the Cousin population of the Seychelles warbler over 25 years (1993–2018). Points refer to *TLR3* allele frequencies in the adult population in a given year, the $TLR3^{A}$ allele in dark green, the $TLR3^{C}$ allele in yellow. Solid lines show linear regressions for the adult population. Dashed lines indicate frequencies in sampled individuals hatched in each year. The shaded grey area (right hand axis) shows the percentage of the adult population (mean: 310 individuals) screened in each year

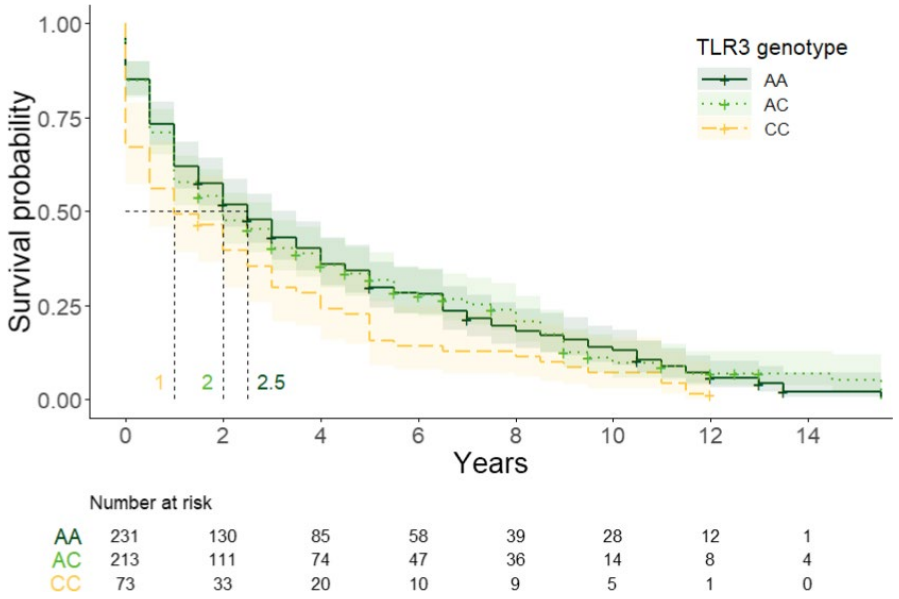

FIGURE 2 Effect of *TLR3* genotype on survival in the Seychelles warbler population on Cousin (n = 517). Lifetime survival probabilities classified into six month periods are shown for individuals with $TLR3^{AA}$ (dark green, solid), $TLR3^{AC}$ (light green, dotted) and $TLR3^{CC}$ (yellow, dashed) genotypes. Shaded areas denote 95% confidence limits. Dotted vertical lines indicate median lifespan (in years) of each genotype. Translocated individuals and individuals still alive at the end of the study are right censored (indicated with the symbol "+")

to those with the $TLR3^{AC}$ or $TLR3^{AA}$ genotypes, with a median age of death of 1, 2, and 2.5 years respectively (COXME, p = .024, Figure 2, Table 1). Thus, individuals with at least one copy of the $TLR3^{A}$ allele had an increased probability of survival than those without (p = 0.025, Table S2). Independently – and as found previously in a smaller data set (Brouwer et al., 2010) – individuals with the *Ase-ua4* MHC-I allele had a 25% reduced risk of mortality than those without, corresponding to a median age of death at 3.5 years (compared to 2 years for those individuals without) (COXME, p = .028, Table 1). There was no significant effect of sex, H_s, maternal H_s, or MHC diversity on lifetime survival probability (Table 1), or of the season in which an individual hatched, although individuals hatched in the minor breeding season tended to have increased survival (COXME, p = .062, Table 1).

In males, individuals with different *TLR3* genotypes had significantly different LRS. Males with $TLR3^{AA}$ had greater LRS than those with $TLR3^{AC}$ (p < .001, Table 2, Figure 3a) or $TLR3^{CC}$ (p = .003, Table 2, Figure 3a), with $TLR3^{AA}$ males producing on average twice the number of independent offspring (mean ± SEM: 1.40 ± 0.27) than either $TLR3^{AC}$ (mean ± SEM: 0.63 ± 0.17), or $TLR3^{CC}$ males (mean ± SEM: 0.70 ± 0.21) over their lifetime. There was no significant difference in LRS between $TLR3^{AC}$ and $TLR3^{CC}$ genotypes (p = .86) in males. Thus, males with at least one copy of the $TLR3^{C}$ allele had reduced LRS than those without (p < .001, Table S3). In contrast in females there was no association between *TLR3* genotype and LRS (Figure 3a). In males, LRS decreased with increasing MHC diversity (p = .047, Table 2), whereas in females LRS tended to increase with increasing MHC diversity, although this result was marginally non-significant (p = .064, Table 2). H_s and the presence of *Ase-ua4* did not predict LRS for either sex (Table 2).

As survival was strongly correlated with *TLR3* genotype, we also investigated whether *TLR3* genotypes predicted reproductive rate after controlling for parental survival – i.e., by including longevity and controlling for breeding ability (survival to recruitment into the adult population). In both sexes, individuals who lived longer (greater longevity) produced significantly more offspring

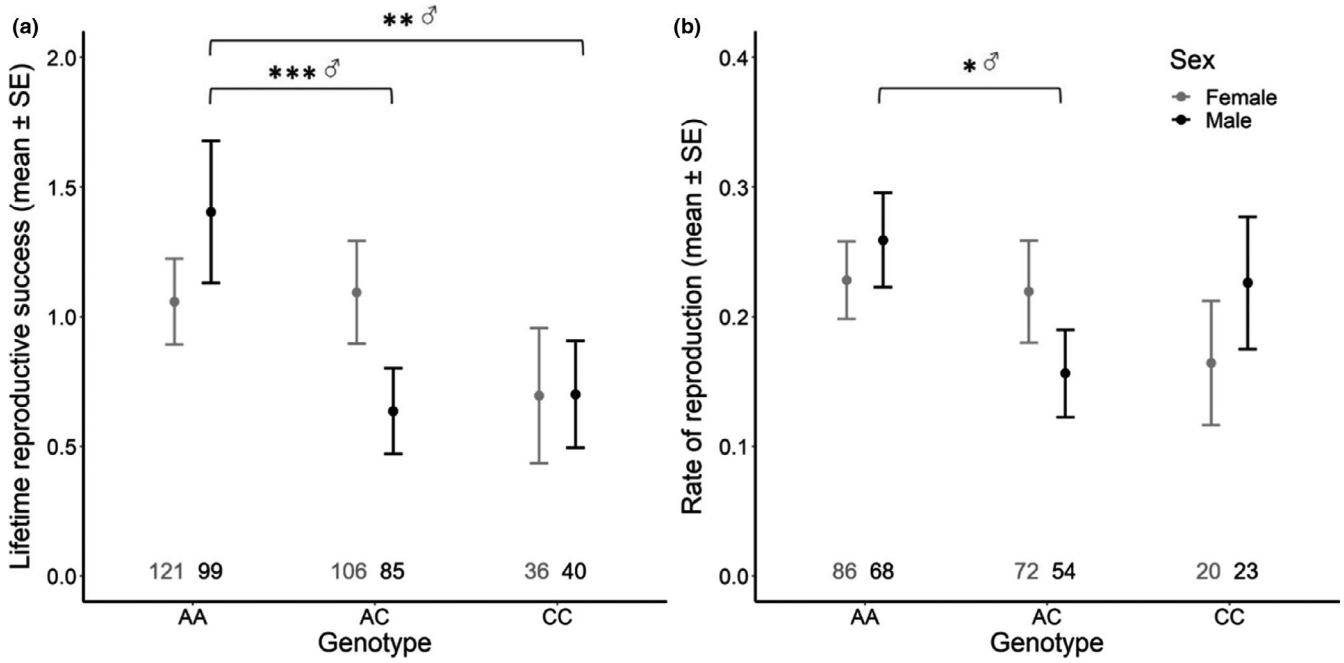

FIGURE 3 Effects of *TLR3* genotype on reproductive success in the Cousin population of the Seychelles warbler: (a) Lifetime reproductive success (offspring surviving >3 months) for all birds; n = 487), (b) Rate of reproduction (i.e. offspring surviving to >3 months/longevity for focal birds that survived to adulthood; n = 323). Data are raw means and standard errors, with female data shown in light grey and males in black separated by genotype, with associated sample sizes at the bottom. ***p < .001, **p < .01, *p < .05

(GLMM, Age p < .001, Table 2). There was also evidence for a negative quadratic effect of longevity in both sexes (GLMM, Age^2 p < .001, Table 2). Males of *TLR3*^{AA} genotype tended to produce more offspring (surviving >3 months; GLMM, p = .049, Table 2, Figure 3b) than those of *TLR3*^{AC} genotype, while *TLR3*^{AA} and *TLR3*^{AC} genotypes did not differ from *TLR3*^{CC} genotypes (p = .38 and .54, respectively). There was no association between the rate of reproduction and *TLR3* genotype or quadratic age in females. H_s, MHC diversity, and the presence of *Ase-ua4* did not predict reproductive rate for either sex (Table 2).

The difference in LRS associated with *TLR3* variation equated to a selection coefficient of 0.34 against *TLR3*^{AC,} and 0.46 against *TLR3*^{CC} genotypes of both sex, over ca three overlapping generations (assuming a generation time of 4 years (Spurgin et al., 2014)), when the selection coefficient of *TLR3*^{AA} genotype was set as 1.

3.3 | Hardy-Weinberg equilibrium in fledglings sampled on Cousin

There was a significant deviation from HWE among fledglings (individuals < 3 months of age) on Cousin, with a deficiency of heterozygotes (n = 591, F_{IS} = 0.12, p = .002, Table S4, Figure S1a). However, there was no deviation from HWE in those individuals that survived until adulthood (individuals >1 year, n = 380, F_{IS} = 0.08, p = .13 Figure S1b). Individuals caught < 3 months of age were then separated into hatch year, and HWE was assessed for each year. The heterozygote deficiency was consistent across most years (indicated by a positive F_{IS}), but with limited power, only 2007 showed a significant deviation from HWE (n = 53, F_{IS} = 0.31, p = .04, Table S4).

3.4 | Spatial and temporal *TLR3* variation across islands

No significant deviation from HWE was observed in any of the different island populations, either pre- or post- translocation (Table S5). All populations showed the same overall trend, with *TLR3*^{C} alleles decreasing in frequency over time (Figure 4), but the rate of change differed between islands (Table 3, Figure 4). As shown above for adults and juveniles, *TLR3*^{C} allele frequencies on Cousin were significantly lower for individuals caught in 2018 compared to 1993–1994 (p < .001, Table 3, Figure 4). Of the translocated populations, only Denis showed a significant decline in *TLR3*^{C} allele frequency between the initial and most recent sample (15 years difference; p = .002; Figure 4; Table 3). *TLR3* allele frequency temporal differences for Frégate (7 years difference), and between the oldest samples from the source population (Cousin) and the contemporary samples from Aride and Cousine (20 or 28-year difference respectively) were not significant (Figure 4; Table 3).

Focusing on the most recent samples, we found significant *TLR3* differentiation between Denis and Aride (p = .001; Table 3), Denis and Cousine (p = .009; Table 3), and Aride and Cousin (p = .022; Table 3). Denis had the lowest frequency of *TLR3*^{C} alleles (22%) while Aride had the highest (39%) (see Figure 4). All other pairwise comparisons were not significant (Table 1).

TABLE 2 Reproductive success in male and female Seychelles warblers on Cousin Island in relation to *TLR3* genotype: (a) Lifetime reproductive success for all birds, (b) Reproductive success controlling for longevity for birds that survived to adulthood

		Male (a: n = 224; b: n = 145)							Female (A: n = 263; B: n = 178)						
Response	**Factor**	ω	β	**SE**	**Adjusted SE**	z	p		ω	β	**SE**	**Adjusted SE**	z	p	
(a) LRS - Count of offspring surviving >3 months (independence)	Intercept		**1.10**	**0.21**	**0.21**	**5.16**	**<.001**	***		**0.95**	**0.15**	**0.15**	**6.38**	**<.001**	***
	Zero-inflated intercept		**0.49**	**0.16**	**0.16**	**2.96**	**.003**	**		**0.53**	**0.14**	**0.14**	**3.68**	**<.001**	***
	TLR3: ^{AC}	**1**	**−0.69**	**0.19**	**0.19**	**3.63**	**<.001**	***	0.15	0.06	0.15	0.15	0.40	.693	
	TLR3: ^{CC}		**−0.74**	**0.25**	**0.25**	**2.97**	**.003**	**		−0.16	0.26	0.26	0.62	.536	
	Individual H_s	0.26	0.04	0.17	0.17	0.23	.815		0.58	−0.24	0.14	0.14	1.64	.101	
	MHC Diversity	**0.71**	**−0.29**	**0.14**	**0.14**	**1.99**	**.047**	*	0.68	0.26	0.14	0.14	1.85	.064	.
	Ase-ua4	0.29	0.12	0.23	0.23	0.53	.599		0.43	0.20	0.16	0.16	1.23	.217	
(b) Reproduction – Count of offspring surviving >3 months (independence)	Intercept		0.00	0.15	0.15	0.03	.979			−0.03	0.11	0.11	0.25	.804	
	Zero-inflated intercept		**−3.43**	**1.05**	**1.06**	**3.25**	**.001**	**		−5.10	7.22	7.27	0.70	.483	
	Longevity	**1**	**3.31**	**0.30**	**0.31**	**10.81**	**<.001**	***	**1**	**3.23**	**0.27**	**0.28**	**11.68**	**<.001**	***
	Longevity^2	**1**	**−1.33**	**0.22**	**0.22**	**6.02**	**<.001**	***	**1**	**−1.51**	**0.26**	**0.26**	**5.81**	**<.001**	***
	TLR3: ^{AC}	**0.49**	**−0.34**	**0.17**	**0.17**	**1.97**	**.049**	*	0.13	−0.01	0.13	0.13	0.06	.955	
	TLR3: ^{CC}		−0.19	0.21	0.22	0.88	.382			−0.20	0.22	0.22	0.90	.368	
	Individual H_s	0.27	0.03	0.17	0.17	0.20	.845		0.25	−0.05	0.14	0.14	0.35	.724	
	MHC diversity	0.25	−0.03	0.14	0.14	0.18	.858		0.30	0.09	0.13	0.13	0.74	.462	
	Ase-ua4	0.28	0.10	0.18	0.18	0.57	.570		0.26	−0.06	0.14	0.14	0.43	.668	

Note: Zero-inflated GLMMs were used to generate conditional model-averaged values for all predictors featuring in the top model set (ΔAICc ≤ 7).

Model-averaged estimates (β), their standard error (SE), adjusted SE, z value, p-value, and relative importance (ω) are shown for all predictors featuring in the top model set (ΔAICc ≤ 7).

Estimates are in reference to *TLR3* = ^{AA}, *Ase-ua4* = Present.

Significant terms are in bold and underlined.

***p < 0.001; **p < 0.01; *p < 0.05.

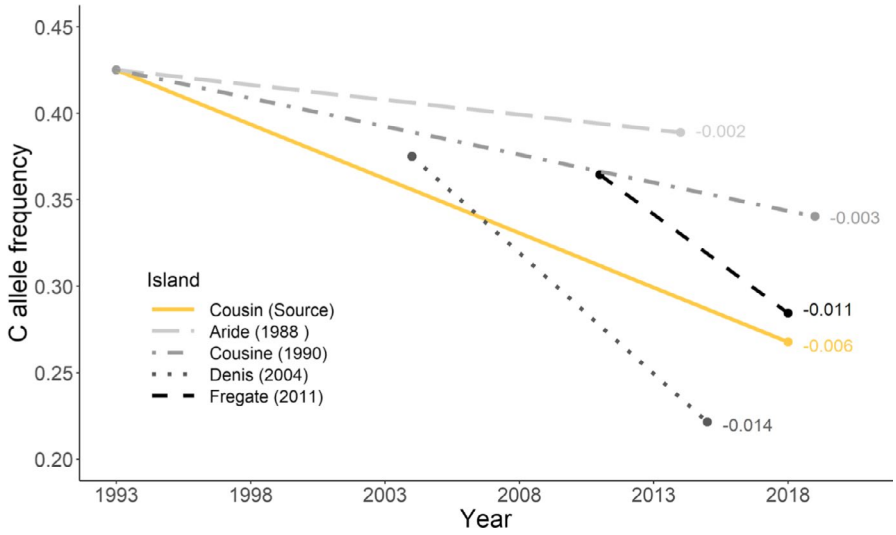

FIGURE 4 Change in the minor allele frequency (C) of the nonsynonymous *TLR3* SNP between two time points in the five isolated island populations of the Seychelles warbler. Points refer to $TLR3^{C}$ allele frequencies for all caught birds at each time point with lines added to emphasize the rate of change. The first time point for Cousin, Aride and Cousine is the 1993–1994 Cousin source population (n = 120), whereas the first time points for Denis (2004, n = 56) and Frégate (2011, n = 59) Islands are the translocated individuals. The second time point indicates the most recent sampling event for each island: Cousin (2018, n = 196), Aride (2012 and 2016, n = 54), Cousine (2019, n = 72), Denis (2015, n = 158) and Frégate (2018, n = 58). The translocation year is indicated in the legend. Values represent annual change in frequency of $TLR3^{C}$ allele

TABLE 3 Allelic differentiation of one *TLR3* SNP in the five isolated island populations of the Seychelles warbler between: (a) two time points for the same island, and (b) between different pairs of islands using the most recently sampled data

	Population comparisons		χ^2	SE	*p*-value
(a) Old versus recent population samples	**Cousin (1993–1994)**	**Cousin (2018)**	**19.44**	**0.00**	**<.001**
	Cousin (1993–1994)	Cousine 2019	4.51	0.01	.105
	Cousin (1993–1994)	Aride (2012/16)	1.13	0.01	.568
	Denis (Translocated)	**Denis (2015)**	**12.09**	**0.00**	**.002**
	Frégate (Translocated)	Frégate (2018)	3.07	0.01	.216
(b) Between most recent samples on different islands	Cousin (2018)	Cousine (2019)	4.51	0.01	.105
	Cousin (2018)	**Aride (2012/16)**	**7.66**	**0.00**	**.022**
	Cousin (2018)	Denis (2015)	3.69	0.01	.158
	Cousin (2018)	Frégate (2018)	0.41	0.00	.816
	Aride (2012/16)	Cousine (2019)	1.35	0.01	.510
	Aride (2012/16)	**Denis (2015)**	**13.74**	**0.00**	**.001**
	Aride (2012/16)	Frégate (2018)	4.28	0.00	.118
	Cousine (2019)	**Denis (2015)**	**9.41**	**0.00**	**.009**
	Cousine (2019)	Frégate (2018)	2.11	0.01	.349
	Denis (2015)	Frégate (2018)	3.21	0.01	.201

Note: The first time point for Cousin, Aride and Cousine are from the 1993–1994 Cousin source population, whereas the first time point for Denis and Frégate are from the translocated individuals. The second time point indicates the most recent sampling event for each island. Significant terms are in bold and underlined.

4 | DISCUSSION

We detected spatial and temporal changes in *TLR3* variation in the Seychelles warbler. On Cousin, we found a consistent decline in the minor allele frequency of the nonsynonymous $TLR3^{C}$ allele in the adult population, from 40% in 1993, to 29% in 2018 (Figure 1). Importantly, differential survival was associated with *TLR3* genotype; individuals with the $TLR3^{CC}$ genotype had 37% increased mortality risk compared to those with $TLR3^{AC}$ or $TLR3^{AA}$ genotypes. Furthermore, males - but not females - with $TLR3^{CC}$ or

TLR3^{AC} genotypes had lower LRS than those with the *TLR3*^{AA} genotype (Figure 3a). Even when controlling for longevity, males with the *TLR3*^{AC} genotype had reduced reproduction compared to those with the *TLR3*^{AA} genotype (Figure 3b). Notably, the *TLR3* genotypes of nestlings/fledglings deviated from Hardy-Weinberg expectations. Lastly, although we found differences in the *TLR3* minor allele frequency among the island populations (Figure 4), they all showed the same pattern of a decrease in the minor allele frequency.

The temporal pattern in our data - with the *TLR3*^{C} allele declining in the population on Cousin over a 25-year period - could be driven by a number of evolutionary forces. However, the lack of migration in or out of Cousin (Komdeur et al., 2004), means it cannot be caused by gene flow. Importantly, our results show that individuals of either sex that were homozygous for *TLR3*^{C} had lower survival and that *TLR3*^{AC} males had a lower rate of reproduction. These differences in survival (and to a lesser degree reproductive rate) resulted, at least in males, in a considerable reduction in LRS; males with one or two copies of the *TLR3*^{C} allele had ca. half the reproductive success of those with none (*TLR3*^{AC}: 0.63, *TLR3*^{CC}: 0.70, compared to *TLR3*^{AA}: 1.4 average independent offspring over their lifetime). These results indicate that selection is occurring and may explain the observed change in the *TLR3*^{C} allele frequency over time. Both *TLR3*^{AC} and *TLR3*^{CC} individuals had relatively large selection coefficients of 0.34 and 0.46, respectively. However, it should be noted that the added complication of overlapping generations in a relatively long-lived species could act to dilute the observed selective benefit of *TLR3*^{AA} genotypes in the short term. While purifying selection in TLRs is the predominant selective mechanism in this multigene family (Alcaide & Edwards, 2011), signatures of positive (or balancing) selection have been detected at the codon level in various wild vertebrate species (Areal et al., 2011; Khan et al., 2019; Liu et al., 2019). Indeed, previous work in the Seychelles warbler detected evidence of past positive selection at this *TLR3* locus (Gilroy et al., 2017). The present study now shows that this *TLR3* locus is under strong positive selection (through both survival and reproductive success differences) in the contemporary Cousin population.

Even if selection is acting upon the *TLR3* locus in the Seychelles warbler genetic drift will also occur. Other studies have shown that genetic drift can override the effect of selection in driving immune gene variation (Miller & Lambert, 2004b; Quemere et al., 2015; Sutton et al., 2011), including TLR variation (Gonzalez-Quevedo et al., 2015; Grueber et al., 2013). However, in the Seychelles warbler the temporal change in allele frequencies at the *TLR3* locus, aligned as it is with the differential fitness of the *TLR3*^{C} allele, suggest that selection is currently the prevailing force acting upon this locus in this population. Furthermore, a previous study showed that neither neutral microsatellite diversity, nor functional MHC allelic richness, changed over an 18-year time period in the Cousin population, while the mean MHC diversity per individual increased over that time (Wright, Spurgin, et al., 2014). This lack of a change at these other loci may suggest that the effect of genetic drift is limited in this already genetically depauperate (Hansson & Richardson, 2005; Richardson & Westerdahl, 2003) population over the timeframe observed here.

While various studies have linked TLR variation with pathogen infection (Quemere et al., 2015; Tschirren et al., 2013), few have found direct links between TLR variation and fitness in wild populations. In the pale-headed brushfinch (*Atlapetes pallidiceps*), decreased survival was associated with high overall TLR diversity (Hartmann et al., 2014), whilst in song sparrows (*Melospiza melodia*) there was no relationship between survival and TLR heterozygosity (Nelson-Flower et al., 2018), although in both cases the effect of specific alleles was not tested. In the Stewart Island robin (*Petroica australis rakiura*), early life mortality was reduced in individuals with the *TLR4*^{BE} genotype, compared to other *TLR4* genotypes, despite it being a synonymous substitution (Grueber et al., 2013). Finally, in Attwater's prairie-chicken (*Tympanuchus cupido attwateri*) the presence of a specific *TLR1B* allele was associated with reduced survival (Bateson et al., 2016). Like the latter two studies, we found the presence of a specific allele to confer differential survival; the *TLR3*^{A} allele conferred a selective advantage via increased survival, predominantly in early life. Given the importance of *TLR3* as an innate immune receptor (Barton, 2007), and that the SNP investigated causes a functional difference in the binding region, it is likely that the survival differences seen here are due to differential pathogen recognition.

In this study, we also found some evidence of *TLR3* genotypes conferring differential reproductive success in male, but not female, warblers. To our knowledge, this is the first-time variation at a TLR gene has been associated with reproductive success in a wild population. In vertebrates, longevity is generally strongly positively correlated with LRS (Clutton-Brock, 1988), indeed we found longevity to be the greatest predictor of reproductive success in the Seychelles warbler. However, even after controlling for fitness effects associated with offspring genotype, ability to breed, and longevity we found an effect of male *TLR3* genotype. Combined with differential survival, this resulted in *TLR3*^{AA} males having considerably greater overall LRS than other genotypes. This observed difference in the reproductive output of males, but not females, could be driven by male-male competition – with males in better condition (through differential immune response due to the *TLR3* variation) better able to outcompete others and gain more social or extra-group offspring. For example, specific alleles at both immune and non-immune loci have been associated with increased competitive ability and increased reproductive success in male vertebrates (Johnston et al., 2013; Sepil et al., 2013).

If female choice is occurring based on the *TLR3* variant in the Seychelles warbler this could explain how only male, and not female, individuals had differential reproduction associated with different *TLR3* genotypes. Previous studies, on both the Seychelles warbler (Richardson et al., 2005; Wright et al., 2016) and other vertebrate taxa, have focused on MHC-based female mate choice (reviewed in Kamiya et al., 2014; Milinski, 2006). As we found a *TLR3* heterozygote deficiency in offspring, it is possible that assortative mating could be taking place, whereby individuals mate with individuals similar to themselves more frequently than expected by chance (Sin et al., 2015). Likewise, as *TLR3* heterozygous individuals do not have greater

fitness than *TLR3* homozygous individuals, mate choice is unlikely to be based on *TLR3* heterozygosity. Further investigation should focus on 'good genes' or assortative mating as potential candidate mechanisms in driving the differential reproduction observed in this study.

A third possibility that could explain the pattern of reproductive success linked to *TLR3* variation is that the heterozygote deficit in offspring is due to selection on those offspring. For example, males with $TLR3^{AA}$ genotypes are unable to produce $TLR3^{CC}$ offspring (whoever they breed with), so those males will never suffer from reduced reproductive success caused by the higher mortality of $TLR3^{CC}$ offspring, and thus will have higher LRS. Nonetheless, if this were the sole determinant of the differential reproductive success found in this study, one would expect an equivalent outcome for females. However, there was no effect of *TLR3* genotype on female overall LRS or rate of reproduction, despite females not differing from males in terms of survival linked to the *TLR3* variation. To differentiate between the three non-mutually exclusive mechanisms outlined above, future studies could determine if differences in competitive ability such as body condition and immune responses, and/or differential patterns of mating success are occurring based on this *TLR3* variation.

That there is contemporary positive selection acting upon the *TLR3* locus in the Seychelles warbler provides insight into the evolutionary mechanisms acting upon this important immune locus. The decline in the $TLR3^{C}$ allele, and corresponding increase in the $TLR3^{A}$ allele demonstrated in the current study only represents a snap-shot view of positive selection acting upon this locus. A previous study by Gilroy et al., (2017) including six other species closely related to the Seychelles warbler only found the A variant at this site, suggesting that the $TLR3^{A}$ allele may be ancestral. Although further phylogenetic analysis across a wide range of bird species would be needed to confirm this. That a selective beneficial polymorphism does exist at this locus despite the considerable bottleneck this species has undergone (Hansson & Richardson, 2005; Richardson & Westerdahl, 2003), may indicate that balancing selection is acting on this locus over the long-term. Given the role this locus plays in the innate immune response, this is likely to be pathogen-mediated. Of the three main mechanisms by which balancing selection is thought to maintain immune variation (reviewed in Spurgin & Richardson, 2010), our study shows that this is not caused by heterozygote advantage (Doherty & Zinkernagel, 1975); $TLR3^{AC}$ individuals did not gain greater LRS or have increased survival than the homozygote genotypes. The variation observed could potentially be driven by rare allele advantage (Slade & McCallum, 1992), or fluctuating selection (Hill et al., 1991), or both. However, differentiating the relative importance of these two mechanisms in driving genetic variation, and separating them from other evolutionary mechanisms is complicated and beyond the scope of the present study (reviewed in Spurgin & Richardson, 2010).

In the present study, we identified a decrease in the $TLR3^{C}$ allele frequency over time across all five island populations (Figure 4) though they did differ in rate of change. These temporal patterns of $TLR3^{C}$ loss suggest that whatever selective agent is acting on Cousin is present on the other islands. Given their very close proximity, and similarity to Cousin - compared to the more isolated islands of Denis and Frégate - the weaker effect on Aride and Cousine is surprising as one may expect close and environmentally similar islands to contain similar pathogens. For example, Cousine (the closest island to Cousin) is the only island to have retained (after translocation) the single strain of the *Haemoproteus nucleocondensus* pathogen that is present in the original Cousin population (Fairfield et al., 2016). A similar pattern of spatio-temporal change in *TLR1LA* diversity between translocated populations of the New Zealand South Island saddleback (*Philesturnus carunculatus)*, was put down to the distribution of malaria parasites (Knafler et al., 2017). However, the distribution of the haemoproteus pathogen found in the Seychelles warbler (not on Aride, Denis or Frégate) means that this cannot be the selective agent here. Work is now needed to identify the pathogen responsible, and determine why the distribution, or impact of this pathogen, differs among the islands.

The avian *TLR3* is orthologous to mammalian *TLR3* and recognises viral dsRNA (including avian pox and influenza viruses) (Brownlie & Allan, 2011; Chen et al., 2013; Hutchens et al., 2008). Therefore, it is likely that the selective agent is a virus. Despite this, we have found no obvious evidence of any viral illness in the Seychelles warbler in over 30 years of study. Furthermore, while viruses such as avian pox are common in many parts of the world (van Riper & Forrester, 2007) there are no reports of this, or any other virus, circulating in the passerines in the Seychelles (Hutchings, 2009). Influenza A has been reported in Procellariformes (petrels and shearwaters) in the Seychelles (Lebarbenchon et al., 2015), but whether this could be passed to the warblers is unknown. It is possible that we just do not see visible signs of a pathogen that is circulating in the warblers because of mild virulence or evolved host tolerance (Råberg, 2014, Hammers et al., 2016). Furthermore, individuals may only show visible symptoms during the acute phase of infection when they are also least active, consequently they may be unlikely to be observed before recovery or death (LaPointe et al., 2009).

Even if there are no virulent pathogens currently in the populations, maintaining immunogenetic variation could have important consequences for the future success of this species. If selection continues, the SNP investigated here will go to fixation, and potentially important immunogenetic variation will be lost in the system. This is particularly important given the reduced diversity already present at this, and other innate immune genes, in the Seychelles warbler (Gilroy et al., 2016, 2017). The innate immune response is often the organism's first line of defence against pathogens and plays an important role in the evolution to novel disease outbreaks (Bonneaud et al., 2012). Thus, knowing the underlying variation present, and understanding the mechanisms driving evolutionary change at these key functional sites could be important for future species conservation. This is important in small populations and/or those of conservation concern which often have reduced genetic variation. Managing genetic variation in such populations could be important for their adaptive potential, while monitoring pathogen presence may be important to identify and control disease outbreaks - both of which may be crucial for the population's long term survival.

5 | CONCLUSION

We found strong evidence that selection, acting through both survival and to a lesser degree reproduction, was associated with *TLR3* locus variation in the contemporary Cousin population. This suggests that an unknown pathogen is present in the Seychelles warbler population, driving evolution at this *TLR3* locus. It is possible that this current positive selection may be part of a much longer-term pattern of balancing selection, but only further monitoring will be able to determine this.

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AUTHOR CONTRIBUTIONS

The study was conceived by C. S. D. and D. S. R. C. S. D. and D. S. R. conducted laboratory work. H. L. D. conducted the parentage analyses. C. S. D., D. S. R., H. L. D., J. K., M. H. and T. B. performed fieldwork. C. S. D. performed analyses and drafted the manuscript with supervision from D. S. R.. D. S. R., H. L. D., J. K. and T. B. managed the long-term Seychelles warbler project. All authors contributed critically to the work and approved the final manuscript for publication.

DATA AVAILABILITY STATEMENT

All metadata, along with R scripts used to run analyses, are available in the Dryad Digital Repository, https://doi.org/10.5061/dryad.m905qfv06.

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SUPPORTING INFORMATION

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

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