



Assessment of the dunnocks' introduction to New Zealand using innate immune-gene diversity

Carlos Esteban Lara, et al. [full author details at the end of the article]

Received: 26 April 2020 / Accepted: 17 August 2020 / Published online: 24 August 2020
© Springer Nature Switzerland AG 2020

Abstract

Immunity genes are proposed to be informative about evolutionary processes acting upon introduced populations (e.g., showing signatures of selection). This is because immunity genes are expected to be under pathogen-mediated selection, and this type of selection can be more pronounced when individuals are exposed to new environmental conditions. Here we assessed innate immune genetic diversity, via Toll-like receptors (TLRs), to quantify genetic differentiation between a deliberately introduced population of dunnocks (*Prunella modularis*) in New Zealand and its source population in the United Kingdom. We also asked whether the introduced population shows signatures of intergenerational and current selection in TLR. We expect intergenerational and current selection patterns because New Zealand dunnocks have been exposed to new environmental conditions following their introduction around the late 1800s, which may have driven pathogen-mediated selection. Counter to our expectation, we found only weak and non-significant genetic differentiation between the introduced and source populations. Further, the levels of genetic differentiation (G'_{ST}) found in TLRs were similar to those found in microsatellites across the populations. Dunnocks, in general, have been under strong purifying selection over evolutionary time, but we found little evidence to support signatures of contemporary selection in TLR in the introduced population. Notably, however, we found a statistically significant heterozygosity advantage for males in TLR3, which lends support to possible current selection acting upon the introduced population. Overall, it appears that New Zealand dunnocks have retained a high proportion of the immunogenetic diversity of the source population, and that such diversity has probably been sufficient to defend against the potential pathogens found in New Zealand. Our results may explain—at least in part—why the introduction of dunnocks has been so successful; dunnocks have become one of the most common birds in New Zealand.

Keywords Avian evolution · Biological introductions · Natural selection · Population genetics · Toll-like receptors

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10682-020-10070-0>) contains supplementary material, which is available to authorized users.

Introduction

Past intentional biological introductions can serve as unplanned experiments that help us to track the genetic effects that introduced populations experience. Evaluating these genetic effects is important to determine the underlying causes of the growth or decline of the introduced population. For instance, the most common genetic effect following a population introduction is a genetic bottleneck, which can reduce genetic diversity, promote inbreeding depression, and lead to rapid accumulation of deleterious alleles (Charlesworth et al. 1993; Stockwell et al. 1996; Briskie and Mackintosh 2004; Dlugosch and Parker 2008). Yet, often genetic diversity losses are not observed even when few founders (i.e. individuals) are introduced (Merilä et al. 1996; Santos et al. 2013). Further, by studying the variation in genetic diversity over time, it is possible to assess the role of evolutionary forces, such as genetic drift or natural selection, acting upon introduced populations (Grueber et al. 2013; González-Quevedo et al. 2015).

Variation in population genetic processes impacting introduced populations have primarily been evaluated via neutral genetic diversity (e.g., microsatellites, see Dlugosch and Parker 2008). Neutral markers are commonly employed to measure genetic distance between populations (e.g. genetic distances between native and introduced population), and they are very informative about genetic structure and drift, as they are typically not subject to selection. On the other hand, we expect that functional genetic diversity may be more informative of non-neutral evolutionary processes than neutral loci, as the diversity of coding and other functional genomics regions is driven by selection. Toll-like receptor genes (TLRs), part of the innate immune system, are an ancient family of evolutionarily conserved transmembrane receptors that act as the first line of defense against pathogen-associated molecular patterns (PAMPS), which are derived from pathogens (Aderem and Ulevitch 2000; Blasius and Beutler 2010). TLR sequences are expected to evolve under pathogen-mediated selection (PMS), a co-evolutionary arms-race between hosts and pathogens, promoting genetic changes that should be traceable in TLR genetic diversity (Barreiro et al. 2009; Kloch et al. 2018). As a consequence of PMS, TLRs can become polymorphic and such diversity can be maintained through balancing selection (Alcaide and Edwards 2011). However, episodic events of positive selection over long timescales (Alcaide and Edwards 2011), purifying selection and genetic drift also contribute to the patterns of genetic variation observed in immune genes (González-Quevedo et al. 2015; Grueber et al. 2015; Gilroy et al. 2016; Morrison et al. 2020).

The biological introduction of bird species to New Zealand are particularly ideal to evaluate contemporary genetic processes, because there is access to the historical records from the Acclimatisation Societies of New Zealand. In this study, we are interested in the introduction of the dunnock (*Prunella modularis*, Prunellidae), a small passerine bird native to Eurasia. Historical records from the Otago Acclimatisation Society indicate that in the Otago region (where one of our study populations is located) a total of 185 individuals, released in small groups, were introduced from United Kingdom between 1865 and 1907 (Santos 2012; Santos et al. 2013). Since their introduction, dunnocks have spread successfully and colonized all habitat types under 1600 m and are considered as one of the most common bird species in the South Island of New Zealand (Kikkawa 1966; Davies 1992).

The introduction of dunnocks to New Zealand resulted in a new population that has been isolated from its source for over 30 generations and, most likely, has been exposed to novel environmental conditions. The ecosystem in New Zealand differs substantially from the dunnocks' ancestral conditions in United Kingdom, as New Zealand has been under

substantial isolation (over 25 Ma) in the cool-temperate south Pacific (McDowall 2008), which, along with biogeographical and biological patterns has generated a distinct biota compared to north-temperate continental areas (Goldberg et al. 2008). Pathogens in both countries likely differ as a consequence of distance-decay and species sorting (Green and Bohannan 2006; Hanson et al. 2012; Székely and Langenheder 2014). So, there seems to be a plausible scenario to test whether there has been an immunogenetic response in dunnocks, via selection, to the novel environment.

In this study, our first goal is to evaluate genetic differentiation between the introduced population of dunnocks from Dunedin (New Zealand) and a population of dunnocks from Cambridge (United Kingdom). Our second goal is to test for signatures of selection acting upon the introduced population with a major emphasis on short evolutionary scales (inter-generational and current selection). Both goals were examined using sequences of eight TLR genes and 16 microsatellites. For our first goal, we hypothesized that the introduction process could have caused a genetic bottleneck, so a plausible outcome is a reduction immune genetic diversity in New Zealand dunnocks compared to the source population (Santos et al. 2013). For our second goal, according to theory, we predict that over evolutionary time purifying selection is the main force shaping TLR variation (Mukherjee et al. 2009, 2014). We also predict that if the microbe-specific molecular signatures (i.e. pathogens) found in New Zealand have challenged the introduced dunnock population, we could detect signatures of (1) intergenerational selection by observing deviations from Hardy–Weinberg Equilibrium (HWE) and/or from Tajima's D, and (2) current selection via heterozygosity-fitness correlations.

Methods

Study populations

The introduced population, hereafter the Dunedin population, consists of approximately 70 breeding adult individuals per year (from 2009 and January 2016), which are resident and territorial at the Dunedin Botanic Garden (45°51'25" S–170°31'08" E, 8 m a.s.l., New Zealand). We closely monitored the breeding biology of this population over six breeding seasons (Holtmann et al. 2017; Santos et al. 2015). The dunnock breeding season in Dunedin starts in September and finishes in late December (austral spring to early summer). We have previously observed two main social mating systems in this population: monogamous pairs (45%) and polyandrous trios (54%), and their territory sizes were on average 0.24 and 0.25 ha, respectively (Santos and Nakagawa, 2013). The Dunedin Botanic Garden consists of 7.1 ha of a diversity of human-made habitats, such as open lawns, hedgerows, flower beds, and open woodland. The climate in Dunedin under the Köppen system can be classified as *Cfb* (temperate oceanic climate) with an average temperature of 10.8 °C and a rainfall of 775 mm per year (climate-data.org).

The source or ancestral population, hereafter named the Cambridge population, consists of approximately 80 breeding adult individuals per year, and it is located in the Cambridge Botanic Garden (52°11'37" N–0°07'37" E, 17 m a.s.l.), United Kingdom. This population was monitored over nine breeding seasons (1981–1990) by Professor Nick Davies and colleagues (Davies 1986; Burke et al. 1989; Davies 1992). The dunnock breeding season in Cambridge starts in April and finishes in July (boreal spring and summer). The mating system in Cambridge was more diverse as it included monogamous pairs (42.5%),

polyandrous trios (29.5%), polygynandrous groups (23.6%), and polygynous groups (4.3%) (Burke et al. 1989). Compared to the Dunedin population, territory sizes in Cambridge are similar for monogamous pairs (0.29 ha), but larger for polyandrous trios (0.59 ha). The Cambridge Botanic Garden is 16 ha and also consists of a mosaic of open woodland, hedgerows, shrubs; all human-made habitats (Davies 1992). The climate in Cambridge, also following the Köppen classification system, can be classified as *Cfb* (temperate oceanic climate) with a slightly lower average temperature of 9.7 °C and slightly less rainfall per year of 553 mm (climate-data.org), compared to the weather in Dunedin. Note that we cannot determine the exact location of the UK source population used to establish the New Zealand population, but we assume that dunnocks from Cambridge are representative of this source, because dunnocks, as other passerine bird species in the UK, have shown very little population structure and very low genetic differentiation among sites (Kekkonen et al. 2011).

DNA extraction and TLR genotyping

We analyzed blood samples from dunnocks between 2009 and 2016 in the Dunedin population ($N_{\text{[Dunedin]}}=55$), and Professor Terry Burke (University of Sheffield) kindly provided us with blood samples from the Cambridge population ($N_{\text{[Cambridge]}}=43$), collected between 1983 and 1990. We extracted DNA following an ammonium acetate protocol (Nicholls et al. 2000). We used primers that targeted the TLR extracellular domain of each gene, as this region of the gene is associated with pathogen recognition (Alcaide and Edwards 2011; Grueber et al. 2012; Grueber and Jamieson 2013), and it is also the most polymorphic part of the gene (Wang et al. 2016). We successfully amplified TLR1LB and TLR4 with primers developed by Alcaide and Edwards (2011) and by Grueber and Jamieson (2013), respectively (Table S1). Using partial dunnock sequences obtained using published primers (Alcaide and Edwards 2011; Grueber et al. 2012), for TLR1LA, TLR3, TLR5, and TLR15, we generated redesigned, optimized primers for dunnock with Codon Code Aligner version 5.0 (Table S1). We attempted to amplify TLR7 and TLR21 using primers from Alcaide and Edwards (2011) and Grueber and Jamieson (2013), and attempted to redesign primers as above, but none produced clear DNA sequences in our samples (Table S1).

We conducted polymerase chain reactions (PCRs) in a total volume of 12 μL , which contained dried DNA (10 ng), 6.0 μL MyTaq Mix 2x (Bioline Ltd), and 500 nM of each primer (Sigma-Aldrich). PCRs were performed on an Eppendorf Mastercycler Pro S using a three-step process: (i) denaturation for 15 min at 95 °C, followed by 12 cycles of denaturation for 30 s at 94 °C, annealing with touch down (65 °C to 52 °C for TLR1LA or 70 °C to 58 °C for all other genes) for 90 s, and extension for 60 s at 52 °C; (ii) 25 cycles of denaturation for 30 s at 94 °C, annealing for 90 s at 58 °C (52 °C for TLR1LA), and extension for 60 s at 52 °C; and (iii) a final extension for 90 min at 60 °C. Amplified products were visualized on a 1% agarose 1 \times TAE gel stained with SYBR safe DNA gel stain (Invitrogen). A 10 μL aliquot of the PCR product was cleaned up with 1 μL of ExoSAP-IT, mixed and incubated at 37 °C for 30 min, followed by inactivation at 80 °C for 15 min. The final PCR products were sequenced on an ABI 3730xl Genetic Analyzer.

We confirmed correct amplification of the target sequence using *Geneious* version R7 (Biomatters) by aligning our data to the TLR sequences obtained by Alcaide and Edwards (2011) and Grueber and Jamieson (2013). We generated consensus sequences for each gene for each individual, from forward and reverse reads, in *Geneious* using the software's default settings. We confirmed single nucleotide polymorphisms (SNPs) by visual

evaluation of chromatograms. We did not find stop codons or frameshift mutations in any of the sequences. We only sequenced a portion of the TLR's ectodomains and the final alignment sizes were: TLR1LA 462 bp, TLR1LB 627 bp, TLR2A 435 bp, TLR2B 300 bp, TLR3 597 bp, TLR4 657 bp, TLR5 618 bp, TLR15 552 bp. We used the Bayesian PHASE algorithms (Stephens and Donnelly 2003) implemented in DnaSP version 5.10.1 using default settings (Librado and Rozas 2009) to infer haplotypes. In addition, we genotyped a set of 16 polymorphic microsatellites for the same individuals following our previously published methods (Santos et al. 2013). The main goal of obtaining microsatellite data was to compare functional genetic diversity (TLRs) with neutral genetic diversity (as the former is subject to selection and drift, whereas the latter is only subject to drift).

Genetic diversity and differentiation among populations

We conducted our sequence and genotype analysis in R version 3.6.1 (R Core Team 2020). As measures of genetic diversity for TLRs we calculated haplotype diversity (H_d), nucleotide diversity (π), mean nucleotide diversity (k), and number of SNPs, with the packages *adegenet* (Jombart 2008) and *pegas* (Paradis 2010). To measure the extent of population differentiation between Dunedin and Cambridge, we quantified TLR genetic differentiation using G'_{ST} (Hedrick 2005). We calculated this metric and its respective confidence intervals (to test for statistical significance) using the *mmod* package (Winter 2012) and its chao bootstrapping function (10,000 bootstraps). Similarly, we obtained genetic differentiation for microsatellites using G'_{ST} and also applied the same bootstrapping process. As G'_{ST} is a standardized (scaled) metric of genetic differentiation, we contrasted the 10,000 G'_{ST} distributions (obtained via bootstrapping) for TLRs and microsatellites to determine whether the level of differentiation between the two types of genetic markers was different. Finally, to visualize genetic relationships between the two populations for each TLR locus, we built haplotype networks in *pegas* (Paradis 2010).

Signatures of selection

First, to examine signatures of selection over evolutionary time acting upon TLRs in dunnocks, we used the ratio between the number of non-synonymous and synonymous substitutions, d_N/d_S (Nei and Gojobori 1986). We obtained d_N/d_S ratios and their confidence intervals for each TLR and for each population based exclusively in the polymorphic sequences. We employed the SLAC method on the Datamonkey webserver (Delpont et al. 2010—accessed December 2019). We assumed purifying selection when $d_N/d_S < 1$ and positive selection when $d_N/d_S > 1$ (Nei and Gojobori 1986).

Second, to infer intergenerational selection, we used two approaches. First, we calculated observed heterozygosity (H_O) and expected heterozygosity (H_E) per gene per population and tested for Hardy–Weinberg Equilibrium (HWE) in each TLR gene as well as in each microsatellite locus both using *pegas* (Paradis 2010) with 1,000,000 iterations. Statistical significance of HWE was corrected for false discovery rates (Benjamini and Hochberg 1995) using the function *fdr* implemented in the *mcp.project* R package. We infer balancing selection when heterozygosity excess was present and when it deviated from HWE. We also used the Tajima's D neutrality test (calculated in *pegas*) to test for balancing or purifying selection. Statistically significant positive Tajima's D values are interpreted as evidence of balancing selection, while negative Tajima's D is interpreted as purifying selection.

Third, in addition to our sequence-based and genotype-based inferences around recent past selection, we examined current selection on TLRs in the Dunedin population, as heterozygosity advantage, using heterozygosity-fitness correlations (HFCs). Our reproductive fitness metric of interest (response variable) was number of chicks produced per individual per breeding season ($n_{[\text{individuals}]} = 55$, $n_{[\text{observations}]} = 476$, $n_{[\text{females}]} = 26$, $n_{[\text{males}]} = 29$), monitored during a six-year period (2009–2015, see: Holtmann et al. 2016, 2017; Santos and Nakagawa 2013; Santos et al. 2015). We fitted a Bayesian generalized linear mixed model (BLMM) using Markov chain Monte Carlo (MCMC) techniques implemented in the *MCMCglmm* R package (Hadfield 2010) with a *Poisson* error distribution (*log-link* function). We included TLR standardized heterozygosity (SH), a metric of multilocus heterozygosity (Coltman et al. 1999) as a fixed effect. To calculate SH we used the package *Rhh* implemented in R (Alho et al. 2010). We also include sex and the individual's age as fixed effects. Note that we included sex and age because these two proxies may influence fitness, the former given the social conflict among sexes in dunnocks (Davies 1986; Santos et al. 2015), and the later given the reproductive senescence with aging in passerine birds (Brommer et al. 2007; Bouwhuis et al. 2012). SH and age were z-transformed so that the regression coefficients were on a comparable scale (Schielzeth 2010). We included individual identity, mating system, and breeding season as random effects. We also tested the role of each individual TLR locus by fitting eight additional BLMMs using the same model structure described above. The only difference is that instead of using SH as a covariate, we separately included in each BLMM, TLR as a 1/0 predictor (1=heterozygote, 0=homozygote). With these additional BLMMs, we attempted to capture individual-locus and specific-sex effects. Note that the covariate SH represents a real slope (one for males and one for females), whereas specific-locus heterozygosity (1/0 predictor) applied to the eight additional BLMMs represents the difference between two intercepts [$H_O - H_E$]. For all the BLMMs, we report estimates of regression coefficients as the mean of 1000 posterior samples along with Highest Posterior Density (HPD) intervals (95%) and considered the effects to be statistically significant if the HPD intervals did not overlap zero. To obtain 1000 posterior samples, we ran each BLMM for 2,600,000 MCMC iterations with a burn-in period of 600,000 iterations and a thinning interval of 2000. We used default priors for the fixed and for the random effects. We expect that if balancing selection does occur, this would generate a positive correlation between multilocus standardized heterozygosity of TLRs and fitness, and/or between each specific-locus and fitness.

Results

Genetic diversity and differentiation among populations

We found that six TLRs (TLR1LA, TLR1LB, TLR2, TLR4, TLR15) were polymorphic in both dunnock populations, whereas TLR3 and TLR5 showed low numbers of polymorphisms (Fig. 1). In general, measures of TLR genetic diversity were similar in both populations, but slightly lower in Dunedin than in Cambridge (Table 1), reflecting what could have been a very minor bottleneck effect in functional genetic diversity. This slightly lowered diversity in the Dunedin population is reflected in the average number of haplotypes, referred here also as number of alleles, that were lower in Dunedin (10.37) compared to Cambridge (11.00 haplotypes). It is also reflected in the average haplotype diversity (0.636 in Dunedin vs. 0.639 in Cambridge, from Table 1) and in the average nucleotide diversity

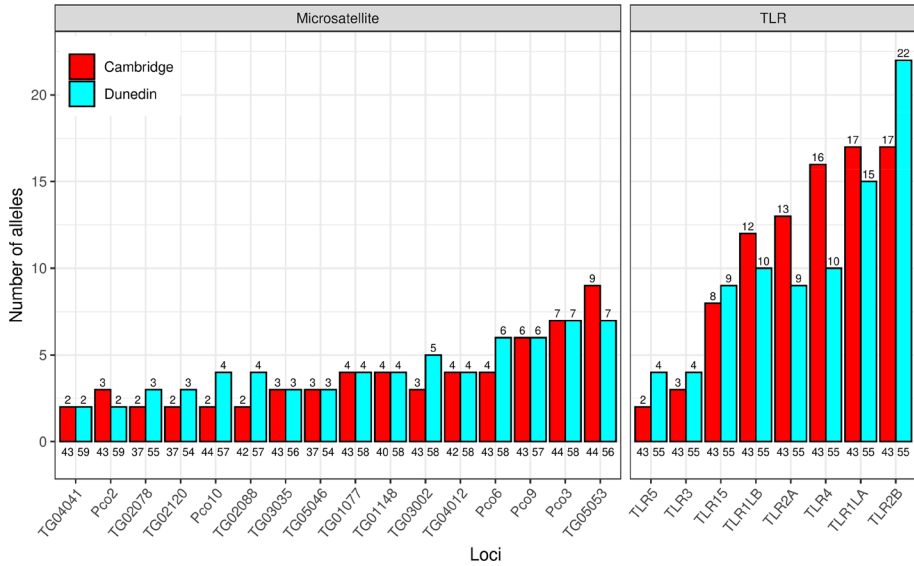


Fig. 1 Number of alleles for 16 microsatellites and eight Toll-like receptor genes (TLRs) in two populations of dunnocks, *Prunella modularis*. Cyan corresponds to the Dunedin population and red corresponds to the Cambridge population. Number of alleles are presented above the bars and sample size (number of individuals) under the bars

Table 1 Genetic diversity measurements of eight Toll-like receptor genes (TLRs) in two populations ($n_{\text{Dunedin}} = 55$, $n_{\text{Cambridge}} = 43$) of dunnocks, *Prunella modularis*

Loci	Population	H_d	π	k	s	nsyn:syn
TLR1LA	Cambridge	0.920	7.603	3.513	11	3:8
	Dunedin	0.886	7.263	3.356	12	4:8
TLR1LB	Cambridge	0.690	4.232	2.654	12	7:5
	Dunedin	0.717	3.479	2.181	12	5:7
TLR2A	Cambridge	0.703	3.091	1.461	8	5:3
	Dunedin	0.707	3.545	1.542	7	4:3
TLR2B	Cambridge	0.894	7.720	2.316	8	6:2
	Dunedin	0.881	7.768	2.346	10	7:3
TLR3	Cambridge	0.154	0.262	0.157	2	1:1
	Dunedin	0.139	0.238	0.142	3	2:1
TLR4	Cambridge	0.871	2.936	1.929	12	4:8
	Dunedin	0.818	2.949	1.938	9	3:6
TLR5	Cambridge	0.046	0.074	0.046	1	0:1
	Dunedin	0.106	0.173	0.107	3	1:2
TLR15	Cambridge	0.831	3.622	1.999	9	2:7
	Dunedin	0.836	4.064	2.244	10	1:9
Mean	Cambridge	0.639	3.692	1.759	7.88	3.50:4.38
	Dunedin	0.636	3.685	1.732	8.25	3.38:4.88

H_d =haplotype diversity; π =nucleotide diversity $\times 1000$; k=average number of nucleotide differences; s=number of SNPs; nsyn:syn=non-synonymous vs. synonymous SNPs

(3.685 Dunedin vs. 3.692 Cambridge, from Table 1). In contrast, we found a few more SNPs in the Dunedin population (66 SNPs) compared to the Cambridge population (63 SNPs). Of those SNPs, however, Dunedin and Cambridge had a very similar number of non-synonymous mutations (27 and 28, respectively). Microsatellites, on the other hand, showed a lower average number of alleles (either including both populations or separately averaging the totals found in Dunedin and in Cambridge) compared to the TLR average allelic diversity (Fig. 1). Also, the average number of alleles in microsatellites was slightly higher in Dunedin (4.18 alleles) compared to Cambridge (3.75 alleles), which opposes a potential bottleneck effect, at least in neutral genetic diversity. However, Fig. S1 shows a small, but non-statistically significant, decline in heterozygosity in microsatellites.

Overall, we found low and non-significant genetic differentiation of TLRs between the Dunedin and the Cambridge population (Global G'_{ST} = 0.0209, 95% CI = -0.0009 to 0.0427, Table 2). This pattern of non-significant genetic differentiation between the two populations also held for each locus, when evaluated separately (Table 2). Further, haplotype networks showed no apparent genetic structure between the two populations (Fig. 2). In terms of microsatellites, we found statistically significant global genetic differentiation of G_{ST} (0.0469, 95% CI = 0.0203 to 0.0734) between the two populations. Note, however, that 15 (out of 16) microsatellites loci did not present statistically significant genetic differentiation (Table S2). Finally, we found that genetic differentiation for TLRs (TLR G'_{ST} global) was not statistically different from that observed in microsatellites (microsatellites G'_{ST} global). In other words, the contrast between the 10,000 distributions of TLR G'_{ST} global minus the 10,000 distributions of microsatellites G_{ST} global (distributions obtained via bootstrapping), overlapped with zero [-0.0535, 0.0140], see details in Fig. S2).

Signatures of selection

Selection over evolutionary time

Except for the mean d_N/d_S of TLR2 in Cambridge, all the TLRs in both populations scored low means of d_N/d_S (< 1) (Fig. 3). Further, four TLRs (TLR1LA, TLR2A, TLR4, TLR15) also had their 95% confidence below 1 ($d_N/d_S < 1$) (Fig. 3). These findings reflect that TLRs have been primarily subject to purifying selection over an evolutionary

Table 2 Estimates of genetic differentiation for eight Toll-like receptor genes (TLRs) and global differentiation of G'_{ST} between two populations ($n_{[Dunedin]} = 55$, $n_{[Cambridge]} = 43$) of dunnocks (*Prunella modularis*)

Loci	G'_{ST}
TLR1LA	0.1476 (-0.0017 to 0.2968)
TLR1LB	0.0294 (-0.0319 to 0.0906)
TLR2A	-0.0037 (-0.0626 to 0.0551)
TLR2B	0.0613 (-0.0504 to 0.1729)
TLR3	-0.0003 (-0.0296 to 0.0291)
TLR4	0.0450 (-0.0772 to 0.1672)
TLR5	-0.0018 (-0.0381 to 0.0345)
TLR15	0.0083 (-0.0893 to 0.1060)
Global	0.0209 (-0.0009 to 0.0427)

95% confidence intervals were obtained by bootstrapping with 10,000 iterations

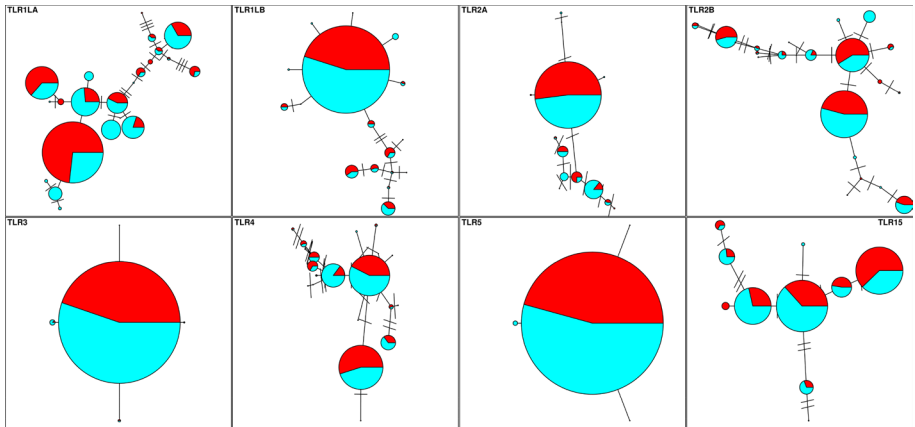
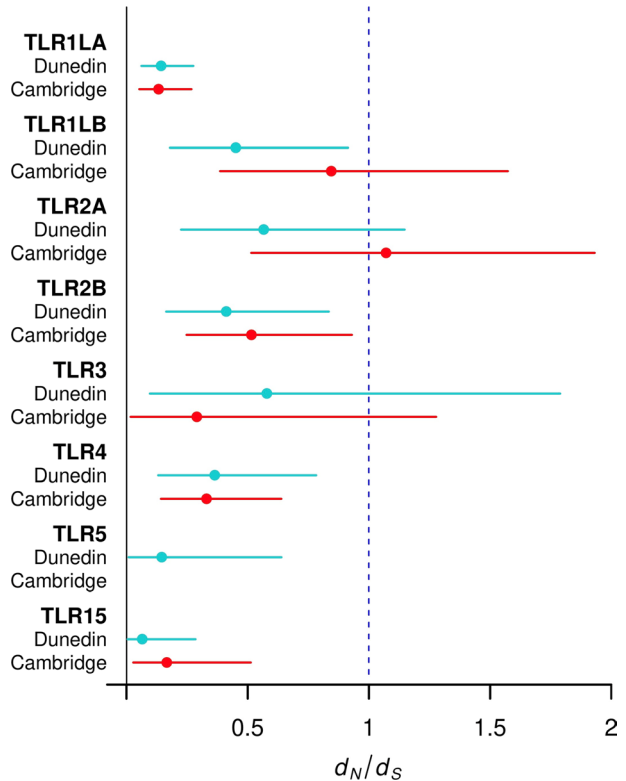


Fig. 2 Network of haplotypes (allelic diversity) for the eight Toll-like receptor genes (TLRs) in two populations ($n_{\text{Dunedin}}=55$, $n_{\text{Cambridge}}=43$) of dunnocks (*Prunella modularis*). Each circle represents a haplotype, and its size is directly proportional to its frequency. Cyan corresponds to the Dunedin population and Red corresponds to the Cambridge population

Fig. 3 d_N/d_S ratios for eight Toll-like receptor genes (TLRs) in two populations ($n_{\text{Dunedin}}=55$, $n_{\text{Cambridge}}=43$) of dunnocks, *Prunella modularis*. Mean d_N/d_S ratio represented as dots with their associated confidence intervals represented as horizontal lines. Cyan corresponds to the Dunedin population and Red corresponds to the Cambridge population. The vertical blue dashed line separates $d_N/d_S > 1$ suggestive of positive selection and $d_N/d_S < 1$ suggestive of purifying selection



timescale. In addition, both populations showed relatively similar patterns in their TLR d_N/d_S ratios and their corresponding confidence intervals (Fig. 3).

Selection over intergenerational time

We found that the average TLR heterozygosity, both observed and expected, was very similar between the two dunnock populations (TLRs: $H_{O[\text{Dunedin}]} = 0.604$ and $H_{O[\text{Cambridge}]} = 0.581$, $H_{E[\text{Dunedin}]} = 0.598$ and $H_{E[\text{Cambridge}]} = 0.602$, see results for each locus in Table 3). We found no statistically significant deviations from Hardy–Weinberg equilibrium in any TLRs in any population (either before or after correction for false discovery rates, Table 3), providing no evidence of short term (intergenerational) selection on genotype frequencies. Also, none of the TLR loci evaluated deviated (statistically significant) from neutrality (Tajima's D), indicating no evidence of purifying nor balancing selection acting on TLRs in the introduced population (Table 3). In microsatellites, we found a relatively low heterozygosity (Table S3, Fig. S1) compared to TLRs. In general, the observed and expected heterozygosity for microsatellites were very similar between the populations (Microsatellites: $H_{O[\text{Dunedin}]} = 0.429$ and $H_{O[\text{Cambridge}]} = 0.496$, $H_{E[\text{Dunedin}]} = 0.429$ and $H_{E[\text{Cambridge}]} = 0.491$, see results for each locus in Table S3, also see Fig. S1). There were no statistically significant deviations from Hardy–Weinberg equilibrium in any microsatellite in any population.

Table 3 Observed versus expected heterozygosity and tests of deviation from Hardy–Weinberg Equilibrium (HWE) for eight Toll-like receptor genes (TLRs) in two populations ($n_{[\text{Dunedin}]} = 55$, $n_{[\text{Cambridge}]} = 43$) of dunnocks, *Prunella modularis*

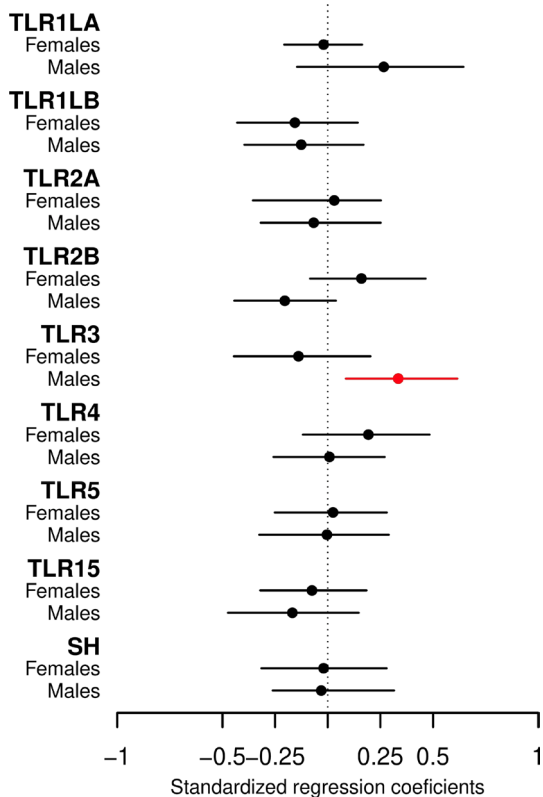
Loci	Population	H_O	H_E	HWE (p values)	HWE (fdr)	Tajima's D (p values)
TLR1LA	Cambridge	0.906	0.909	0.241	0.551	1.613 (0.106)
	Dunedin	0.927	0.878	0.365	0.649	1.244 (0.214)
TLR1LB	Cambridge	0.651	0.681	0.682	0.992	0.302 (0.762)
	Dunedin	0.709	0.710	0.199	0.550	−0.108 (0.914)
TLR2A	Cambridge	0.651	0.689	0.317	0.634	0.291 (0.771)
	Dunedin	0.709	0.700	0.218	0.551	0.374 (0.709)
TLR2B	Cambridge	0.697	0.728	0.119	0.550	1.130 (0.258)
	Dunedin	0.527	0.631	0.008	0.131	1.282 (0.199)
TLR3	Cambridge	0.162	0.152	1.000	1.000	−0.960 (0.337)
	Dunedin	0.145	0.138	1.000	1.000	−1.322 (0.186)
TLR4	Cambridge	0.744	0.787	0.213	0.551	−0.521 (0.602)
	Dunedin	0.854	0.793	0.018	0.142	0.334 (0.739)
TLR5	Cambridge	0.046	0.045	1.000	1.000	−0.910 (0.363)
	Dunedin	0.109	0.104	1.000	1.000	−1.432 (0.152)
TLR15	Cambridge	0.790	0.819	0.409	0.655	0.298 (0.766)
	Dunedin	0.854	0.828	0.956	1.000	0.462 (0.644)

We obtained the p values for HWE from the exact test with 1,000,000 permutations. We estimated false discovery rates for HWE with the fdr method implemented in R

Selection over the current generation

The Bayesian linear mixed model (BLMM) that assessed whether standardized heterozygosity (SH) reflects any advantage in reproductive fitness (HFC), showed no statistically significant associations (Fig. 4 and Table S4—SH BLMM). This finding held true for both sexes (SH BLMM: SH female’s slope = -0.031 [95% CI = -0.262 to 0.315], SH male’s slope = -0.020 [95% CI = -0.315 to 0.280], Fig. 4 and Table S4, values are on the *log-link* scale). In general, for the additional fitted BLMMs that targeted specific-locus effects, we found similar results (i.e. limited evidence to support current signatures of selection). Notably, however, we found statistically significant heterozygote advantage, exclusively for males, in TLR3 (BLMM TLR3: male’s difference [$H_O - H_E$] = 0.335 [95% CI = 0.086 to 0.616], Fig. 4 and Table S4). This heterozygote advantage indicates, in the *original scale* (back-transformed as $= \exp(\text{Intercept males}_{[H_O]} + \text{Diff. males}_{[H_E - H_O]}) - \exp(\text{Intercept males}_{[H_O]})$), that males that are heterozygous at TLR3 have on average 0.167 chicks more than homozygous males. We stress that this effect is the only one with statistical support that let us infer current selection in the Dunedin population.

Fig. 4 Forest plots for the Bayesian linear mixed models (BLMMs) that assessed whether standardized TLR heterozygosity (SH) and specific-TLRs heterozygosity predicted fitness in dunnocks (*Prunella modularis*) within the Dunedin population. Dots represent the posterior mean (i.e. regression coefficients) of the heterozygosity slope (SH model) and the difference in heterozygosity (for the specific-locus models), and horizontal bars represent the 95% Highest Posterior Density (HPD) intervals. The only red dot and red horizontal bar represent the statistically significant difference observed in heterozygosity. Values presented in *log-link* scale. Sample size as follows: $n_{[\text{individuals}]} = 55$, $n_{[\text{observations}]} = 476$, $n_{[\text{females}]} = 26$, $n_{[\text{males}]} = 29$



Discussion

The present study, using sequences of eight Toll-like receptors and genotypes of 16 microsatellites of dunnocks, evaluated genetic differentiation between an introduced population to New Zealand and a source population from United Kingdom, and tested whether signatures of contemporary and current selection are acting upon the introduced population. First, we found that measures of TLR genetic diversity were similar in the introduced population compared to the source population, and that genetic population differentiation was non-statistically significant. For microsatellites, our findings are similar to the ones observed in TLRs (i.e. small genetic differentiation across the populations). Second, we found limited evidence to support either balancing or purifying contemporary selection as we did not observe any statistically significant deviations from HWE or neutrality (Tajima's D). Our evaluation of current selection, via heterozygosity–fitness correlations (HFC), conditionally supported (males only) heterozygote advantage for a single locus (TLR3). Nonetheless, the other BLMMs using standardized multi-locus heterozygosity (SH), or other TLR loci, failed to detect heterozygotes advantage in the introduced population.

Genetic diversity and differentiation among populations

The relatively small reduction in TLR genetic diversity that was observed in the introduced population (Table 1) implies that the dunnock introduction to New Zealand resulted in a minor bottleneck. However, we found limited evidence to support a bottleneck in TLR genetic diversity. The genetic differentiation (G'_{ST}) between the source and introduced population was not statistically significant, and we did not observe a reduction in TLR heterozygosity (Fig. S1). A bottleneck was detectable using microsatellites, as the global G'_{ST} differentiation was statistically significant, however we stress that 15 (out of 16) microsatellites did not show significant differentiation when analyzed separately. Even accepting statistically significant differentiation at microsatellites between the populations, levels of differentiation were very low (Table S2). In addition, there was no reduction in microsatellite heterozygosity from Cambridge to Dunedin, which mirrors the findings reported by Santos et al. (2013) in the same study populations. Altogether, there was no strong support for a bottleneck at functional nor neutral genetic diversity. We conclude that the impact of the introduction at both types of genetic markers was minor.

Our findings of little TLR genetic differentiation between the two populations also imply that it is very plausible that the introduced population has retained most of the original TLR genetic diversity brought from the ancestral source population. We suggest that the number of individuals introduced in the Otago region, estimated by Santos et al. (2013) to be about 185 individuals, and their subsequent rapid population expansion (Kikkawa 1966; Davies 1992), were likely sufficient to bring and maintain virtually all extant TLR diversity from the source population in the United Kingdom.

Signatures of selection

Along with previous studies of TLR sequence variation (Alcaide and Edwards 2011; Grueber et al. 2014; Raven et al. 2017), we found that TLRs in dunnocks display a consistent pattern of purifying selection over an evolutionary timescale, and such pattern was consistent between the two populations for all the loci evaluated (Fig. 3). When considering an intergenerational timescale, on the other hand, we did not find support for our initial

hypothesis that pathogen-mediated selection would strongly shape dunnock immunogenetic variation as a consequence of the new environmental conditions. In other words, we neither observe significant deviations from HWE nor Tajima's D null hypotheses for any TLR region. However, we found that TLR3 may be the subject to current selection and this finding deserves further discussion (see below). Given that we gathered little evidence of recent selection we below consider alternative interpretations of our findings, and then discuss the findings for TLR3.

First, it is possible that introduced dunnocks, instead of facing novel and challenging pathogens, were instead released from co-evolving pathogens present in their ancestral habitat. A lack of exposure to co-evolved pathogens is known as the enemy release hypothesis (Lee and Klasing 2004; White and Perkins 2012), and may partially explain the absence of signatures of recent selection. To test this hypothesis directly, we would need to characterize the pathogens interacting with dunnocks in New Zealand and in the United Kingdom, but such data is currently limited. Yet, we know from a few studies, particularly on bacteria found in dunnocks in Europe, that dunnocks are associated with a high diversity of bacteria, which do not appear to cause disease (e.g., *Yersinia* sp., *Erwinia* sp., *Pseudomonas* sp., *Stenotrophomonas* sp., and *Salmonella* sp., among others, Benskin et al. 2009; Kisková et al. 2012). Similarly, the United Kingdom's dunnock populations do not decline as a consequence of protozoan diseases caused by *Trichomonas gallinae*, whereas other species (*Carduelis chloris* and *Fringilla coelebs*) are susceptible (Robinson et al. 2010). There are no specific studies of such a kind for dunnocks in New Zealand, but studies in New Zealand's native passerine species indicate that bacterial diversity can be lower than bacterial diversity found on England. For instance, an evaluation of the bacteria in several New Zealand bird species only reports *Salmonella* sp. and *Yersinia* sp. (Cork et al. 1995; Ewen et al. 2012). In addition, it has been found that New Zealand has not been invaded by serious exotic viruses (Alley and Gartrell 2019).

Nonetheless, there are reports of some introduced viruses from Europe, such as the most prevalent strains of avian pox viruses in New Zealand that were introduced by the European avian hosts (Ha et al. 2011). This introduced virus may have been in contact with dunnocks even before the introduction to New Zealand, so instead of a release-enemy hypothesis, the arms-race between dunnocks and their co-evolving pathogens may have continued in New Zealand. This idea partially supports our observation of heterozygote advantage at TLR3, as this locus plays a key role in recognizing viruses such as the avian influenza virus (Chen et al. 2013). Hence, TLR3 heterozygous individuals may cope better against these types of RNA viruses. Yet, our findings of a positive association between fitness and TLR3 heterozygosity should be taken with caution, given that the HFC advantage only occurred within males, and we see no reason why females would not show the same pattern. It could be plausible that the intricate sexual conflict among dunnocks (Davies 1986; Santos et al. 2015) caused the observed association.

Another alternative explanation for the lack of signatures of contemporary selection is that even if the pathogens found in New Zealand are challenging dunnocks, the species' high TLR diversity can counteract the selective forces exerted by pathogens. Dunnock TLR diversity is substantially similar to or higher than values reported in many other bird species (González-Quevedo et al. 2015; Grueber et al. 2015; Nelson-Flower et al. 2018). Hence, we may suggest that the current TLR diversity, brought through the introduction process, has most likely been adequate to cope with the possible extant pathogens of New Zealand, but there is limited evidence to fully support this alternative hypothesis.

Lastly, it is possible that the short period of isolation (150 years) has not been enough to fully capture such signatures of contemporary selection. However, we can argue against

this alternative view. As mentioned above, we found a positive HFC effect, that although conditional to one locus and one sex, it is representative evidence of selection in the current generation. In addition, a recent study by Grueber et al. (2013) is a good example of selection acting at TLRs over a short timescale. Grueber et al. (2013) reported a strong association between specific TLR genotypes and individual survival in the South Island robin (*Petroica australis*). Similarly, in our study populations, we have previously found statistically significant differentiation in functional genetic diversity at dopamine *DRD4* and serotonin *SERT* genes, which are candidate genes for personality traits (Holtmann et al. 2016). Taken together, our findings and the ones reported in these two previous studies lend support to the idea that divergence in functional genetic diversity between our two dunnock populations can occur within short timescales. Hence, overall, we believe our results are more indicative of alternative hypotheses, such as the enemy release hypothesis and/or sufficient TLR diversity enough to counteract NZ pathogens as a result of little to no genetic bottleneck.

Conclusions

In conclusion, we showed that TLR genes are diverse in dunnocks, and that the biological introduction of dunnocks to New Zealand has not led to a significant loss of immunogenetic diversity nor neutral genetic diversity. Although we predicted positive selection, as the deliberate introduction of dunnocks in a new environment created a seemingly good scenario, we only captured limited indications of current selection and none for intergenerational selection. This result could be caused by the enemy release hypothesis or sufficient diversity to respond to the pathogens found in New Zealand. While we characterize the introduction of dunnocks in New Zealand via functional genetic diversity, we encountered limitations to fully infer the evolutionary processes due to the lack of knowledge of pathogen profiles. We suggest pathogen profiling should be the focus of future studies to fully disentangle the selective pressures acting upon the immune system of dunnocks and to better understand how TLR diversity is shaped by selection over short timescales. Particularly, it would be interesting to explore the underlying causes of the heterozygote advantage observed at TLR3 and perhaps the association with a possible virus strain. Overall, our observation of retained TLR genetic diversity may explain—at least in part—the remarkable success of dunnocks in colonizing New Zealand.

Acknowledgements We thank T. Burke for providing blood-samples from dunnocks. We are grateful to J. Lim for his assistance in the laboratory, and to G. Knafler and F. Fontúrbel for commenting the manuscript. CEL received funds from Minciencias Colombia (568-2012 and 811-2018) and from the Vicerrectoría de Investigaciones y Posgrados of the Universidad de Caldas (80740-2019, 201010027251). CEG acknowledges the support of San Diego Zoo Global. SN received funds from a Rutherford Discovery Fellowship (New Zealand). The New Zealand Department of Conservation (Permit 36716-FAU) and the University of Otago Animal Ethics Committee (Permits 12/49 and 89/14) approved this research.

Author contributions CEL, SN, CEG, ML, SLJ and GCV conceived and designed the study. CEL, BH and ESAS collected data from dunnocks in New Zealand. CEL, BH, ML and BR conducted laboratory analysis. CEL conducted analysis with advice from SN, ML, BR and GCV. All authors have commented and approved the current version of this article.

Data availability TLR sequence data from this manuscript is available on Genbank (accession numbers: MT847643-MT849210). All the alignments will be deposited in the Open Science Framework—OSF.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.


References

- Aderem A, Ulevitch RJ (2000) Toll-like receptors in the induction of the innate immune response. *Nature* 406:782–787
- Alcaide M, Edwards SV (2011) Molecular evolution of the toll-like receptor multigene family in birds. *Mol Biol Evol* 28:1703–1715. <https://doi.org/10.1093/molbev/msq351>
- Alho JS, Välimäki K, Merilä J (2010) Rhh: an R extension for estimating multilocus heterozygosity and heterozygosity–heterozygosity correlation. *Mol Ecol Resour* 10:720–722. <https://doi.org/10.1111/j.1755-0998.2010.02830.x>
- Alley M, Gartrell B (2019) Wildlife diseases in New Zealand: recent findings and future challenges. *N Z Vet J* 67:1–11. <https://doi.org/10.1080/00480169.2018.1520656>
- Barreiro LB, Ben-Ali M, Quach H et al (2009) Evolutionary dynamics of human toll-like receptors and their different contributions to host defense. *PLoS Genet* 5:e1000562. <https://doi.org/10.1371/journal.pgen.1000562>
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol* 57:289–300
- Benskin H, Wilson K, Jones K, Hartley IR (2009) Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biol Rev* 84:349–373. <https://doi.org/10.1111/j.1469-185X.2008.00076.x>
- Blasius AL, Beutler B (2010) Intracellular toll-like receptors. *Immunity* 32:305–315
- Bouwhuis S, Choquet R, Sheldon BC, Verhulst S (2012) The Forms and fitness cost of senescence: age-specific recapture, survival, reproduction, and reproductive value in a wild bird population. *Am Nat* 179:E15–E27. <https://doi.org/10.1086/663194>
- Briskie JV, Mackintosh M (2004) Hatching failure increases with severity of population bottlenecks in birds. *Proc Natl Acad Sci USA* 101:558. <https://doi.org/10.1073/pnas.0305103101>
- Brommer JE, Wilson AJ, Gustafsson L (2007) Exploring the genetics of aging in a wild passerine bird. *Am Nat* 170:643–650. <https://doi.org/10.1086/521241>
- Burke T, Davies NB, Bruford MW, Hatchwell BJ (1989) Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. *Nature* 338:249–251. <https://doi.org/10.1038/338249a0>
- Charlesworth B, Morgan MT, Charlesworth D (1993) The effect of deleterious mutations on neutral molecular variation. *Genetics* 134:1289
- Chen S, Cheng A, Wang M (2013) Innate sensing of viruses by pattern recognition receptors in birds. *Vet Res* 44:82. <https://doi.org/10.1186/1297-9716-44-82>
- Coltman DW, Pilkington JG, Smith JA, Pemberton JM (1999) Parasite-mediated selection against inbred soay sheep in a free-living, island population. *Evolution* 53:1259–1267. <https://doi.org/10.2307/2640828>
- Cork SC, Marshall RB, Madie P, Fenwick SG (1995) The role of wild birds and the environment in the epidemiology of Yersinia in New Zealand. *N Z Vet J* 43:169–174. <https://doi.org/10.1080/00480169.1995.35883>
- Davies N (1986) Reproductive success of dunnocks, *Prunella modularis*, in a variable mating system. I. Factors influencing provisioning rate, nestling weight and fledging success. *J Anim Ecol* 55:123–138. <https://doi.org/10.2307/4697>
- Davies N (1992) Dunnock behaviour and social evolution. Oxford University Press, Oxford
- Delpont W, Poon AFY, Frost SDW, Kosakovsky Pond SL (2010) Datamonkey 2010: a suite of phylogenetic analysis tools for evolutionary biology. *Bioinformatics* 26:2455–2457. <https://doi.org/10.1093/bioinformatics/btq429>
- Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol Ecol* 17:431–449. <https://doi.org/10.1111/j.1365-294X.2007.03538.x>
- Ewen JG, Armstrong DP, Empson R et al (2012) Parasite management in translocations: lessons from a threatened New Zealand bird. *Oryx* 46:446–456. <https://doi.org/10.1017/S0030605311001281>

- Gilroy DL, van Oosterhout C, Komdeur J, Richardson DS (2016) Toll-like receptor variation in the bottlenecked population of the endangered Seychelles warbler. *Anim Conserv*. <https://doi.org/10.1111/acv.12307>
- Goldberg J, Trewick SA, Paterson AM (2008) Evolution of New Zealand's terrestrial fauna: a review of molecular evidence. *Philos Trans R Soc Lond B Biol Sci* 363:3319–3334. <https://doi.org/10.1098/rstb.2008.0114>
- González-Quevedo C, Spurgin LG, Illera JC, Richardson DS (2015) Drift, not selection, shapes toll-like receptor variation among oceanic island populations. *Mol Ecol* 24:5852–5863. <https://doi.org/10.1111/mec.13437>
- Green J, Bohannan BJM (2006) Spatial scaling of microbial biodiversity. *Trends Ecol Evol* 21:501–507. <https://doi.org/10.1016/j.tree.2006.06.012>
- Grueber C, Jamieson I (2013) Primers for amplification of innate immunity toll-like receptor loci in threatened birds of the Apterygiformes, Gruiformes, Psittaciformes and Passeriformes. *Conserv Genet Resour* 5:1043–1047. <https://doi.org/10.1007/s12686-013-9965-x>
- Grueber CE, Wallis GP, King TM, Jamieson IG (2012) Variation at innate immunity toll-like receptor genes in a bottlenecked population of a New Zealand robin. *PLoS ONE* 7:e45011. <https://doi.org/10.1371/journal.pone.0045011>
- Grueber CE, Wallis GP, Jamieson IG (2013) Genetic drift outweighs natural selection at toll-like receptor (TLR) immunity loci in a re-introduced population of a threatened species. *Mol Ecol* 22:4470–4482. <https://doi.org/10.1111/mec.12404>
- Grueber CE, Wallis GP, Jamieson IG (2014) Episodic positive selection in the evolution of avian toll-like receptor innate immunity genes. *PLoS ONE* 9:e89632. <https://doi.org/10.1371/journal.pone.0089632>
- Grueber C, Knafler G, King T et al (2015) Toll-like receptor diversity in 10 threatened bird species: relationship with microsatellite heterozygosity. *Conserv Genet* 16:595–611. <https://doi.org/10.1007/s10592-014-0685-x>
- Ha HJ, Howe L, Alley M, Gartrell B (2011) The phylogenetic analysis of avipoxvirus in New Zealand. *Vet Microbiol* 150:80–87. <https://doi.org/10.1016/j.vetmic.2011.01.011>
- Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R Package. *J Stat Softw* 33:22. <https://doi.org/10.18637/jss.v033.i02>
- Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JBH (2012) Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat Rev Microbiol* 10:497–506. <https://doi.org/10.1038/nrmicro2795>
- Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution* 59:1633–1638. <https://doi.org/10.1111/j.0014-3820.2005.tb01814.x>
- Holtmann B, Grosser S, Lagisz M et al (2016) Population differentiation and behavioural association of the two 'personality' genes *DRD4* and *SERT* in dunnocks (*Prunella modularis*). *Mol Ecol* 25:706–722. <https://doi.org/10.1111/mec.13514>
- Holtmann B, Santos ESA, Lara CE, Nakagawa S (2017) Personality-matching habitat choice, rather than behavioural plasticity, is a likely driver of a phenotype–environment covariance. *Proc R Soc B Biol Sci*. <https://doi.org/10.1098/rspb.2017.0943>
- Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24:1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>
- Kekkonen J, Seppä P, Hanski IK et al (2011) Low genetic differentiation in a sedentary bird: house sparrow population genetics in a contiguous landscape. *Heredity* 106:183–190. <https://doi.org/10.1038/hdy.2010.32>
- Kikkawa J (1966) Population distribution of land birds in temperate rainforest of southern New Zealand. *Trans R Soc N Z* 7:215–277
- Kisková J, Hrehová Z, Janiga M et al (2012) Bacterial prevalence in the Dunnock (*Prunella modularis*) in sub-alpine habitats of the Western Carpathians, Slovak Republic. *Ornis Fenn* 89:34–43
- Kloch A, Wenzel MA, Laetsch DR et al (2018) Signatures of balancing selection in toll-like receptor (TLRs) genes—novel insights from a free-living rodent. *Sci Rep* 8:8361. <https://doi.org/10.1038/s41598-018-26672-2>
- Lee KA, Klasing KC (2004) A role for immunology in invasion biology. *Trends Ecol Evol* 19:523–529. <https://doi.org/10.1016/j.tree.2004.07.012>
- Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>
- McDowall RM (2008) Process and pattern in the biogeography of new zealand: a global microcosm? *J Biogeogr* 35:197–212
- Merilä J, Björklund M, Baker AJ (1996) The successful founder: genetics of introduced *Carduelis chloris* (greenfinch) populations in New Zealand. *Heredity* 77:410–422. <https://doi.org/10.1038/hdy.1996.161>

- Morrison CE, Hogg CJ, Gales R et al (2020) Low innate immune-gene diversity in the critically endangered orange-bellied parrot (*Neophema chrysogaster*). *Emu Austral Ornithol* 120:56–64. <https://doi.org/10.1080/01584197.2019.1686994>
- Mukherjee S, Sarkar-Roy N, Wagener DK, Majumder PP (2009) Signatures of natural selection are not uniform across genes of innate immune system, but purifying selection is the dominant signature. *PNAS* 106:7073–7078. <https://doi.org/10.1073/pnas.0811357106>
- Mukherjee S, Ganguli D, Majumder PP (2014) Global footprints of purifying selection on Toll-like receptor genes primarily associated with response to bacterial infections in humans. *Gen Biol Evol* 6:551–558. <https://doi.org/10.1093/gbe/evu032>
- Nei M, Gojobori T (1986) Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Mol Biol Evol* 3:418–426
- Nelson-Flower MJ, Germain RR, MacDougall-Shackleton EA et al (2018) Purifying selection in the toll-like receptors of song sparrows *Melospiza melodia*. *J Hered* 109:501–509. <https://doi.org/10.1093/jhered/esy027>
- Nicholls JA, Double MC, Rowell DM, Magrath RD (2000) The evolution of cooperative and pair breeding in thornbills *Acanthiza* (Pardalotidae). *J Avian Biol* 31:165–176. <https://doi.org/10.1034/j.1600-048X.2000.310208.x>
- Paradis E (2010) *pegas*: an R package for population genetics with an integrated–modular approach. *Bioinformatics* 26:419–420. <https://doi.org/10.1093/bioinformatics/btp696>
- R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Raven N, Lisovski S, Klaassen M et al (2017) Purifying selection and concerted evolution of RNA-sensing toll-like receptors in migratory waders. *Infect Genet Evol* 53:135–145. <https://doi.org/10.1016/j.meegid.2017.05.012>
- Robinson RA, Lawson B, Toms MP et al (2010) Emerging infectious disease leads to rapid population declines of common british birds. *PLoS ONE* 5:e12215. <https://doi.org/10.1371/journal.pone.0012215>
- Santos ESA (2012) Discovery of previously unknown historical records on the introduction of dunnocks (*Prunella modularis*) into Otago, New Zealand during the 19th century. *Notornis* 59:79–81
- Santos E, Nakagawa S (2013) Breeding biology and variable mating system of a population of introduced dunnocks (*Prunella modularis*) in New Zealand. *PLoS ONE* 8:e69329. <https://doi.org/10.1371/journal.pone.0069329>
- Santos EA, Jamieson I, Santos LS, Nakagawa S (2013) Low genetic and morphological differentiation between an introduced population of dunnocks in New Zealand and an ancestral population in England. *Biol Invasions* 15:185–197. <https://doi.org/10.1007/s10530-012-0278-x>
- Santos ESA, Santos LLS, Lagisz M, Nakagawa S (2015) Conflict and cooperation over sex: the consequences of social and genetic polyandry for reproductive success in dunnocks. *J Anim Ecol* 84:1509–1519. <https://doi.org/10.1111/1365-2656.12432>
- Schielzeth H (2010) Simple means to improve the interpretability of regression coefficients. *Methods Ecol Evol* 1:103–113. <https://doi.org/10.1111/j.2041-210X.2010.00012.x>
- Stephens M, Donnelly P (2003) A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 73:1162–1169
- Stockwell CA, Mulvey M, Vinyard GL (1996) Translocations and the preservation of allelic diversity. *Conserv Biol* 10:1133–1141
- Székely AJ, Langenheder S (2014) The importance of species sorting differs between habitat generalists and specialists in bacterial communities. *FEMS Microbiol Ecol* 87:102–112. <https://doi.org/10.1111/1574-6941.12195>
- Wang J, Zhang Z, Liu J et al (2016) Ectodomain architecture affects sequence and functional evolution of vertebrate toll-like receptors. *Sci Rep* 6:26705. <https://doi.org/10.1038/srep26705>
- White TA, Perkins SE (2012) The ecoimmunology of invasive species. *Funct Ecol* 26:1313–1323. <https://doi.org/10.1111/1365-2435.12012>
- Winter DJ (2012) *mmod*: an R library for the calculation of population differentiation statistics. *Mol Ecol Resour* 12:1158–1160. <https://doi.org/10.1111/j.1755-0998.2012.03174.x>

Affiliations

Carlos Esteban Lara^{1,2}  · **Catherine E. Grueber**³ · **Benedikt Holtmann**⁴ ·
Eduardo S. A. Santos⁵ · **Sheri L. Johnson**¹ · **Bruce C. Robertson**¹ ·
Gabriel J. Castaño-Villa^{2,6} · **Malgorzata Lagisz**⁷ · **Shinichi Nakagawa**⁷

✉ Carlos Esteban Lara
celarav@gmail.com

¹ Department of Zoology, University of Otago, Dunedin, New Zealand

² Grupo de Investigación GEBIOME, Facultad de Ciencias Exactas y Naturales, Universidad de Caldas, Manizales, Colombia

³ School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Sydney, NSW, Australia

⁴ Behavioural Ecology, Department of Biology, Ludwig-Maximilians-University of Munich, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany

⁵ Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil

⁶ Departamento de Desarrollo Rural y Recursos Naturales, Universidad de Caldas, Manizales, Colombia

⁷ Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South, Sydney, NSW, Australia