



The ectoparasites of hybrid ducks in New Zealand (Mallard x Grey Duck)

Mariana Bulgarella^{a,*}, Mathieu Quenu^b, Lara D. Shepherd^a, Mary Morgan-Richards^b

^a Museum of New Zealand Te Papa Tongarewa, PO Box 467, Wellington, 6140, New Zealand

^b Ecology, College of Science, Massey University, Private Bag 11-222, Palmerston North, 4442, New Zealand

ARTICLE INFO

Keywords:

Anatidae
Chewing lice
Ectoparasites
Hybrids
mtDNA
New Zealand
Transmission
Waterfowl

ABSTRACT

We studied the population genetics of one population sample of hybrid Mallard x Grey Ducks and their lice in New Zealand. We aimed to document the relationship between ectoparasite load and host phenotype, and test for an association between the mtDNA diversity of the lice and their hosts, which is predicted based on maternal care. We found three feather lice species previously described for these hosts: *Anaticola crassicornis* (wing louse), *Anatoecus dentatus* (head louse), and *Trinoton querquedulae* (body louse). No new or rare lice species were uncovered. Most ducks in our sample were more Mallard-like than Grey Duck-like hybrids for the five colour and plumage traits examined. We confirm that based solely on phenotypic characters it is difficult to distinguish between Mallards, hybrids and Grey Ducks. We detected no association between the number of lice and host phenotype for two of the three louse species (while controlling for bird size). However, the Grey Duck-like hybrids had fewer head lice (*A. dentatus*) than their Mallard-like counterparts. Only three of the 40 hosts had mtDNA haplotypes that characterise Grey Ducks. We present the first genetic data of *Anaticola crassicornis*, *Anatoecus dentatus* and *Trinoton querquedulae* from New Zealand waterfowl. We found that the lice mtDNA had greater sequence diversity than the homologous gene for the ducks. A mitochondrial phylogeny for *A. crassicornis* collected from hosts worldwide has been previously published, and we added our novel data to infer evolutionary relationships among worldwide populations of this louse. None of the three lice species showed a close association of parasite and host mtDNA lineage despite lack of paternal care in these duck species.

1. Introduction

Hybridisation between host species has the potential to bring into contact parasites that have diverged in isolation (Detwiler and Criscione, 2010). Different populations of a given parasite species are engaged in slightly different coevolutionary associations with their local hosts (Thompson, 2005). The outcome of secondary contact for the parasites is expected to be as complex as their host interactions; competition, selection, extinction, interbreeding and merging are all possible. And at the same time, the interaction between parasites and new hybrid host genotypes will provide a selective force that might determine the final outcome (Baird et al., 2012). Host specificity is determined by opportunities for host switching or colonization, availability of suitable hosts, and how host switching affects parasite fitness (Poulin, 2007). Although the host range of a parasite is constrained by its history, it is known to vary in accordance to the presence and relative abundances of local host species (Krasnov et al., 2004).

Lice (Insecta: Phthiraptera) are permanent parasites of their hosts (i.e. they spend their whole life cycle on one host) and usually exhibit

high host specificity (Clayton et al., 2004). In birds, feather lice predominantly show vertical transmission from parents to offspring, during brooding and feeding of chicks (Clayton et al., 2016). However, the chewing lice parasitising ducks, geese and swans (Aves: Anseriformes) around the world are among the least specific of all avian lice (Escalante et al., 2016). Whether these parasites are generalists because of the recent radiation of their hosts, because of their hosts' behaviour and ecology or because of the biology of the lice (i.e., increased dispersal capabilities) is still unknown. The diversification of modern ducks is estimated to have occurred by rapid radiation sometime during the Miocene (10–5 Myr ago; Sun et al., 2017), which would probably provide enough time for their lice to diverge (Clayton et al., 2016). An alternative explanation for the broad host range exploited by these lice invokes their host ecology, i.e. many waterfowl species migrate to regions where they form mixed-species flocks allowing parasite transmission, while other species co-occur in bodies of water providing additional opportunities for host transfer (Escalante et al., 2016). In addition, hybridisation between waterfowl species is relatively common (Tubaro and Litjmaer, 2002) providing further opportunities for lice transfer between host species.

* Corresponding author. Museum of New Zealand Te Papa Tongarewa, PO Box 467, Wellington, 6140, New Zealand.

E-mail address: Mariana.Bulgarella@vuw.ac.nz (M. Bulgarella).

<https://doi.org/10.1016/j.ijppaw.2018.09.005>

Received 15 August 2018; Received in revised form 7 September 2018; Accepted 7 September 2018

2213-2244/ © 2018 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Some of the best documented examples of hybridisation in ducks are between the sexually dimorphic Mallard (*Anas platyrhynchos*) and several non-dimorphic related species (Rhymer et al., 1994). Cases include the extensive hybridisation between Mallards and Mexican Ducks (*A. platyrhynchos diazi*) that have caused the two species to be declared conspecific (AOU, 1983). The Hawaiian Duck (*A. wyvilliana*) is currently classified as Endangered by the IUCN Red List (BirdLife International, 2017) mainly due to cross-breeding with introduced Mallards. American Black Duck (*A. rubripes*) numbers have been greatly reduced due to hybridisation with Mallards (Kirby et al., 2004). In New Zealand and Lord Howe Island, hybridisation of the native Grey Duck (*A. superciliosa*) with Mallards has greatly reduced the population size of pure Grey Ducks (Williams, 2017) to the point where the species is on the brink of local extinction (Tracey et al., 2008; Guay et al., 2015). In New Zealand, the Mallard phenotype is also declining as the number of ducks with hybrid phenotype increases (Gillespie, 1985). Currently, wild Mallards and Mallard x Grey Duck hybrids are the most common and widespread species, with approximately 500,000 individuals being hunted each year (McDougall and Amundson, 2017). Thus, implementing hunting regulations, censusing and conducting field work is problematical because of the difficulty distinguishing between pure-Mallard, hybrids and pure-Grey Ducks (Muller, 2008; Williams, 2017). The hybridisation of Mallard and Grey Ducks will have provided an opportunity for their parasites to come into contact and may have resulted in a similar mixing of genotypes in lice as seen in their hosts and/or the opportunity for competitive exclusion of lineages. However, in both these species female ducks provide maternal care (Johnson et al., 1999), while the males desert the female and offspring (Kear, 1970), therefore, transmission of lice might be expected to be primarily from mother to offspring as happens with mitochondrial DNA (mtDNA). Therefore, we hypothesize that although the New Zealand population of Grey and Mallard Ducks are dominated by hosts with intermediate phenotype, their lice populations might reveal less mixing.

In our study we focus on three species of lice that have been recorded on Grey Ducks and Mallards, each from a different genus: *Anaticola crassicornis*, *Anatoecus dentatus*, and *Trinoton querquedulae* (Pilgrim and Palma, 1982; Aksin, 2011; Grossi et al., 2014; Escalante et al., 2016). A fourth louse genus has also been recorded from New Zealand Mallards, *Holomenopon* (Pilgrim and Palma, 1982) but this was not found in our survey, so is not discussed further. These lice are all members of the insect order Phthiraptera. The three species differ in their host microhabitat (wing, head, body), dispersal abilities (DiBlasi et al., 2018), and possess widespread geographic distributions providing excellent examples of ‘ecological replicates’ (Clayton et al., 2016). *Anaticola crassicornis* is a feather wing louse (Johnson et al., 2012). DNA sequence variation suggests that this species could be a complex of cryptic, geographically isolated species (Escalante et al., 2016). *Anaticola crassicornis* collected from Grey Ducks in Australia were genetically similar to lice collected from other Australian ducks but differentiated from both the lineage living on Mallards in Japan and Sweden and the lineage on USA Mallards (Escalante et al., 2016). However, the molecular phylogeny of *A. crassicornis* collected from waterfowl hosts worldwide did not include lice from New Zealand. The head louse, *Anatoecus dentatus*, lives and feeds on the head and neck of its host (Clayton et al., 2016). The species has recently been synonymised with *A. icterodes*. All *A. dentatus* collected from a number of different waterfowl species in Canada were genetically identical, suggesting little host-parasite specialisation (Grossi et al., 2014). The body louse, *Trinoton querquedulae*, is also a generalist and found on Mallard, Grey and many other duck species; however little is known of its genetic diversity worldwide (Singh, 1970; Pilgrim and Palma, 1982).

Most parasite surveys sample fewer than 10 host individuals therefore the detection of new or rare parasite species is unlikely (Clayton et al., 2016). By sampling a large number of hosts, we aimed to avoid this bias.

The objectives of this study were to examine a population sample of hybrid Mallard x Grey Ducks from New Zealand in order to: (1) document the relationship between ectoparasite load and phenotype. Although hybrids are often predicted to have increased numbers of lice compared to parental phenotypes (Fritz et al., 1994), the abundance of lice and mixing of genotypes might not create a stable association and therefore we aimed to provide data as a snapshot that can be used to study changes over time (Wolinska et al., 2008); and (2) test for an association between the mtDNA diversity of the lice and their hosts, which is predicted based on lack of paternal care. Maternal care would result in ectoparasite transmission predominantly by mother-offspring contact with lower rates of horizontal transmission. Although unique species of lice have not been described from the New Zealand Grey Duck, distinct mtDNA lineages might have evolved within the New Zealand region, as seen for Australian *Anaticola crassicornis* (Escalante et al., 2016). If lice lineages had differentiated prior to introduction of Northern Hemisphere ducks (and their parasites) we might detect maternal transmission of Grey Duck mtDNA and maternal transmission of distinct lice mtDNA. Thus, we present the first genetic data for *A. crassicornis*, *A. dentatus* and *T. querquedulae* from New Zealand waterfowl. We also compare *A. crassicornis* mtDNA diversity from New Zealand with that found worldwide.

2. Material and methods

2.1. Duck and lice samples

Duck specimens from Opiki, Manawatu, New Zealand (40.444505 °S, 175.379740 °E) were provided by a local hunter. Forty Mallard-Grey Duck hybrids were shot on 1 May 2015. The carcasses were put in individual plastic Zip Lock® bags and sealed to avoid lice straggling and frozen until use.

To remove and collect as many lice as possible from each duck, we used the Lipovsky (1951) body washing method. Briefly, a frozen bird carcass is placed inside a bucket with 15 L of fresh, warm water with 20 ml of dishwashing liquid. The detergent acts as a wetting agent. Each bird is washed three times, ruffled under water so that the wetting agent penetrates the plumage and loosens the lice. Each bucket of soapy water was then put through a series of sieves (8 mm, 600 µm, 500 µm, and 212 µm). Subsequently, the lice from the three washes of the same individual duck were collected together in a 50 ml vial and stored in 99% ethanol. Thirty-one ducks were washed, and their lice collected using this method (Suppl. Table S1). The body washing method proved an extremely accurate predictor of total abundance for wing and body lice in an evaluation of five different methods to quantify chewing lice in birds (Clayton and Drown, 2001).

Lice were identified using published keys (Price et al., 2003) by visual inspection of their morphology under a microscope (Leica S6D). The total lice number and the number of individuals of each species were recorded for each of the 31 duck specimens washed.

Infestation parameters were estimated following Rózsa et al. (2000). Prevalence was defined as the proportion of birds with lice, with 95% confidence intervals estimated using Sterne's exact method (Reiczigel, 2003). Mean intensity was defined as the mean number of lice per host in the total sample of infested hosts. The mean abundance of lice was calculated by multiplying prevalence and mean intensity. To set 95% confidence intervals of mean intensity and mean abundance we used a bootstrap procedure with 20000 replications (Rózsa et al., 2000). We used the software Quantitative Parasitology v.3 (Reiczigel and Rózsa, 2005) to estimate these parameters.

2.2. Host morphological measurements

Six morphological measurements (± 0.1 mm, unless otherwise specified) were taken from each thawed bird ($n = 37$): wing chord length (WC, carpal joint to longest primary feather unflattened), bill

length (BL, exposed culmen), neck length (NL), total tibia length (TTL, from heel to top of bent knee), tibiotarsus length (TML, from heel to base of toe), and skull length (SK, back of the skull to tip of bill). Birds were sexed using external characteristics as all were adult specimens. Adult males present green necks and/or curled tail feathers and/or red-brown chest feathers, depending on the degree of hybridisation.

Multivariate analysis is often the most effective way of comparing overall body size in birds (Rising and Somers, 1989), however, preliminary analysis revealed that there was no strong correlation between most of the different duck phenotypic variables. Therefore, we ran analyses with each morphological variable separately as each contains different information regarding the bird's size and shape (Supplementary Fig. S1). Skull length and tibia length were not included in further statistical analysis as they were strongly correlated with bill length and tibiotarsus length, respectively.

2.3. Host phenotypic characterisation

We scored the phenotype of each duck using five traits that are known to distinguish Mallard from Grey Ducks (Gillespie, 1985). These phenotypic traits correlate to some extent with the degree of genetic hybridisation (Rhymer et al., 1994; Muller, 2008) so we considered these as a relative measure of mixed ancestry. Each trait was assigned a score from 0 to 5 following Rhymer et al. (1994). A trait with a score of 0 matches the phenotype of a 'pure Grey Duck' and a trait with a score of 5 matches the phenotype of a 'pure Mallard Duck'. The five traits scored were: shape of the face stripes, bill colour, toe and leg colour, speculum colour and the thickness of the two white lines bordering the speculum. All scored traits were used to determine relative hybridisation index for each bird (Rhymer et al., 1994; Green et al., 2003). In addition, we ran a principal component analysis with the five traits. The first principal component correlated positively with each of the five mentioned traits and therefore was used to determine the degree of phenotypic hybridisation of each bird.

To investigate any potential effect of the phenotypic hybridisation level of the duck on the number of lice it was carrying, we used negative binomial regression analysis. We used the `glm()` function in R v.3.4.3 (R Core Team, Vienna, Austria, 2017), with the negative binomial distribution family and a log link function. We chose to use negative binomial regression because Poisson regression is suitable for count data (Long, 1997) and can account for overdispersion (Gardner et al., 1995), which was the case here.

Negative binomial regression models were built with the total count of lice and counts of the three different louse species separately as response variables. The hybridisation level of the duck was used as a covariate along with a proxy for bird size (wing length), to control for potential host body mass effect on parasite load (Clayton and Walther, 2001). Two different measures of phenotypic hybridisation level of the duck were used in separate analyses: additive trait score and the first principal component variable. In both these variables, a low value is associated with Grey Duck phenotype, and a high value with Mallard phenotype. The significance of the relationship between lice abundance and the duck phenotypic hybridisation level was assessed using likelihood-ratio tests between regression models containing and not containing this response variable, and a *p*-value computed from the likelihood-ratio test. Slope values of the regression models were used to assess direction and amplitude of the correlations.

2.4. Duck and lice DNA sequencing

DNA extraction, PCR-amplification and sequencing were performed following standard protocols for both louse and duck samples (e.g., Trewick et al., 2017). For the host ducks ($n = 40$), we amplified two mtDNA regions. The first one comprised 744 bp of the cytochrome c oxidase unit 1 gene (MT-CO1) using the primer pair AWCFl–AWCRint7 (Patel et al., 2010). The second region targeted contains most of

Domain I and all of Domain II of the mtDNA control region (636 bp), sequenced using the overlapping primer pairs L78–H774 (Sorenson and Fleischer, 1996; Sorenson et al., 1999).

For a subset of the lice, we also amplified the MT-CO1 gene. For *A. crassicornis* the primer pair L6625–H7005 (Hafner et al., 1994) rendered a 306 bp fragment for 16 individual lice collected from 6 ducks. For *T. querquedulae* (327 bp, $n = 24$ lice collected from 13 ducks) and *A. dentatus* (299 bp, $n = 24$ lice collected from 6 ducks) the forward primer LCO1490 (Folmer et al., 1994) was used with primer H7005. See Supplementary Table S1 for a list of duck hosts from which the lice were extracted. All sequences were archived in GenBank (accession numbers for *Anaticola crassicornis*: MH635514–MH635529, *Anatocetus detatus*: MH635530–MH635553. *Trinoton querquedulae*: MH635597–MH635620, duck control region: MH635554–MH635593, duck CO1: MH635594–MH635596 and MH635621–MH635657).

We downloaded from GenBank 76 CO1 sequences of *Anaticola crassicornis* collected in several waterfowl hosts around the world (accession numbers: KT587796–KT587871; Escalante et al., 2016). We aligned these sequences to the 16 *A. crassicornis* sequences from New Zealand to compare variability and determine to which of the 'crassicornis' clades from Escalante et al. (2016) the New Zealand *A. crassicornis* belong.

2.5. Genetic analyses

Sequences were aligned using the default alignment algorithm implemented in the software Geneious v.10.2.3 (<http://www.geneious.com>; Kearse et al., 2012). Allelic networks for each locus for the ducks and the three louse genera were inferred using the median-joining algorithm in NETWORK v.5 (<http://www.fluxus-engineering.com>; Bandelt et al., 1999).

Nucleotide site diversity (π) with confidence intervals was calculated for the CO1 gene data for the three lice species and for the host ducks with Arlequin v.3.5.2.2 (Excoffier and Lischer, 2010).

Phylogenetic trees for lice and ducks were inferred using the MrBayes v.3.2.6 (Huelsenbeck and Ronquist, 2001) plug-in in Geneious. Clade probabilities for the ducks and the three lice species were obtained from the posterior distribution. Bayesian analyses were replicated twice, each with four Markov chains of 2 million generations. Trees were sampled every 2500 generations, of which the first 0.5 million generations were discarded as burnin. To show the associations between the lice and the host duck CO1 gene trees we used the software Tree-Map v3.0b (Charleston and Robertson, 2002).

3. Results

3.1. Louse prevalence, abundance, intensity and host phenotype

Out of the 40 birds, 30 were males and 10 were females. Five traits that distinguish Mallard and Grey Ducks were scored and none of the specimens were phenotypically pure Grey Duck (e.g. score = 1) and only one was phenotypically pure Mallard (e.g. score = 25, male 12859). Most ducks in our sample were phenotypically more Mallard-like than Grey Duck-like hybrids (average score: 19) for the five traits examined (range: 11–24).

All ducks washed were host to two or three species of feather lice. All hosts were infested with *A. crassicornis* and *A. dentatus* but two ducks did not present any *T. querquedulae*. No new lice species were discovered despite our larger than usual sample size of 31 host individuals. Mean intensity and mean abundance for the three lice combined was 62.94 (Table 1). The louse *A. crassicornis* was the most abundant, with one duck being host to 131 *A. dentatus* individuals and had 190 lice in total. The least common louse species observed was the body louse *T. querquedulae* (Table 1).

Louse abundance was associated with host wing length but not tibiotarsus, bill length or sex. Wing length is a good proxy for body

Table 1
Descriptive statistics of ectoparasite load in 31 hybrid ducks from New Zealand with confidence intervals (CI).

Louse species	Total number of hosts	No. infected hosts	Range of lice detected	Prevalence (95% CI)	Mean intensity (95% CI)	Mean abundance (95% CI)
<i>Anatocercus dentatus</i>	31	31	1–131	100% (0.89–1)	24.65 (18.10–37.58)	24.65 (18.13–37.81)
<i>Anaticola crassicornis</i>	31	31	5–73	100% (0.89–1)	33.03 (27.74–38.94)	33.03 (27.71–38.84)
<i>Trinoton querquedulae</i>	31	29	0–16	93.5% (0.790.98)	5.48 (4.48–6.79)	5.13 (4.06–6.52)
Combined	31	31	11–190	100% (0.89–1)	62.94 (53.19–78.16)	62.94 (53.23–78.48)

Table 2
Effect of relative hybridisation level (determined by colour and plumage) on parasite load of New Zealand Mallard x Grey Duck hybrids. Two methods of summarising phenotype were used, either simple additive score of five traits or first Principal Component score (PC1) based on the same five traits. Three species of lice were recorded per host. Negative binomial regression models incorporated host size (wing length). Likelihood-ratio test (LR) between models with and without phenotype determined effect.* = output values when excluding the duck that presented 131 *A. dentatus*.

Count variable	Hybridisation variable used to inform the model	Slope value for the hybridisation variable	p-value for the LR test
Total lice number	Additive score	0.04	0.09
	PC1	0.08	0.08
<i>A. crassicornis</i> number	Additive score	–0.0008	0.97
	PC1	0.02	0.70
<i>A. dentatus</i> number	Additive score	0.09/*0.06	0.02/*0.13
	PC1	0.17/*0.11	0.03/*0.12
<i>T. querquedulae</i> number	Additive score	0.01	0.70
	PC1	0.01	0.91

size in avian species that fly (Owen and Cook, 1977; Yom-Tov et al., 2006). No significant relationship between the phenotypic hybridisation level of the host and abundance of *A. crassicornis*, *T. querquedulae* or total parasite load was found (Table 2, summary of the values of slope parameters of the negative binomial regression models and p-values associated with likelihood-ratio tests). However, a significant

effect of the phenotypic hybridisation level of the bird on the parasite load was found for the number of *A. dentatus* recorded (Fig. 1, Table 2). The Grey Duck-like hybrid phenotype had fewer *A. dentatus* lice than their Mallard-like counterparts (positive slope value in the regression model). This significant result was influenced by one particular host with many lice as confirmed when removing this ‘outlier’ (Table 2).

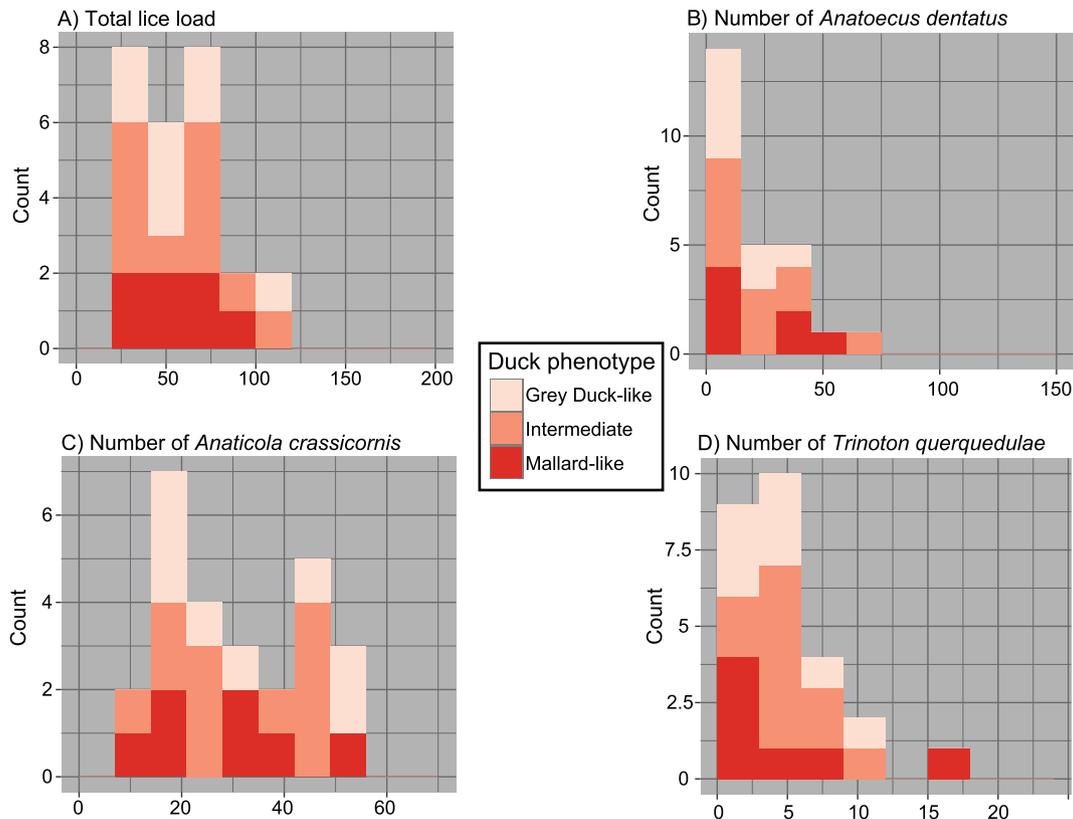


Fig. 1. Ectoparasite abundance on Mallard x Grey Duck hybrids in New Zealand. Histograms of A) total lice load; B, C, D) abundance per host for each of three feather lice species, with the phenotypic-hybridisation level of each duck shown in different colours. For representation purposes, ducks were considered to be Grey Duck-like for principal component 1 (PC1) score below –1.5, intermediate for a PC1 score between –1.5 and 1.5 and Mallard-like for a score above 1.5.

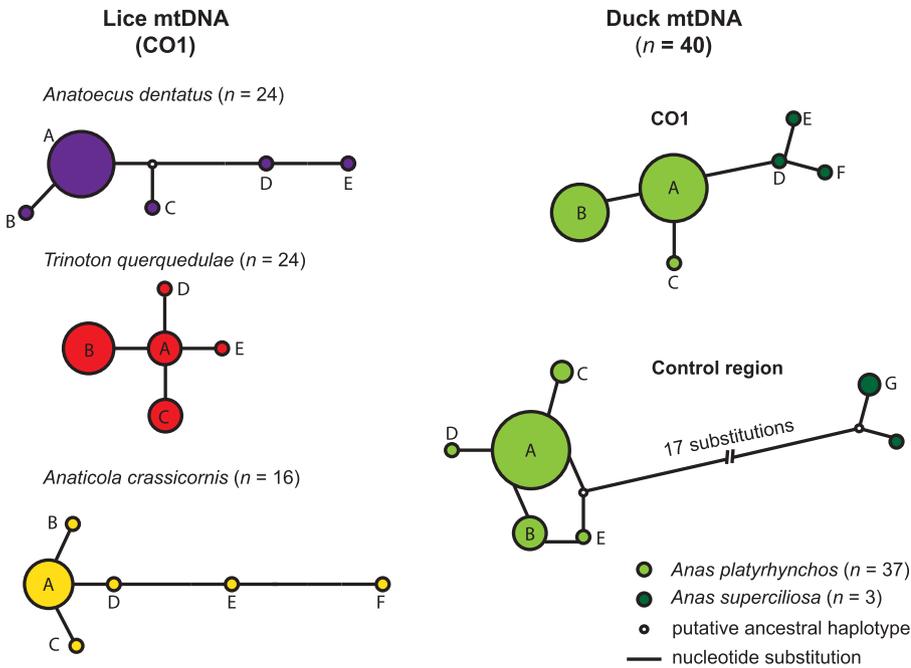


Fig. 2. On the left, unrooted parsimony networks for the three species of lice found on Mallard x Grey Duck hybrids showing the relationships of CO1 haplotypes. On the right, unrooted parsimony networks for the 40 hybrid host ducks showing the relationships of CO1 haplotypes (top) and control region (bottom). The areas of the circles are proportional to the number of haplotypes observed. The capital letters indicate the different haplotypes found.

3.2. mtDNA variation

3.2.1. Hosts

Even though almost all ducks showed phenotypic characters indicative of Mallard x Grey Duck hybrids, only three of the 40 individuals had mtDNA haplotypes that characterise Grey Ducks

(12850, 12854 and 12875; Fig. 2). Two of these three birds were more Grey Duck-like than the average within our sample, but they did not include the specimen with the strongest phenotypic resemblance to a Grey Duck (phenotype scores of 14 and 16; Supplementary Table S1). The host CO1 DNA fragment contained less sequence variation than the control region segment sequenced (maximum DNA sequence

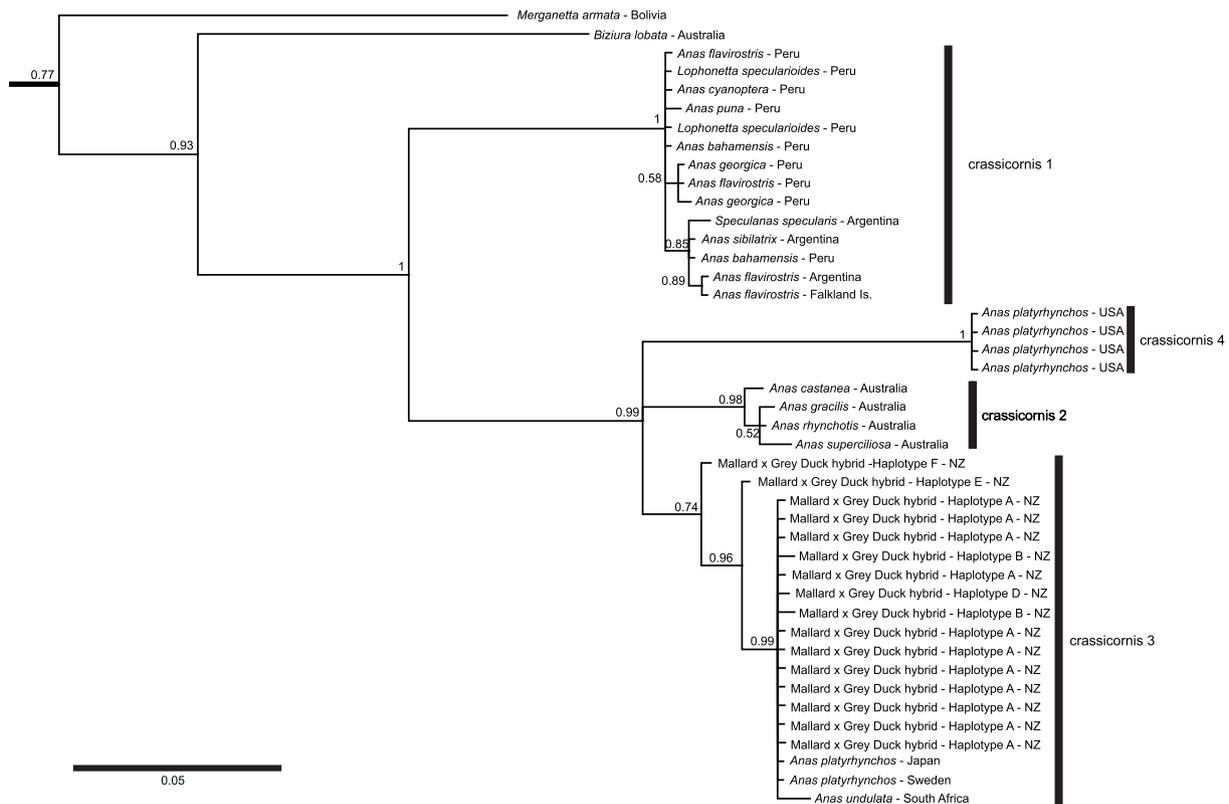


Fig. 3. Bayesian phylogeny of *Anaticola crassicornis* based on 378 bp of CO1 gene from Escalante et al. (2016) but with the addition of 16 new sequences from New Zealand hosts. The values above branches are posterior probabilities. The scale bar indicates nucleotide substitutions per site along the branch lengths. Haplotypes names correspond to those shown in Fig. 2. For simplicity we are showing the portion of the tree of interest, the full tree with all downloaded sequences can be found in the Supplementary Fig. S2. NZ = New Zealand.

divergence among any pair of haplotypes was 0.4% for CO1 and 3.1% for control region).

3.2.2. Parasites and duck-lice associations

The mtDNA CO1 sequences showed that the same duck could host lice of the same species but with different mitochondrial haplotypes. For example, duck 12850 had all three species of lice and for each louse species at least two different mtDNA haplotypes were recorded (Fig. 3, Supplementary Table S1). mtDNA haplotypes sampled within *A. dentatus* varied by a maximum of 1.3% DNA sequence divergence, within *T. querquedulae* by a maximum of 0.6%, and within *A. crassicornis* by a maximum of 0.9% DNA sequence divergence. Nucleotide site diversity (π) calculated for CO1 for *A. crassicornis* was: 0.0057 ± 0.0039 , for *A. dentatus*: 0.0036 ± 0.0028 , and for *T. querquedulae*: 0.0032 ± 0.0024 while the host ducks presented a nucleotide site diversity (π) of 0.0012 ± 0.001 . Therefore, for all three parasite species, CO1 sequence divergence was higher and for *A. crassicornis* π was significantly higher than observed within the same gene in their hosts. One duck with the mtDNA of Grey Duck had 11 lice sequenced (6 *A. dentatus*, 2 *T. querquedulae* and 3 *A. crassicornis*) that represented 8 different haplotypes, 5 of them exclusive to ducks with Grey Duck mtDNA (Fig. 3, Supplementary Table S1). In our sample, more hosts had Mallard rather than Grey Duck mtDNA, therefore some lice haplotypes were only observed on ducks with Mallard mtDNA (e.g. *A. crassicornis* haplotypes B, C & D). However, each louse species had one haplotype observed in lice living on both mtDNA duck hosts (Mallard-like and Grey Duck-like). Thus, none of the three lice species showed a close association of parasite and host mtDNA lineage. Despite our host sample being strongly male-biased, the four females whose lice were sampled seem to have higher lice haplotype diversity than found on lice extracted from males although we cannot confirm this trend due to the small sample size.

The global phylogeny of *A. crassicornis* showed that all New Zealand *A. crassicornis* samples grouped in a monophyletic clade with lice collected from Mallard (*Anas platyrhynchos*) in Japan and Sweden and from Yellow-billed Duck (*Anas undulata*) from South Africa (clade ‘crassicornis 3’ from Escalante et al., 2016, Fig. 3). None of our *A. crassicornis* mtDNA sequences matched those from *A. crassicornis* from USA-collected Mallards (clade ‘crassicornis 4’, Fig. 3).

The associations between host and parasite CO1 gene trees for 15 duck hosts and 61 of their lice from three species are illustrated with a tanglegram (Fig. 4). Two hybrid ducks with Grey Duck-mtDNA (12850 and 12854) presented lice with mostly the same haplotypes as those found on hybrid ducks with Mallard-like mtDNA. We did not detect divergent lice lineages that would suggest extant New Zealand endemic

diversity. There are few lice haplotypes exclusive to either host mtDNA lineage, thus we observed no close association of host mtDNA and parasite lineage.

4. Discussion

4.1. Parasite load and phenotype

Studies of parasite load of wild bird populations have found that the size of the host and their social groups have a positive influence on lice numbers (Hoi et al., 1998; Galloway and Lamb, 2017) but that this relationship can depend on the parasite species (Whiteman and Parker, 2004). We found an association between host body size (measured by wing length) and number of lice so included this variable in our glm analysis. After controlling for body size, we found an association between abundance of one of the three louse species and phenotypic hybrid index. The head louse *A. dentatus* was more common on Mallard-like host individuals than Grey Duck-like hybrids despite our small sample size for Grey Duck-like hybrids. Studies of the parasites of hybrid animal populations have detected changes in parasite abundance that might be due to selection on both novel host genotypes and novel parasite interactions (Wolinska et al., 2008; Baird et al., 2012), therefore the current advantage that Grey Duck-like hybrids exhibit (lower abundance of *A. dentatus*) might be short lived. Comparative studies over time and space of the relationship between host phenotype and abundance of *A. dentatus* are warranted. However, the total ectoparasite load observed in our study was not influenced by the hybrid-index of the host, suggesting little variation in resistance or tolerance to lice by hybrid ducks (Whiteman et al., 2007), whether Grey Duck-like or Mallard-like.

Additionally, our study has confirmed the difficulty of identifying pure-Mallards, hybrids and pure-Grey Ducks solely based on phenotypes (Gillespie, 1985; Muller, 2008; Williams, 2017). The majority of ducks now present in New Zealand are Grey x Mallard hybrids. Thus, conducting fieldwork, population estimations and implementing hunting regulations are difficult tasks that interfere with proper conservation measures for the disappearing New Zealand Grey Duck.

4.2. Host-parasite population genetics

DNA sequence variation (maximum haplotype divergence and population nucleotide diversity) was higher in all three lice species than observed within the same gene in their host ducks. The higher parasite diversity might be the result of their host hybridisation that resulted in contact of parasite populations that had diverged in isolation. Previous

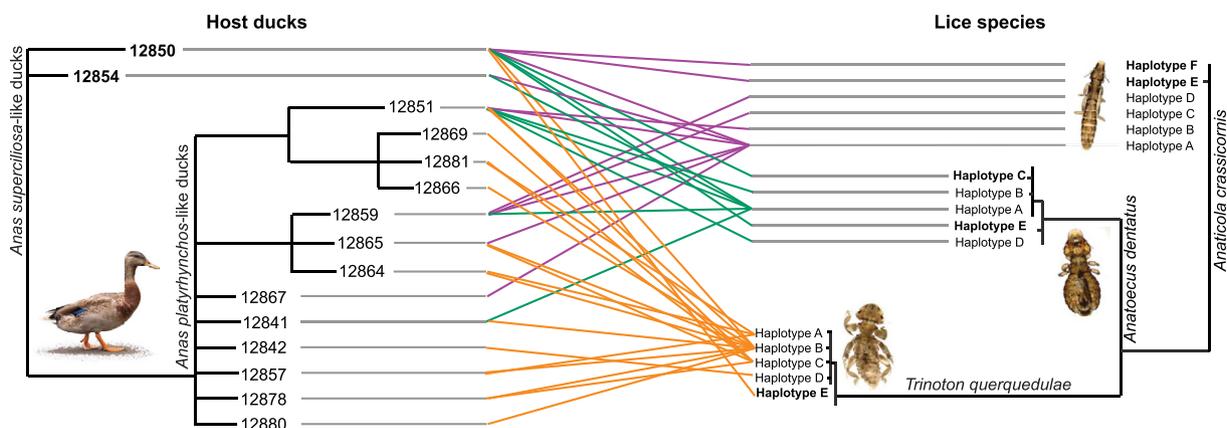


Fig. 4. Tanglegram showing the associations between the CO1 gene trees for the host ducks (on the left, $n = 15$) and the three species of louse (on the right, $n = 61$) from Manawatu, New Zealand. For lice, only the different haplotypes are shown. The two hosts with Grey Duck mtDNA are shown in bold as well as the louse haplotypes exclusive to them. Thin lines indicate host–parasite associations. Lice photos are illustrative and not to scale.

studies showed that homologous mitochondrial genes and nuclear protein-coding genes in lice evolve 2–15 times faster than their hosts (Clayton et al., 2016). Examples include rodents and *Fahrenholzia* lice (Light and Hafner, 2007), lice and their primate hosts (Reed et al., 2004), pigeons and *Columbicola* lice (Johnson et al., 2003), and swiftlets and *Dennyus* lice (Page et al., 1998). However, the population size of all three lice species is larger than their hosts (on average each individual duck is host to three lice species with between 5 and 33 lice each). There is a positive correlation between genetic diversity and population size due to the slower action of genetic drift in a large population compared to a small population (Charlesworth, 2009). When we sample from hosts and parasites as done here, we expect to detect more standing genetic variation in the large parasite populations compared to their hosts. Therefore, as well as the possibility that hybridisation has elevated genetic diversity, both rapid rates of substitution and large population sizes could explain the genetic diversity we observed in the lice population samples.

We found that lice phylogenies are not congruent with their respective host phylogenies. The lack of host specificity of waterfowl lice is well-known. In brief, for the three lice species found in New Zealand waterfowl, the wing louse *Anaticola crassicornis* has been recorded from 24, the body louse *Trinoton querquedulae* from 68 and the head louse *Anatoecus dentatus* from 69 host species worldwide (Price et al., 2003). Horizontal lice transmission is thought to be common in the numerous aggregations that waterfowl exhibit on bodies of water in winter (Clayton et al., 2016); live lice has been observed on moulted feathers (Eichler, 1963); and walking on water has been reported for specimens of *Trinoton*. Individuals of this genus have tarsal setae that enable them to walk or run quickly across the surface tension of water allowing them to switch hosts with ease (Stone, 1969).

Anaticola crassicornis collected from New Zealand forms a monophyletic group with *A. crassicornis* collected from *A. platyrhynchos* in Sweden and Japan and with *A. undulata* from South Africa, which confirms a Eurasian origin of the ducks and their lice. The first introduction of Mallards into New Zealand was from a British stock imported via Australia in the second half of the 19th century (Knox, 1969; Heather et al., 2000). In the 1930s and 1940s Mallards of American origin were imported (some as eggs) at a much larger scale than previously (Knox, 1969; Heather et al., 2000). However, the host mtDNA in New Zealand populations suggest that the British-sourced introductions contributed more to current diversity (Guay et al., 2015), and this would explain why we did not sample any *A. crassicornis* with mtDNA haplotypes from the USA clade which forms a separate, monophyletic clade in the phylogeny. With our short DNA sequences, the New Zealand *A. crassicornis* did not unequivocally group with the Australian *A. crassicornis* as might be expected either from host switching in Australia or from the common origin of New Zealand and Australian Grey Ducks.

It is important to highlight that we needed to compare 16 *A. crassicornis* mtDNA sequences to find two rare haplotypes (E and F) that represent standing genetic variation in New Zealand louse populations. These two haplotypes were probably detected because of increased sampling effort in New Zealand compared to the Old World. As it has been pointed out many times, sampling mtDNA from one individual may not be representative of the species as a whole (Peters et al., 2005) and when documenting diversity of parasites on hybridising hosts larger sample sizes are required.

5. Conclusion

We studied the population genetics of one population sample of hybrid Mallard x Grey Ducks and their lice in New Zealand. We found no association between the ectoparasite load and host phenotype for two of the three louse species, with Grey Duck-like hybrids hosting fewer head lice (*A. dentatus*) than their Mallard-like counterparts. We uncovered no association between the mtDNA diversity of the lice and

that of their duck hosts. This study presents the first molecular diversity data for three species of lice found in New Zealand Anatidae, with our results highlighting the importance of sampling more than one individual per species.

Acknowledgements

We thank Kevin Stafford who shot the ducks and Cleland Wallace who produced the duck illustration. Ellie Bradley helped wash ducks and Benjamin Bridgemen assisted with extracting duck DNA. This project was funded by Massey University and the Royal Society of New Zealand Marsden Fund (09-MAU-037). We thank Ricardo Palma and two anonymous reviewers for their useful comments that improved our original manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ijppaw.2018.09.005>.

References

- Aksin, Z., 2011. Chewing lice (insecta: Phthiraptera) on mallards (*Anas platyrhynchos*) in Turkey. *J. Anim. Vet. Adv.* 10, 1656–1659.
- American Ornithologists' Union, 1983. Check-list of North American Birds, sixth ed. Am. Ornithol. Union, Washington, D.C., USA.
- Baird, S.J.E., Ribas, A., Macholán, M., Albrecht, T., Piálek, J., Göüy de Bellocq, J., 2012. Where are the wormy mice? A reexamination of hybrid parasitism in the European house mouse hybrid zone. *Evolution* 66, 2757–2772.
- Bandelt, H.-J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16, 37–48.
- BirdLife International, 2017. *Anas wyvilliana* (amended version of 2016 assessment). The IUCN Red List of Threatened Species 2017: e.T22680199A112386802. <https://doi.org/10.2305/IUCN.UK.2017-1.RLTS.T22680199A112386802.en>, Accessed date: 28 July 2018.
- Charleston, M.A., Robertson, D.L., 2002. Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny. *Syst. Biol.* 51, 528–535.
- Charlesworth, B., 2009. Fundamental concepts in genetics: effective population size and patterns of molecular evolution and variation. *Nat. Rev. Genet.* 10, 195–205.
- Clayton, D.H., Drown, D.M., 2001. Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera). *J. Parasitol.* 87, 1291–1300.
- Clayton, D.H., Walther, D.A., 2001. Influence of host ecology and morphology on the diversity of Neotropical bird lice. *Oikos* 94, 455–467.
- Clayton, D.H., Bush, S.E., Johnson, K.P., 2004. Ecology of congruence: past meets present. *Syst. Biol.* 53, 165–173.
- Clayton, D.H., Bush, S.E., Johnson, K.P., 2016. *Coevolution of Life on Hosts: Integrating Ecology and History*. University of Chicago Press, Chicago, USA.
- Detwiler, J.T., Criscione, C.D., 2010. An infectious topic in reticulate evolution: introgression and hybridization in animal parasites. *Genes* 102–123.
- DiBlasi, E., Johnson, K.P., Stringham, S.A., Hansen, A.N., Beach, A.B., Clayton, D.H., Bush, S.E., 2018. Phoretic dispersal influences parasite population genetic structure. *Mol. Ecol.* 27, 2770–2779.
- Eichler, W.D., 1963. Arthropoda. Insecta. Phthiraptera 1. Mallophaga. In *Bronn's Klassen und Ordnungen des Tierreichs, Leipzig*, vol. 5. pp. 290 (III).
- Escalante, G.C., Sweet, A.D., McCracken, K.G., Gustafsson, D.R., Wilson, R.E., Johnson, K.P., 2016. Patterns of cryptic host specificity in duck lice based on molecular data. *Med. Vet. Entomol.* 30, 200–208.
- Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Res.* 10, 564–567.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.
- Fritz, R.S., Nichols-Orians, C.M., Brunsfeld, S.J., 1994. Interspecific hybridization of plants and resistance to herbivores: hypotheses, genetics, and variable responses in a diverse community. *Oecologia* 97, 106–117.
- Galloway, T.D., Lamb, R.J., 2017. Abundance of chewing lice (Phthiraptera: Ischnocera and Amblycera) increases with the body size of their host woodpeckers and sapsuckers (Aves: Piciformes: Picidae). *Can. Entomol.* 149, 473–481.
- Gardner, W., Mulvey, E.P., Shaw, E.C., 1995. Regression analyses of counts and rates: Poisson, overdispersed Poisson, and negative binomial models. *Psychol. Bull.* 118, 392–404.
- Gillespie, G.D., 1985. Hybridization, introgression and morphometric differentiation between mallard (*Anas platyrhynchos*) and grey duck (*Anas superciliosa*) in Otago, New Zealand. *Auk* 102, 459–469.
- Green, J., Wallis, G., Williams, M., 2003. Determining the Extent of Grey Duck x Mallard Hybridisation in New Zealand. Department of Conservation, Wellington, New Zealand.

- Grossi, A.A., Sharanowski, B.J., Galloway, T.D., 2014. *Anatoecus* species (Phthiraptera: Philopteridae) from Anseriformes in North America and taxonomic status of *Anatoecus dentatus* and *Anatoecus icterodes*. *Can. Entomol.* 146, 598–608.
- Guay, P.-J., Williams, M., Robinson, R.W., 2015. Lingering genetic evidence of North American mallards (*Anas platyrhynchos*) introduced to New Zealand. *New Zeal. J. Ecol.* 39, 103–109.
- Hafner, M.S., Sudman, P.D., Villablanca, F.X., Spardling, T.A., Demastes, J.W., Nadler, S.A., 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. *Science* 265, 1087–1090.
- Heather, B.D., Robertson, H.A., Onley, D.J., 2000. *The Field Guide to the Birds of New Zealand*. Viking, Auckland, New Zealand, pp. 78–79.
- Hoi, H., Darolova, A., König, C., Kristofik, J., 1998. The relation between colony size, breeding density and ectoparasite loads of adult European bee-eaters (*Merops apiaster*). *Ecoscience* 5, 156–163.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- Johnson, K.P., McKinney, F., Sorenson, M.D., 1999. Phylogenetic constraint on male parental care in the dabbling ducks. *Proc. Roy. Soc. Lond. B* 266, 759–763.
- Johnson, K.P., Cruickshank, R.H., Adams, R.J., Smith, V.S., Page, R.D.M., Clayton, D.H., 2003. Dramatically elevated rate of mitochondrial substitution in lice (Insecta: Phthiraptera). *Mol. Phylogenet. Evol.* 26, 231–242.
- Johnson, K.P., Shreve, S.M., Smith, V.S., 2012. Repeated adaptive divergence of micro-habitat specialization in avian feather lice. *BMC Biol.* 10, 52. <https://doi.org/10.1186/1741-7007-10-52>.
- Kear, J., 1970. The adaptive radiation of parental care in waterfowl. In: Crook, J.H. (Ed.), *Social Behavior in Birds and Mammals*. Academic Press, London, pp. 357–392.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649.
- Kirby, R.E., Sargeant, G.A., Shutler, D., 2004. Haldane's rule and American black duck × mallard hybridization. *Can. J. Zool.* 82, 1827–1831.
- Knox, G.A., 1969. *The Natural History of Canterbury*. Canterbury Branch of the Royal Society of New Zealand. A. H. & A. W. Reed, Wellington, New Zealand.
- Krasnov, B.R., Mouillot, D., Shenbrot, S.I., Khokhlova, I.S., Poulin, R., 2004. Geographical variation in host specificity of fleas (Siphonaptera) parasitic on small mammals: the influence of phylogeny and local environmental conditions. *Ecography* 27, 787–797.
- Light, J.E., Hafner, M.S., 2007. Phylogenetics and host associations of *Fahrenholzia* sucking lice (Phthiraptera: Anoplura). *Syst. Entomol.* 32, 359–370.
- Lipovsky, L.J., 1951. A washing method of ectoparasite recovery with particular reference to chiggers (Acarina: Trombiculidae). *J. Kans. Entomol. Soc.* 24, 151–156.
- Long, J.S., 1997. *Regression Models for Categorical and Limited Dependent Variables*. Sage Publications, Thousand Oaks, California, USA.
- McDougall, M.B., Amundson, C.L., 2017. Harvest dynamics and annual survival of mallards and grey ducks. *J. Wildl. Manag.* 81, 449–460.
- Muller, W., 2008. Hybridisation, and the Conservation of the Grey Duck in New Zealand. Ph.D. Thesis. Canterbury University, New Zealand.
- Owen, M., Cook, W.A., 1977. Variations in body weight, wing length and condition of Mallard *Anas platyrhynchos platyrhynchos* and their relationship to environmental changes. *J. Zool.* 183, 377–395.
- Page, R.D.M., Lee, P.L.M., Becher, S.A., Griffiths, R., Clayton, D.H., 1998. A different tempo of mitochondrial DNA evolution in birds and their parasitic lice. *Mol. Phylogenet. Evol.* 9, 276–293.
- Patel, S., Waugh, J., Millar, C.D., Lambert, D.M., 2010. Conserved primers for DNA barcoding historical and modern samples from New Zealand and Antarctic birds. *Mol. Ecol. Res.* 10, 431–438.
- Peters, J.L., McCracken, K.G., Zhuravlev, Y.N., Lu, Y., Wilson, R.E., Johnson, K.P., Omland, K.E., 2005. Phylogenetics of wigeons and allies (Anatidae: Anas): the importance of sampling multiple loci and multiple individuals. *Mol. Phylogenet. Evol.* 35, 209–224.
- Pilgrim, R.L.C., Palma, R.L., 1982. A list of the chewing lice (Insecta: Mallophaga) from birds in New Zealand. *Notornis* 29, 1–32.
- Poulin, R., 2007. *Evolutionary Ecology of Parasites*. Princeton University Press, New Jersey, U.S.A.
- Price, R.D., Hellenenthal, R.A., Palma, R.L., Johnson, K.P., Clayton, D.H., 2003. *The Chewing Lice: World Checklist and Biological Overview*, vol. 24 Illinois Natural History Survey Special Publications.
- R: A Language and Environment for Statistical Computing R Core Team 2017. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>.
- Reed, D.L., Smith, V.S., Hammond, S.L., Rogers, A.R., Clayton, D.H., 2004. Genetic analysis of lice supports direct contact between modern and archaic humans. *PLoS Biol.* 2 e304.
- Reiczigel, J., 2003. Confidence intervals for the binomial parameter: some new considerations. *Stat. Med.* 22, 611–621.
- Reiczigel, J., Rózsa, L., 2005. *Quantitative Parasitology 3.0*. Budapest. Distributed by the authors. Available at: <http://www.zoologia.hu/qp/>.
- Rhymer, J.M., Williams, M.J., Braun, M.J., 1994. Mitochondrial analysis of gene flow between New Zealand mallards (*Anas platyrhynchos*) and grey ducks (*Anas superciliosa*). *Auk* 111, 970–978.
- Rising, J.D., Somers, K.M., 1989. The measurement of overall body size in birds. *Auk* 106, 666–674.
- Rózsa, L., Reiczigel, J., Majoros, G., 2000. Quantifying parasites in samples of hosts. *J. Parasitol.* 86, 228–232.
- Singh, G., 1970. *The Morphology and Systematics of Mallophaga Infesting New Zealand Anatidae*. Ms. Thesis. Victoria University of Wellington, Wellington, New Zealand.
- Sorenson, M.D., Fleischer, R.C., 1996. Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. *Proc. Natl. Acad. Sci. U.S.A.* 93, 15239–15243.
- Sorenson, M.D., Ast, J.C., Dimcheff, D.E., Yuri, T., Mindell, D.P., 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Mol. Phylogenet. Evol.* 12, 105–114.
- Stone, W.B., 1969. *The Ecology of Parasitism in Captive Waterfowl at the Burnet Park Zoo*. PhD diss. Syracuse University, NY, USA.
- Sun, Z., Pan, T., Hu, C., Sun, L., Ding, H., Wang, H., et al., 2017. Rapid and recent diversification patterns in Anseriformes birds: inferred from molecular phylogeny and diversification analyses. *PLoS One* 12 (9), e0184529. <https://doi.org/10.1371/journal.pone.0184529>.
- Thompson, J.N., 2005. *The Geographic Mosaic of Coevolution*. University of Chicago Press, Chicago, USA.
- Tracey, J.P., Lukins, B.S., Haselden, C., 2008. Hybridisation between mallard (*Anas platyrhynchos*) and grey duck (*A. superciliosa*) on Lord Howe Island and management options. *Notornis* 55, 1–7.
- Trewick, S.A., Pilkington, S., Shepherd, L., Gibb, G.C., Morgan-Richards, M., 2017. Closing the gap: avian lineage splits at a young, narrow seaway imply a protracted history of mixed population response. *Mol. Ecol.* 26, 5752–5772.
- Tubaro, P.L., Litjmaer, D.A., 2002. Hybridization patterns and the evolution of reproductive isolation in ducks. *Biol. J. Linn. Soc.* 77, 193–200.
- Whiteman, N.K., Parker, P.G., 2004. Body condition and parasite load predict territory ownership in the Galapagos hawk. *Condor* 106, 915–921.
- Whiteman, N.K., Kimball, R.T., Parker, P.G., 2007. Co-phylogeography and comparative population genetics of the threatened Galápagos hawk and three ectoparasite species: ecology shapes population histories within parasite communities. *Mol. Ecol.* 16, 4759–4773.
- Williams, M., 2017. The changing relative abundance of grey duck (*Anas superciliosa*) and mallard (*A. platyrhynchos*) in New Zealand. *Notornis* 64, 211–228.
- Wolinska, J., Lively, C.M., Spaak, P., 2008. Parasites in hybridizing communities: the Red Queen again? *Trends Parasitol.* 24, 121–126.
- Yom-Tov, Y., Yom-Tov, S., Wright, J., Thorne, C.J.R., Du Feu, R., 2006. Recent changes in body weight and wing length among some British passerine birds. *Oikos* 112, 91–101.