Population Genomics Advances and Opportunities in Conservation of Kiwi (*Apteryx* spp.)

Kristina M. Ramstad and Luke T. Dunning

Abstract Kiwi (*Apteryx* spp.) are highly threatened flightless birds endemic to New Zealand. They are members of the most basal extant avian lineage, the paleognaths, and exhibit a suite of traits that are unusual in birds. Despite their iconic and imperiled status, there have been only four genomic studies of kiwi to date with only two of these aimed at improving conservation. There is, therefore, massive opportunity to use genomic techniques to elucidate the genetic basis and consequences of the strange ecology and evolution of kiwi and to inform their intensive management. In this chapter, we review genomic studies in paleognaths, assess prospects for the future of kiwi genomics, and define some lessons for population genomics and conservation of at-risk taxa generally. We also present an analysis of genomic signatures associated with the evolution of Apterygidae and the genes involved in diversification of kiwi via comparison of 3,774 orthologous protein coding genes among 28 avian species. We found strong signals of selection in genes associated with dwarfism, neurogenesis, retinal development, and temperature regulation. Our results provide clues as to why kiwi have such small body size (relative to other paleognaths), large egg size (relative to their body size), excellent olfaction, and poor vision. The data further suggest that coping with highly divergent temperature regimes may be a defining feature of the spotted kiwi clade which includes the only kiwi species that inhabits the alpine zone. Considerable genomic resources are now available for kiwi, including whole-genome sequences, transcriptome assemblies, thousands of SNP markers, and numerous candidate genes. There is also a myriad of outstanding questions about kiwi that genomic studies can inform. The challenge now is to bring these new genomic tools to bear on conservation and management of kiwi.

K. M. Ramstad (✉)
Department of Biology and Geology, University of South Carolina Aiken, Aiken, SC, USA
e-mail: kristinar@usca.edu

L. T. Dunning
Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK
e-mail: l.dunning@sheffield.ac.uk

1   Introduction

The fields of molecular ecology and conservation genetics have seen dramatic advances over the last decade as high-throughput sequencing has been widely adopted. The transition from genotyping a handful of loci to sequencing whole genomes has enabled researchers to shift their focus from neutral loci that capture patterns of population dynamics to identifying functional loci of adaptive significance (Allendorf 2017; Luikart et al. 2019; Shafer et al. 2015). Genomic approaches have also yielded an unprecedented ability to explore the genetic architecture of adaptation in non-model organisms by coupling reference genomes with resequencing data from multiple individuals (Berg et al. 2016; Hartmann et al. 2017; Hohenlohe et al. 2010a; Zhang et al. 2014a; Zhou et al. 2014). The increased number of markers used in whole-genome, and to a lesser extent reduced representation (e.g., RADseq), studies has greatly improved the power of traditional population genetic analyses aimed at estimating effective population size (N_e), inbreeding coefficients, population structure, dispersal, admixture, and introgression (Allendorf 2017; Berg et al. 2016; Bernatchez et al. 2017).

Conservation genomics is an extension of conservation genetics and seeks to apply genomic techniques to practical management of small populations of at-risk species. While there is widespread appreciation for the practical benefits of conservation genomics (Allendorf et al. 2010; Andrews et al. 2016; Funk et al. 2012; Hohenlohe et al. 2010b; Ryder 2005; Steiner et al. 2013), the real-world applications have been slow in coming (Funk et al. 2018), and there has been a persistent gap between theory and practice. Wildlife managers often view genomic research as too costly, confusing, and unreliable to add value to conservation programs. In addition, advancements are still needed, particularly in analytical techniques, as our ability to analyze data is being outpaced by data collection (Andrews and Luikart 2014; Benestan et al. 2016; Shafer et al. 2015, 2016; Steiner et al. 2013). Despite these challenges, an increasing number of case studies demonstrate the benefits of applying genomic techniques to species conservation (e.g., Tasmanian devil, *Sarcophilus harrisii*, Margres et al. 2018; greater sage grouse, *Centrocercus urophasianus*, Oyler-McCance et al. 2019; ungulates, Martchenko et al. 2018; Burmese roofed turtle, *Batagur trivittata*, Çilingir et al. 2017; Pacific salmon, *Oncorhynchus* spp., Prince et al. 2017; plateau deer mouse, *Peromyscus melanophrys*, Vega et al. 2017), and the gap between basic genomic research and applied conservation is narrowing.

Genomics of small populations, which we define here as those having an N_e of less than 200 (and thus a census size of likely <1,000; Jamieson and Allendorf 2012; Palstra and Ruzzante 2008), differs in a number of fundamental ways from genomics of large populations. Small populations often exist in fragmented, isolated, inaccessible, and wild habitats; the distribution of such species is often patchy due to

extirpation of populations or whole lineages. Low census size and rarity often mean few individuals can be sampled, and tissue samples often must be collected using non-invasive techniques (Hoffman et al. 2013; Hoffman 2011; Ramstad et al. 2016). Thus, the quantity, type (e.g., lethal sampling for different tissues for RNA expression analysis), and quality of samples (e.g., feathers or hair rather than tissue or blood) for genomic analysis are limited for at-risk species. Applying traditional population genetic measures can be difficult for at-risk species as well. Long generation intervals and overlapping generations make estimating N_e difficult (Waples et al. 2014), and low sample sizes make it difficult statistically to find loci under selection or associated with ecological traits (Margres et al. 2018; Shen et al. 2011). In addition, rare species with small populations are vulnerable to elevated levels of both interspecific hybridization and inbreeding depression (Edmands 2007). At-risk species are also often phylogenetically distinct, making it unlikely that a closely related reference genome will be available (Miller et al. 2012). All of these issues are a product of the biology and history of these endangered populations and still complicate matters despite the advent of genomics.

Genomics, and in particular whole-genome sequencing, initially focused on a few key model species and then expanded to include abundant and economically important species (Bernatchez et al. 2017). These early studies typically included a single individual or handful of individuals to maximize sequencing depth for de novo genome assembly. In recent years, however, genomic research has included more genome resequencing to assess the variation within and among populations, and there have been increasing numbers of projects aimed at sequencing the genomes of endangered species (e.g., koala, *Phascolarctos cinereus*, Johnson et al. 2018; giant panda, *Ailuropoda melanoleuca*, Li et al. 2010; Chinese alligator, *Alligator sinensis*, Wan et al. 2013). More reference genomes are becoming publicly available each year, and increased efficiency and decreased cost of collecting genomic data are allowing unprecedented depth and breadth of sampling of genomic variation at the population level. Techniques such as Rapture (Ali et al. 2016) and RADcap (Hoffberg et al. 2016) allow both to be accomplished simultaneously by assaying variation at the same suite of thousands of SNPs reliably across different genomic libraries.

In this chapter, we use kiwi (family Apterygidae) as a case study to illustrate the application of conservation genomic techniques to species of conservation concern. Avian taxa are excellent systems for comparative genomic and molecular evolution studies because they have relatively small genomes that show highly conserved synteny (Zhang et al. 2014a). We review how recent genomic research has significantly improved our understanding of the evolution, taxonomy, and ecology of kiwi. We combine existing genomic data for kiwi and present an analysis detecting genes under positive selection in the different lineages, illustrating that meaningful conservation genomic insights do not always require new data. Finally, we outline the conservation impact of this research and identify future genomic research that may aid conservation of this charismatic species. Although focused on kiwi, much of the research discussed is applicable to any taxon of conservation concern and so provides an example of how researchers might initially apply genomic techniques to non-model species.

1.1 Evolutionary and Ecological Significance of Kiwi

Kiwi are one of the most instantly recognizable bird species and a national icon of New Zealand. They represent the most basal extant avian lineage, the paleognaths, and possess a suite of traits that are rare or absent in other birds (Fig. 1). Paleognaths make up less than 1% of extant avian species and differ from all other birds (neognaths) by having a primitive and reptile-like palate (the paleognath palate), a distinct pelvic structure (a large and open ilioischiatic fenestra), and an unusual pattern of grooves on their bills (rhamphotheca; Cracraft 1974; Huxley 1867; Parkes and Clark 1966). The paleognaths include five extant and two extinct lineages of flightless ratites and the flighted tinamous (Harshman et al. 2008), all of which are restricted to the southern hemisphere (Fig. 1). Flightlessness is relatively rare (<1%) among extant birds (McCall et al. 1998), but is found in seven of eight paleognath lineages which together comprise the ratites. Not surprisingly, ratites share a number of traits associated with flightlessness that are not typically found in neognaths or tinamous. For example, they lack a keeled sternum and have reduced flight muscles, furcula (wishbones), and wings (Cracraft 1974; Fowler 1991). Ratites also tend to be large-bodied and herbivorous and have "hairlike" feathers (Fowler 1991).
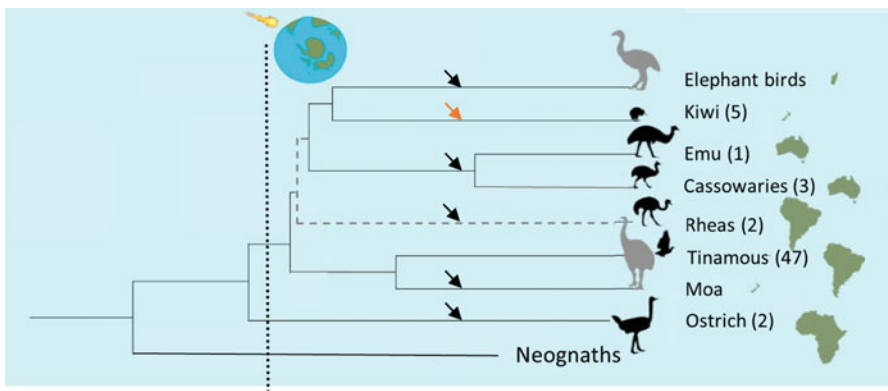

Fig. 1 Phylogeny of the paleognaths, modified from Maderspacher (2017) and based on Yonezawa et al. (2017). More recent studies (Cloutier et al. 2019; Sackton et al. 2018) place rhea as sister taxa to kiwi/emu/cassowary (dashed lines) but do not change the position of kiwi. Paleognaths make up less than 1% of extant avian species and are the most basal lineage of extant birds. Extant paleognaths include five flightless lineages (the ratites) and one volant lineage (tinamous). The dotted line indicates the Cretaceous-Paleogene extinction event that occurred approximately 65 Mya, arrows indicate hypothesized loss of flight either coupled with gigantism (black) or not (orange; Mitchell et al. 2014), and silhouettes in gray and black indicate extinct and extant lineages, respectively. Geographic distribution of each taxon is indicated on the far right and number of extant species per lineage is in parentheses. Ostriches (family Struthionidae) are found in Africa, kiwi (family Apterygidae) and extinct moa (order Dinornithiformes) are found in New Zealand, emu (*Dromaius novaehollandiae*) and cassowary (*Casuarius* spp.) are native to Australia and New Guinea, rhea (family Rheidae) and tinamous (family Tinamidae) are found in South America, and the extinct elephant birds (family Aepyornithidae) were endemic to Madagascar (Harshman et al. 2008; Mitchell et al. 2014)

Kiwi diverged from other paleognaths approximately 60 million years ago (Mitchell et al. 2014; Phillips et al. 2010) and five species of kiwi comprising two clades are currently recognized (Fig. 2). The little spotted (LSK; *Apteryx owenii*) and great spotted kiwi (GSK; *A. haastii*) belong to the spotted clade, and North Island brown kiwi (NIB; *A. mantelli*), Okarito brown kiwi or rowi (*A. rowi*), and tokoeka (*A. australis*; Holzapfel et al. 2008) comprise the brown kiwi clade. The brown and spotted kiwi clades diverged from one another approximately 5 million years ago (Weir et al. 2016; Fig. 2) making kiwi an ancient lineage with deep evolutionary divergence between species.

Kiwi possess a set of traits not found in other paleognaths. These include paired functional ovaries (there is typically a single functional ovary in birds and non-avian reptiles) that produce extremely large eggs (up to >25% of the weight of the female; Kinsky 1971; Sales 2005). For example, NIB kiwi eggs are approximately 400% larger than the allometrically expected value for a bird of their body size (Prinzinger and Dietz 2002). These enormous eggs have unusually high yolk content (~65% compared to 30–40% on average) and antimicrobial properties relative to other birds (Sales 2005). Kiwi have small eyes and a small optic lobe and lack color vision (Le Duc et al. 2015; Sales 2005). In contrast, they have a highly enlarged olfactory
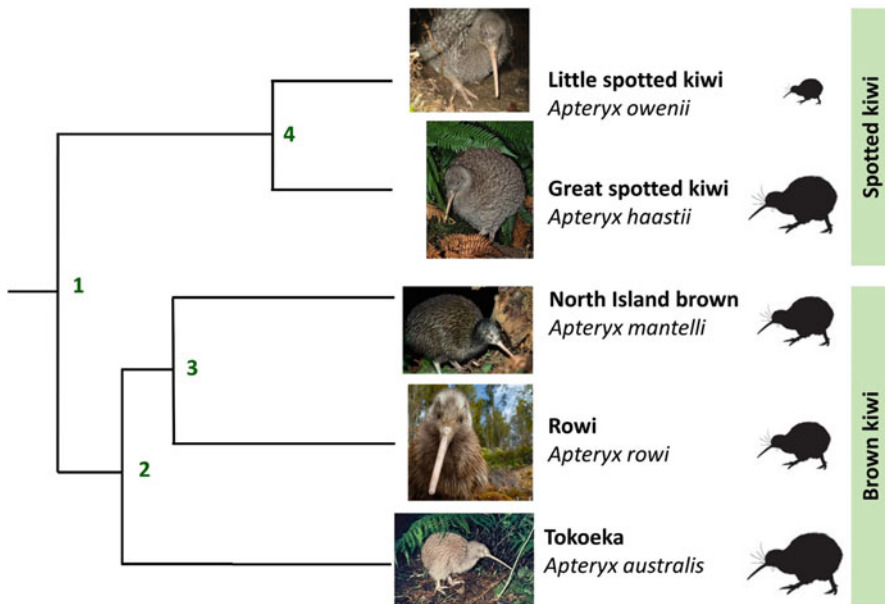

Fig. 2 Phylogeny of family Apterygidae based on Weir et al.'s (2016) analysis of 1,710 mtDNA SNPs and 1,000 nuclear SNPs. Five species of kiwi in two clades (spotted and brown) are currently recognized with all divergence events having occurred within the last 8 million years. Node dates (95% CI) are (1) 3.85 Mya (1.87–7.00), (2) 1.56 Mya (0.76–2.83), (3) 1.12 Mya (0.54–2.02), and (4) 0.55 Mya (0.27–0.99; Weir et al. 2016). Bird silhouettes are scaled to mean body weight based on data in Table 1. Photo credits: *A. owenii* Andrew Digby, *A. haastii* and *A. mantelli* Tui De Roy, *A. rowi* Grant Maslowski, and *A. australis* John Kendrick

bulb and exhibit strong olfaction; they are the only bird in the world with nostrils on the end of their bill (Castro et al. 2010; Corfield et al. 2008). The enlarged olfactory bulb results in kiwi having unusually large brain size relative to body size, on par with that observed in parrots and songbirds (Corfield et al. 2008).

Kiwi behavior differs significantly from other paleognaths as well. They are almost entirely nocturnal (Heather and Robertson 2005), a behavior that is found in less than 3% of all avian species and none of the other paleognaths (Le Duc et al. 2015; Martin 1986). They are also fossorial, meaning they are adapted to digging and nest and shelter in underground burrows (Sales 2005). Kiwi form long-term monogamous pair bonds and have an extremely long incubation period (Fowler 1991; Sales 2005). They typically lay one to two eggs per clutch which are then incubated for 65–85 days either by both parents (rowi, tokoeka, GSK) or solely by the male (LSK and NIB kiwi; Sales 2005). The eggs have thin shells and are prone to cracking and microbial invasion during their long underground incubation period (Calder 1979). Chicks are precocial – they hatch fully feathered and reach maturity within 2–5 years (Sales 2005) – and kiwi can live to more than 50 years of age (life expectancy of a territorial adult LSK is 45 years (95% CI 27–83 years; Robertson and Colbourne 2004)). Age of reproductive senescence is unknown; the oldest known LSK was at least 32 years old in 2013 and was still producing chicks annually (Ramstad et al. 2013; Taylor et al. 2017).

Finally, while ratites are the largest birds in the world (Kummrow 2014), kiwi are the smallest of the ratites (Davies 2003; Sales 2005). It has been estimated that the elephant bird could weigh in excess of 400 kg (Amadon 1947) and moa up to 300 kg (Amadon 1947; Bunce et al. 2003). In contrast, kiwi mean weight is between 1 and 4 kg, a trait that is shared with the small-bodied and flighted tinamous (Davies 2003; Sales 2005; Table 1; Fig. 1). Along with small body size, kiwi display the lowest basal metabolic rate observed in birds, low body temperature (2–4°C lower than expected of similar-sized neognathous birds), and slow growth rate (Sales 2005).

Table 1 Mean body weight of the five currently recognized kiwi species by sex (number of birds weighed)

Species	Sex	Weight (kg)	Sources
Little spotted	M (51)	1.14	Jolly and Daugherty (2002)
Apteryx owenii	F (41)	1.35	
Great spotted	M (39)	2.31	McLennan and McCann (2002)
A. haastii	F (29)	3.19	
North Island brown	M (34)	2.04	McLennan et al. (2004) and Miles et al. (1997)
A. mantelli	F (22)	2.66	
Rowi	M (49)	1.92	Tennyson et al. (2003)
A. rowi	F (51)	2.65	
Tokoeka	M (71)	2.6	Edmonds (2015)
A. australis	F (27)	3.3	

In summary, kiwi are unique among extant paleognaths in that they have larger eggs and brains relative to body size, are nocturnal and fossorial, form long-term monogamous pair bonds, and have an extremely long incubation period. In addition, they are the only extant paleognath that is both flightless and small-bodied. Their basal position in the avian phylogeny and unusual phenotypic traits make them an important taxon for studies of avian evolution.

1.2 Kiwi Are Highly Threatened and Intensively Managed

Flightlessness, ground nesting, small body size, and large egg size make kiwi highly vulnerable to predation, while their life history (long generation interval, limited dispersal ability, low reproductive rate, intense parental investment) means adult mortality has a tremendous impact on their population growth and persistence (Sæther and Bakke 2000). Four of the five species of kiwi are listed as Threatened (Vulnerable) by the International Union for Conservation of Nature (IUCN 2019); the exception is LSK which are considered Near Threatened and are genetically imperiled (Ramstad et al. 2013; Taylor et al. 2017). Numerous diseases, parasites, and pathogens threaten kiwi (Sales 2005; White et al. 2016), but their principal threat is predation by introduced mammalian predators, including stoats, rats, possums, and dogs (Germano et al. 2018). Indeed, kiwi populations are declining at a rate of approximately 2% per year with chicks having a 5–6% chance of survival in wild and unmanaged landscapes (Germano et al. 2018; Holzapfel et al. 2008; McLennan et al. 1996). While there are approximately 70,000 kiwi at present, 76% are living in unmanaged areas, and kiwi numbers continue to decline (Germano et al. 2018).

Significant efforts have been made to conserve kiwi, including extensive mammal trapping and poisoning, establishing predator-free kiwi sanctuaries, translocation programs, and the captive incubation and rearing program called Operation Nest Egg (Colbourne et al. 2005). These efforts have been spearheaded by the New Zealand Department of Conservation (DOC) and involve countless community groups, trusts, zoos, sanctuaries, and volunteers throughout New Zealand (Germano et al. 2018). Over 100 Māori iwi (tribes) are also actively involved in kiwi conservation. Māori are the indigenous people of New Zealand and consider kiwi a tāonga (treasure) for which they are kaitiaki (guardians). Obtaining a permit to work with kiwi, or collect or use archived samples, requires consultation with iwi which can be time-consuming and sensitive. Conservation efforts have been successful; where managed, kiwi populations are growing at a rate of 2% or more. The current goal of kiwi management is to reverse the 2% decrease seen in unmanaged sites to a 2% annual increase across all kiwi species and to reach 100,000 kiwi by 2030 while also restoring the former distribution of kiwi and maintaining their genetic diversity (Germano et al. 2018).

Significant funding is put toward controlling predators at the landscape scale (100,000 of hectares) to conserve kiwi and other native species. For example, Predator Free New Zealand invests $5 million NZD annually toward their goal of

ridding New Zealand of its most damaging introduced predators by 2050 (Germano et al. 2018). The DOC Battle for Our Birds campaign put $21.3 million NZD toward controlling a boom in rat and stoat numbers associated with beech forest masting in 2017. In addition, the 2018 government budget included an additional $20 million NZD for predator control (Germano et al. 2018). The beloved and iconic status of kiwi has resulted in significant governmental funds being allocated to kiwi conservation specifically as well. For example, Operation Nest Egg costs approximately $75,000 NZD annually and the New Zealand government allocated $11.2 million NZD between 2015 and 2019 and another $6.8 million NZD annually thereafter for the Save Our Iconic Kiwi program (Germano et al. 2018).

2 Review of Relevant Genomic Research

2.1 Genomic Studies of Paleognaths

Recent comparative genomic studies have transformed our understanding of paleognath and ratite evolution (Cloutier et al. 2019; Grealy et al. 2017; Mitchell et al. 2014; Sackton et al. 2018; Yonezawa et al. 2017). Prior to these studies, it was thought that ratites were monophyletic, kiwi were most closely related to moa, and ratites evolved via allopatric speciation associated with the breakup of Gondwana (Cracraft 1974). These conclusions were based on the geographic distribution of species and geological history, with continental rafting thought to be the driving force in their diversification (Cracraft 1974). Ratites were thought to have arisen from a common flightless and large-bodied ancestor that independently rafted to their current locations as Gondwana divided (Paton et al. 2002).

High-throughput sequencing was used to recover the mitochondrial genome and approximately 12,500 bp of nuclear exon sequence of the extinct elephant bird of Madagascar and for the first time showed they are sister taxa to kiwi (Grealy et al. 2017; Mitchell et al. 2014; Fig. 1). This result was contrary to expectations based on continental vicariance because Madagascar and New Zealand had never been directly connected and elephant birds and kiwi diverged approximately 50 Mya, which was well after the breakup of Gondwana. Mitchell et al. (2014) hypothesized that flighted dispersal, potentially from Antarctica which was warm and covered in trees at the time (Pross et al. 2012), gave rise to the current distribution of ratites. The presence of small, potentially volant paleognaths in New Zealand during the early Miocene and the fact that paleognaths in the early Tertiary were capable of long-distance flight support this hypothesis (Mayr 2009; Mitchell et al. 2014; Worthy et al. 2013). Following dispersal, every major ratite lineage would have lost flight independently (at least six times in total; Fig. 1). The revised paleognath phylogeny suggests also that gigantism evolved at least five times among ratites and that the large egg size to body size observed in kiwi was likely inherited from an ancestor kiwi shared with elephant birds (Mitchell et al. 2014; Yonezawa et al. 2017).

Parallel loss of flight and gigantism appear to have been associated with an explosive radiation event approximately 69–52 million years ago after the Cretaceous-Paleogene mass extinction (Grealy et al. 2017; Mitchell et al. 2014; Yonezawa et al. 2017). The extinction of dinosaurs would have provided an opportunity for large, flightless, herbivorous birds to evolve. Mitchell et al. (2014) further hypothesized that kiwi may have evolved to fill the alternative niche of a nocturnal, ground-dwelling, and small omnivore because the now extinct moa were already occupying the large herbivore niche when the ancestors of kiwi colonized New Zealand.

The most recent paleognath phylogenies are based on whole-genome datasets and challenge the typology of the previous trees based on concatenated data (Cloutier et al. 2019; Sackton et al. 2018). Cloutier et al. (2019) used over 41 million base pairs of aligned sequence data including over 20 thousand loci from three types of non-coding nuclear markers. Their tree places rheas, and not tinamous, as the sister taxa to the kiwi and emu + cassowary branch. Sackton et al. (2018) found the same result after constructing a phylogeny based on 284 thousand conserved non-exonic elements in 14 paleognath species, including 11 newly assembled genomes. The position of kiwi, however, remains unchanged. Collectively, these recent genomic studies overturned decades of morphological and genetics studies and revolutionized our view of avian evolution. Ratites have been used as an example of vicariance for decades, a scenario which is highly unlikely based on recent genomic studies, but is still in nearly all introductory biology textbooks. While there is general agreement on the order and timing of kiwi evolution, the tree typology and timing of divergence among paleognath lineages are still a matter of great debate.

2.2 Genomic Studies of Kiwi

Despite their scientific and cultural value, a great deal remains unknown about kiwi due to their secretive nature, nocturnal habits, long generation interval, and highly protected status. Four genomic studies to date have focused on understanding the evolution of kiwi specifically.

The first kiwi transcriptome was published in 2010 and based on a single NIB individual (Subramanian et al. 2010). Over 1,543 conserved protein-coding regions were identified using the chicken genome as a scaffold, and 702 genes were used to estimate the divergence time between paleognaths and neognaths. Subramanian et al. (2010) estimated that the split occurred 132 million years which was consistent with previous estimates based on mitochondrial genomes (Brown et al. 2008; Pereira and Baker 2006). A mixture of tissues from a male embryo was sequenced for this study. Thus, differential expression between tissues and sex chromosomes (females are the heterogametic sex in birds) could not be assessed, and most of the genes identified were highly constrained and associated with protein synthesis, structural proteins, and developmental gene regulation.

The first kiwi genome was published in 2015 and based on three NIB kiwi (Le Duc et al. 2015). The authors annotated this genome with the NIB transcriptome of Subramanian et al. (2010) and other well-annotated avian genomes and tested for evidence of positive selection specific to NIB kiwi in 4,152 genes orthologs among 8 bird species. They found that several opsin genes are pseudogenized in kiwi and dated the loss of color vision to 30–38 million years ago after kiwi arrived in New Zealand. They also found that kiwi have a highly diverse suite of odorant receptor genes relative to all other birds, including other ratites, reflecting their greater reliance on smell than sight. A number of genes associated with metabolism and energy expenditure also show signs of selection in NIB. The authors attributed all of these findings to the kiwi's nocturnal lifestyle.

A more recent paper presented transcriptomes of 16 individual kiwi (8 rowi and 8 LSK) and showed gene expression differences between species and sexes (Ramstad et al. 2016). The study used non-lethal sampling of the two rarest kiwi species and focused on finding variation within and between species that could be useful for conservation genomic studies. More than 7,900 protein coding transcripts were identified based on homology with chicken. Though only whole blood was sequenced, the genes identified were related to diverse functions including growth, development, disease resistance, reproduction, and behavior. Importantly, a wealth of SNP markers within these transcripts differentiate between rowi and LSK (66,909) and between individuals within these rare species (LSK, 12,384; rowi, 29,313). Many of these SNPs are likely neutral as they are positioned in untranslated regions or reflect synonymous substitutions. The study defined a suite of functional genes potentially under selection in kiwi to aid in future studies of evolution and adaptation as well.

Ramstad et al. (2016) also found significant expression differences between male and female kiwi in 150 transcripts most of which showed male-biased expression ($n = 94$) and were syntenic with the Z chromosome ($n = 79$). Given incomplete dosage compensation in birds, male-to-female expression ratios and high SNP densities found in a minority of these chrZ genes suggest a large pseudoautosomal region in kiwi sex chromosomes. This pattern was previously known in emu and ostrich (Ogawa et al. 1998; Zhou et al. 2014), suggesting a common pattern of homomorphy, recombination, and gene dosage among living paleognaths.

Also in 2016, Weir and colleagues presented a genomic analysis of 1,710 bp of mtDNA sequence and 6,332 SNPs that resolved the taxonomy and provided evidence of historical demography of kiwi (Weir et al. 2016). They confirmed the long suspected presence of four extant genetic lineages within each of tokoeka and NIB kiwi, thus bringing the total number of extant kiwi lineages to 11. Divergence between some of these lineages may rise to the level of species ($F_{ST} = 0.21$–0.63) and provides evidence of greater divergence and diversity within extant kiwi than previously appreciated. Weir et al. (2016) also identified up to six additional extinct kiwi lineages (two tokoeka, two rowi, and one or two LSK) and showed there was an intense period of speciation associated with Pleistocene glaciation that accounts for 80% of kiwi diversification events. Habit fragmentation due to glacial ice resulted in numerous isolated refugia, particularly in the South Island of New Zealand to which

ten of these lineages were restricted. Kiwi diversification rates during the Pleistocene are greater than the most explosive radiations previously documented, including Galapagos finches and Tanganyika cichlids (Weir et al. 2016).

Fully assembled and annotated genomes of rowi, GSK, and LSK have become available since the analysis we present here (Sackton et al. 2018), as well as the complete mitochondrial genome of NIB (Liu et al. 2017) and the first nuclear genome assembly of a moa (Cloutier et al. 2018). Additional paleognath genomes have become available recently as well (Sackton et al. 2018), to add to the more than 50 avian genomes previously available (Zhang et al. 2014a). Analysis of these new genome assemblies has (1) shown that loss of flight in paleognaths is due to changes in regulatory sequences and not in protein coding sequences (Sackton et al. 2018), (2) allowed reevaluation of avian sex chromosome evolution (Xu et al. 2018), and (3) uncovered an anomaly zone in paleognaths that has been a likely the source of the difficulty in resolving their phylogeny (Cloutier et al. 2019). The focus, however, has remained almost entirely on evolution and not conservation per se. Of the papers reviewed above, only two discuss the utility of their results for conservation (Ramstad et al. 2016; Weir et al. 2016), one is focused on an extinct lineage (Cloutier et al. 2018), and none have been applied to paleognath management.

In the section below, we test for positive selection in each of three kiwi species, two kiwi clades and the kiwi lineage broadly. Previous work tested for positive selection in only NIB kiwi and so was not able to distinguish whether selection was acting before or after NIB diverged from the other kiwi species. There are numerous outstanding questions for kiwi genomics, including:

1. Is there a genomic signature associated with the evolution of Apterygidae?
2. What genes were involved in the diversification of kiwi species?
3. How can genomic data help with kiwi conservation?

3   Analysis of Genes Under Selection in Kiwi

3.1   Leveraging Publicly Available Data to Study Evolution in Kiwi

We aligned protein coding sequences from the NIB kiwi genome (Le Duc et al. 2015) and rowi and LSK reference blood transcriptomes (Ramstad et al. 2016) with ortholog alignments from diverse avian species. High-quality alignments are essential to accurately infer positive selection (Jordan and Goldman 2011; Ramstad et al. 2016). We therefore utilized the 8,295 bird ortholog alignments from the Avian Genome Consortium (Zhang et al. 2014a). The initial alignments contained sequences from 48 species. To reduce computational time, we removed a subset of sequences from the overrepresented neoaves for a final data set of 25 species (Table 2). We used a previously published stringent phylogenetic annotation pipeline to identify orthologous kiwi genes and generate subsequent high-quality

Table 2 Species included in the positive selection analysis

Order	Family	Common group	Common species	Species
Passeriformes	Estrildidae	Finches	Zebra finch	*Taeniopygia guttata*
Psittaciformes	Strigopidae	Parrots	Kea	*Nestor notabilis*
Falconiformes	Falconidae	Falcons	Peregrine falcon	*Falco peregrinus*
Cariamiformes	Cariamidae	Seriemas	Red-legged seriema	*Cariama cristata*
Bucerotiformes	Bucerotidae	Hornbills	Rhinoceros hornbill	*Buceros rhinoceros*
Strigiformes	Tytonidae	Owls	Barn owl	*Tyto alba*
Accipitriformes	Accipitridae	Eagles	Bald eagle	*Haliaeetus leucocephalus*
Pelecaniformes	Pelecanidae	Pelicans	Dalmation pelican	*Pelecanus crispus*
Sphenisciformes	Spheniscidae	Penguins	Emperor penguin	*Aptenodytes forsteri*
Gaviiformes	Gaviidae	Loons	Red-throated loon	*Gavia stellata*
Phaethontiformes	Phaethontidae	Tropicbirds	White-tailed tropicbird	*Phaethon lepturus*
Gruiformes	Gruidae	Cranes	Grey crowned crane	*Balearica regulorum*
Opisthocomiformes	Opisthocomidae	Hoatzin	Hoatzin (aka Stinkbird)	*Opisthocomus hoazin*
Cuculiformes	Cuculidae	Cuckoos	Common cuckoo	*Cuculus canorus*
Columbiformes	Columbidae	Doves	Pigeon	*Columba livia*
Phoenicopteriformes	Phoenicopteridae	Flamingos	American flamingo	*Phoenicopterus ruber*
Galliformes	Phasianidae	Landfowl	Chicken	*Gallus gallus*
Anseriformes	Anatidae	Waterfowl	Pekin duck	*Anas peking*
Piciformes	Picidae	Woodpeckers	Downy woodpecker	*Dryobates pubescens*
Accipitriformes	Cathartidae	New World vultures	Turkey vulture	*Cathartes aura*
Pelecaniformes	Ardeidae	Herons	Little egret	*Egretta garzetta*
Pelecaniformes	Threskiornithidae	Ibis	Crested ibis	*Nipponia nippon*
Caprimulgiformes	Trochilidae	Hummingbirds	Anna's hummingbird	*Calypte anna*
Tinamiformes	Tinamidae	Tinamous	White-throated tinamou	*Tinamus guttatus*
Struthioniformes	Struthionidae	Ostrich	Common ostrich	*Struthio camelus*

(continued)

Table 2 (continued)

Order	Family	Common group	Common species	Species
Apterygiformes	Apterygidae	Kiwi	North Island brown kiwi	*Apteryx mantelli*
Apterygiformes	Apterygidae	Kiwi	Little spotted kiwi	*Apteryx owenii*
Apterygiformes	Apterygidae	Kiwi	Rowi	*Apteryx rowi*

Protein coding sequences were retrieved from the North Island brown kiwi genome (Le Duc et al. 2015) and reference blood transcriptomes for rowi and little spotted kiwi (Ramstad et al. 2016). Ortholog alignments for all other species listed are from the Avian Genome Consortium (Zhang et al. 2014a) available at http://avian.genomics.cn/en/jsp/database.shtml

alignments (Dunning et al. 2017). Only bird orthologs with all three kiwi species were considered, and if a kiwi ortholog was represented by more than one sequence for a species, then the longest sequences were retained for downstream analysis. Finally, the alignment procedure was repeated, with an additional TCS residue filtering (Chang et al. 2014) step so that only the highest confidence residues were retained, before Gblocks trimming and phylogeny inference (Dunning et al. 2017).

We tested for positive selection in each of the bird orthologs by optimizing several codon models (site and branch-site models) using codeml (Yang 1997). For each gene, the best-fit model was identified by comparing the corrected Akaike information criterion (AICc) of the null model which assumes no positive selection (M1a), a branch-site model that assumes a shift to relaxed selection on a defined foreground branch (model BSA), and a branch-site model that assumes a shift to positive selection on the foreground branch (BSA1). We defined several different foreground branches to test (base of kiwi, NIB, and LSK; NIB and rowi; LSK and rowi; NIB; LSK; rowi) and included a kiwi clade model to allow for bouts of recurrent selection during the diversification of this lineage. We rejected the null model if the ΔAICc score for the best fit model was more than 5.22 units less than the M1a model. A ΔAICc threshold of 5.22 is equivalent to a *P*-value threshold of 0.01 for a likelihood ratio test comparing the models using 2 degrees of freedom (df). Finally, we tested for a significant shift to positive selection if the null model could be rejected by preforming a likelihood ratio test (df = 1) between the two branch-site models, with *P*-values <0.01 considered significant. We preformed gene ontology over representation analysis using the PANTHER webserver (Mi et al. 2009) with the complete biological process set for the chicken.

3.2 Genes Under Selection in the Kiwi Lineage

Out of the 8,295 bird orthologs from the Avian Genome Consortium, 3,774 had orthologs in all 3 kiwi datasets and were used in subsequent analyses (Fig. 3a). The null model was not rejected for 3,548 of these orthologs, while 149 rejected the null
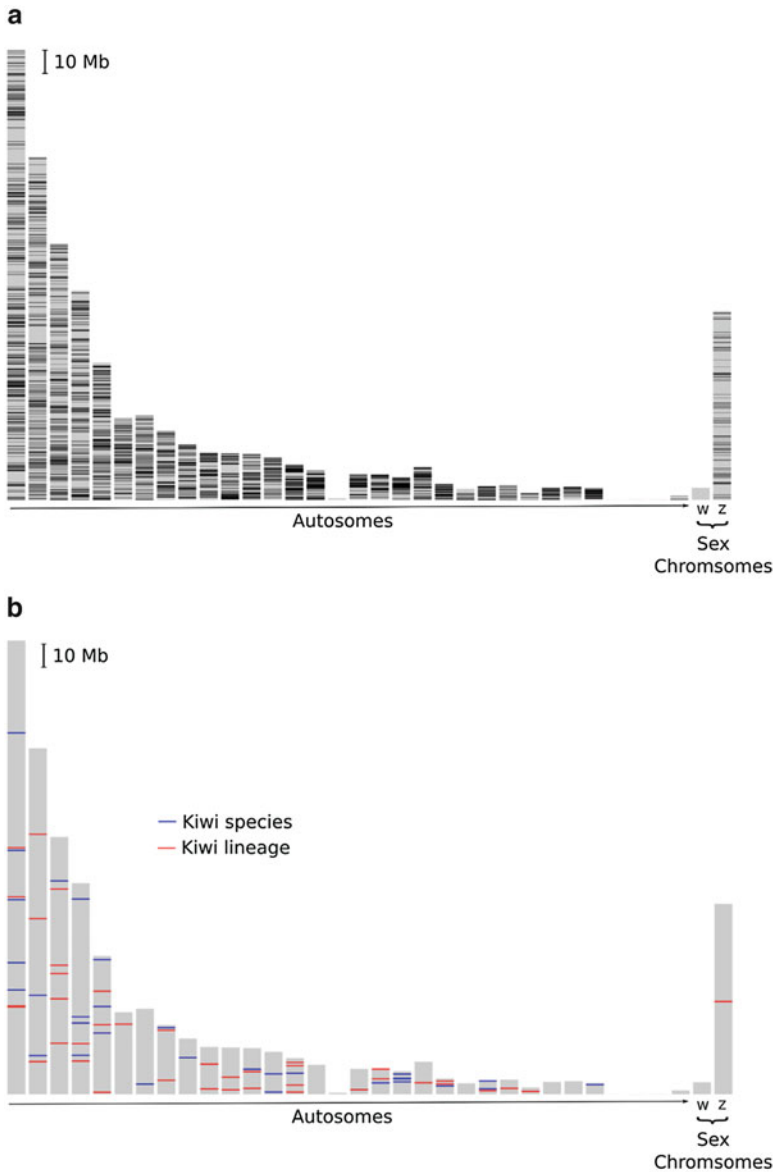
Fig. 3 Highly conserved genome synteny among birds means that we can use the well-annotated chicken genome as a reference. (**a**) Positive selection signatures were assessed at 3,774 orthologous protein coding sequences (black lines) spread throughout the genome and identified in 28 avian species that include 3 species of kiwi (NIB, rowi, and LSK) and represent 23 avian orders. (**b**) A total of 77 genes show signatures of positive selection in nearly every chromosome both among kiwi species and between kiwi and other paleognath lineages

Table 3 Number and functions of genes under positive selection in kiwi

Lineage	Number of orthologs	Overrepresented gene ontologies
Kiwi branch	30	Endochondral bone morphogenesis, inflammatory response, response to stress, neurogenesis
Kiwi clade (i.e., recurrent selection)	12	T cell proliferation, negative regulation of gene expression (epigenetic), Notch receptor processing
LSK and NIB	4	Regulation of cell morphogenesis
LSK and rowi	5	Nucleotide-excision repair, DNA damage recognition
Brown kiwi clade (NIB and rowi)	6	Response to UV, cardiac muscle tissue morphogenesis
Spotted kiwi clade (LSK)	5	Diet-induced thermogenesis, cerebral cortex development
NIB	11	Positive regulation of glycolytic process, ATP metabolic process
Rowi	4	Epithelial cell morphogenesis, activation of innate immune response

Branch-site codon models were used for 3,774 genes to identify those evolving under positive selection, and a comparison of corrected Akaike information criterion (AICc) values was used to determine the branch of the phylogeny on which this occurred

model but did not show significant positive selection. A signal of positive selection was detected in the remaining 77 genes, which are found on nearly every kiwi chromosome and spread apparently randomly throughout the genome (Fig. 3b). The majority of orthologs showing significant positive selection occurred on the branch separating kiwi from other species ($n = 42$), and 12 of these genes exhibited recurrent selection in the kiwi clade (signal present in both the lineage leading to kiwi and among kiwi species; Table 3). Positive selection was found in six genes in brown kiwi (NIB and rowi) and another five kiwi genes in LSK suggesting divergent selection between the brown and spotted kiwi clades. However, a similar number of genes showed positive selection in both LSK and rowi but not in NIB (five genes) and in both LSK and NIB but not in rowi (four genes). Another 11 and 4 genes showed positive selection exclusively within NIB and rowi, respectively.

The genes under selection are functionally diverse and include those associated with bone morphogenesis, stress and immune response, neurogenesis, metabolism, retinal development, and longevity (Table 3). Among the 42 genes showing positive selection in the branch leading to kiwi, we found several genes associated with small body size, short limbs, and skeletal abnormalities. For example, the thyroid hormone receptor (*TRIP11*) gene causes achondrogenesis type IA (ACG), an autosomal recessive disorder that can result in congenital chondrodysplasia (malformation of bones and cartilage; Parwanto 2017). Individuals with ACG are characterized by small body size, short limbs, and skeletal abnormalities. The protein encoded by the aggrecan (*ACAN*) gene is an integral part of the extracellular matrix in cartilaginous tissue. Mutations in this gene are associated with disturbed chondroskeletal development resulting in skeletal dysplasia or dwarfism (Dateki 2017).

The evolution of Apterygidae is also associated with positive selection in genes associated with stress resistance. The eukaryotic translation initiation factor (*EIF2AK1*) gene is involved in downregulating protein synthesis in response to oxidative stress, heme deficiency, osmotic shock, and heat shock (Krishna and Kumar 2018). The telomere maintenance 2 (*TELO2*, also known as *Tel2P* or *CLK2*) gene encodes a protein involved in cellular resistance to stressors that cause DNA damage, such as ionizing and ultraviolet radiation (Hurov et al. 2010). *TELO2* is also involved in telomere length regulation and, potentially, aging (Hurov et al. 2010; Lim et al. 2001). We also found signals of positive selection in genes that influence the effects of acetylcholine in the central and peripheral nervous system (cholinergic receptor, *CHMR5*; Hurov et al. 2010; Lim et al. 2001; Yamada et al. 2001) as well as genes associated with insulin regulation of metabolism (forkhead box K1, *FOXK1*; Sakaguchi et al. 2018), retinal degeneration (Bardet-Biedl syndrome 10, *BBS10*; Álvarez-Satta et al. 2017), and neurogenesis (nicastrin, *NCSTN*; Xie et al. 2014).

3.3   Genes Under Selection in Brown and Spotted Kiwi Clades

Selection signals found in six genes in the brown kiwi clade (NIB and rowi) are associated with immune response, metabolism, and neural development. The transactivator (*CIITA*) gene is essential for MHC class II gene expression. Mutations in this gene cause bare lymphocyte syndrome, where the immune system is severely compromised and cannot effectively fight infection (Steimle et al. 1993), as well as pathogenesis of several lymphomas (Steidl et al. 2011). Two of the genes identified are associated with metabolism of vitamin B (*THNSL1* B6, *ABCD4* B12) and thus normal brain development and proper functioning of nervous and immune systems. We also found evidence of positive selection among brown kiwi in the *WDR81* gene, which encodes a transmembrane protein predominantly expressed in the brain. Mutations in this gene are associated with severe progressive photoreceptor loss and the autosomal recessive Uner Tan syndrome, a degenerative disease of the nervous system (Traka et al. 2013). The *GEMIN4* and *UBE4B* (ubiquitination factor E4B) genes code for proteins involved in multiple pathological processes and several forms of cancer (Liu et al. 2012; Zhang et al. 2014b).

Signals of positive selection in the spotted kiwi clade suggest selection associated with temperature. The genes under selection in spotted kiwi were the mono-carboxylate transporter *SLC16A3* gene, the *TTF2* transcription termination factor gene, the *TRPV1* (transient receptor potential cation channel) gene, and the *NIPAL3* and *TACC3* genes. *TRPV1* is also known as the capsaicin receptor gene and is involved in detecting scalding heat and pain and regulating body temperature (Xu et al. 2007). Also, one of the gene ontology terms overrepresented among these genes was diet-induced thermogenesis or the amount of energy expended above the basal metabolic rate due to the cost of processing food. The two spotted kiwi species inhabit locales with very different temperature regimes. Little spotted

kiwi have historically been restricted to lowland areas. In contrast, GSK are the only kiwi that occupy elevations up to 1,500 m above sea level and can survive in areas where soils can be frozen or covered in snow for days (McLennan and McCann 2002). Thus, our data suggests that selection for coping with highly divergent temperature regimes may be a defining feature of the spotted kiwi clade. This hypothesis should be tested in the future by broadly comparing LSK and GSK diversity at the *TRPV1* and other candidate genes associated with thermal tolerance.

3.4   What Makes a Kiwi a Kiwi?

There is surely a great deal of important functional diversity that we did not detect in our tests as we only looked at a subset of protein coding genes. In particular, the use of blood samples limits us to genes expressed in this tissue; we could not test for selection in the opsin genes, for example. Finally, our data cannot differentiate between historical and recent selection and certainly cannot predict what genes will be important in the future. These data do, however, provide insight into the genomic signature associated with the evolution of Apterygidae and indicate candidate genes that could now be surveyed more broadly in kiwi to improve our understanding of kiwi evolution and management.

Recent genomic studies inform the ongoing debate of why kiwi have such a small body size relative to other ratites and such large eggs relative to their body size. The phyletic dwarfing hypothesis explained this pattern as being due to the kiwi ancestor having a large body size with a proportionately large egg and kiwi experiencing a subsequent reduction in body size, but not egg size, after arrival in New Zealand (Calder 1979; Cracraft 1974; Gould 1986). This made sense when kiwi were thought to be most closely related to moa or emu and cassowary (large-bodied ratites with proportionate eggs) and to have attained their present distribution via vicariance. The alternative view was that regardless of how kiwi arrived in New Zealand, they were small-bodied ancestrally and then evolved the large egg, presumably because there was an advantage to producing highly precocial young to avoid avian predators in New Zealand (e.g., aerial raptors, including the Haast eagle, and terrestrial *Aptornis* or adzebills, Worthy et al. 2013).

Genomic results suggest both hypotheses may be correct. These data show that kiwi's closest relatives are elephant birds (Mitchell et al. 2014) which, like kiwi, have disproportionately large egg size relative body size (Dickison 2007). Also, our data show positive selection among kiwi in genes associated with skeletal morphology and dwarfism. Together, these findings suggest that kiwi have disproportionately large eggs because they inherited the allometry from a common ancestor shared with elephant birds, but also experienced changes in body size after dispersal to New Zealand. Predation could have maintained a large egg size (precocial chicks) while also promoting small body size (to allow the use of burrows for protection). Competition with moa would likely also have promoted small body size to reduce niche overlap as suggested by Mitchell et al. (2014).

4   Conservation Impacts and Research Needed

4.1   Can Genomics Enhance Conservation of Kiwi?

Kiwi exemplify a number of genomic difficulties often encountered when working with small populations. For example, they have experienced significant genetic bottleneck effects (Miller et al. 2011; Ramstad et al. 2010, 2013), hybridization (Ramstad, unpublished data), and extreme inbreeding (Taylor et al. 2017) and exhibit patterns of isolation by distance (White et al. 2018), sex-biased dispersal, and strong social organization (Ramstad, unpublished data). Below we describe how genomics can be applied to some of these issues in conservation of kiwi and small populations more broadly.

Genetic Population Structure and Connectivity   In small populations, drift over-whelms gene flow and selection in defining the genetic population structure of the species. Genomic data can provide more precise estimates of divergence between populations (F_{ST}) than limited genetic data from a handful of markers (typically microsatellites) and identify populations that have previously gone undetected (Luikart et al. 2019; Oyler-McCance et al. 2019). In kiwi, numerous small and extant subpopulations have recently been identified, some that almost certainly represent new species, and will require specific management in the future (Weir et al. 2016; White et al. 2018).

In addition, fine-scale genetic structuring is possible within populations (Beck et al. 2008; Kanno et al. 2011). Such structure can be sex, age, or stage biased and may indicate important social or geographic barriers to gene flow. There is now evidence of fine-scale genetic structure in the last remnant population of the rarest kiwi species, rowi, which exhibits sex-biased dispersal and isolation by distance within their 14 km spatial range (Ramstad, unpublished data). Genomic research will help refine these findings, allow us to look for similar patterns in other kiwi species, and significantly enhance our understanding of what population units and dispersal behaviors are important to conserve in kiwi.

Genetic Bottleneck Effects and $\mathbf{N_e}$ Effective population size (N_e) is a critical parameter for understanding the magnitude and effects of drift, and therefore the rate of genetic erosion, in small populations. Many populations with large census size are functionally small from a conservation genomics perspective due to high reproductive skew, overlapping generations, differences in timing of reproduction between sexes, or an isolation by distance pattern of genetic structure (Neel et al. 2013; Waples et al. 2013, 2014). Genomics can be used in combination with advances in theory and statistical techniques, such as coalescent models and approximate Bayesian computation, to provide reliable estimates of current and historical N_e and changes in population size (Salmona et al. 2019). For example, Nunziata et al. (2017) used RADseq data to estimate changes in population size in two salamander species (*Ambystoma* spp.) and found a pattern that agreed with 37 years of population size estimates based on mark-recapture data.

Likewise, genomic data and coalescent-based demographic models can be used to estimate the timing and magnitude of genetic bottleneck effects. In kiwi, genomic data resolved a significant genetic bottleneck effect and subsequent explosive diversification associated with glaciation (Weir et al. 2016). Microsatellite data suggest that genetic bottleneck effects and low N_e are common and sometimes recent in kiwi (Ramstad, unpublished data; Ramstad et al. 2013; Taylor 2014). Genomic techniques would improve estimates of the timing and magnitude of bottlenecks, as well as provide more robust estimates of current N_e for many small populations of kiwi. For example, the ratio between N_e and census size (N_C) of Kapiti Island LSK was found to be exceptionally low for terrestrial vertebrates (0.03 using 15 microsatellite loci) and suggests that genetic diversity might still be eroding in this population, despite its large census size (Ramstad et al. 2013). The accuracy of this estimate is unclear, however; it may be downwardly biased due to residual gametic disequilibrium from the Kapiti Island founding event, overlapping generations, population subdivision, social structure, or simply an explosive increase in N_C but not N_e in this closed population (Ramstad et al. 2013).

The Inbreeding Effect of Small Population Size When populations shrink, the likelihood of inbreeding, or mating with a close relative, increases (Crow and Kimura 1970). Many small populations would benefit from estimates of inbreeding coefficients (F) to aid in selecting unrelated founders for translocation and mating pairs for captive breeding programs. F is typically estimated from a pedigree, but these are notoriously difficult to construct (Taylor et al. 2015), especially in small populations that have low genetic diversity. Unless all individuals in a population are sampled over many generations, then estimates of F based on pedigrees (F_P) are often highly imprecise and downwardly biased (Taylor et al. 2015). Many populations cannot be sampled to near completion, making it essentially impossible to produce a robust pedigree or precise and unbiased estimates of inbreeding coefficients for small populations in the wild. The advent of genomics makes it possible to precisely measure F of any individual without a pedigree (Kardos et al. 2016). F can be estimated for both unmapped (using the diagonal elements of a genomic relatedness matrix) and mapped (the proportion of the genome that includes runs of homozygosity) loci (Kardos et al. 2016). The latter can measure F virtually without error because an individual can reliably be scored as heterozygous or homozygous at nearly every position in the genome (Kardos et al. 2016). Indeed, marker-based estimates predict F better than F_P in recently bottlenecked and partially isolated small populations ($N_e = 75$, Kardos et al. 2015). In some cases, F estimated with as few as 1,000 SNPs was more closely correlated with multilocus heterozygosity than F_P estimated from a 20-generation pedigree (Kardos et al. 2015).

While strong inbreeding is known to occur in some kiwi populations (Taylor et al. 2017), no pedigrees have been built to date for any naturally occurring kiwi population. A pedigree based on 30 microsatellite loci is underway for rowi (Ramstad, unpublished data) and is possible only because nearly the entire extant population has been genotyped (Taylor 2014, 2015). Rowi are actively managed through captive incubation and translocation of founders to predator-free

sanctuaries. Reliable estimates of inbreeding coefficients and relatedness among birds will help managers choose founders for these new populations and avoid inbreeding in rowi. More precise estimates of F based on runs of homozygosity would make it easier to detect inbreeding depression and to identify loci making a large contribution to inbreeding depression. This technique will no doubt become commonplace in conservation genomics once genome resequencing is occurring more routinely.

Mating Systems and Social Organization Kiwi are thought to be monogamous, but a robust test of this assumption is lacking (but see Ziesemann 2011), and extra pair fertilization has been detected in many other bird species that were thought to be monogamous (Wink and Dyrcz 1999). Very little is known of natal dispersal in kiwi, how territories and pairs are established, and the potential for kin recognition. There is, however, clearly variation among kiwi in their mating and social systems. For example, LSK and NIB kiwi live solely in pairs, while small groups of tokoeka often occupy and fiercely defend a single territory. Robust genomic testing of relatedness among individual kiwi relative to their spatial distribution and behavior could elucidate the social and mating systems of the individual species. This information is critical for establishing new populations via translocation, selecting individuals for captive incubation and rearing programs, and effectively reintroducing captive-reared juveniles to the populations they were collected from as eggs.

Hybridization and Outbreeding Depression Another potential threat to kiwi is hybridization, which can result in genomic extinction of species that have been reduced to one or few small populations. In some cases, however, hybridization is an important source of genetic diversity for what would otherwise be genetically depauperate species (Caniglia et al. 2018; Pimm et al. 2006). Genomic sequencing has greater power to differentiate between hybridization and incomplete lineage sorting than traditional genetic approaches (vonHoldt et al. 2018). For example, it is very difficult to determine the taxonomy of "species" with 0.5–2% genomic divergence (Roux et al. 2016). However, genomic data can provide a robust estimate of the fraction of the genome derived from admixture or hybridization between closely related species and the specific genes that have been introgressed (Wayne and Shaffer 2016).

Among kiwi, hybrids have been detected between GSK, LSK, and rowi based on mtDNA and nuclear DNA (Ramstad, unpublished data). To date, four F1 LSK x rowi hybrids have been found in the last remaining remnant population of rowi. As a precautionary measure, the hybrids were taken from Okarito sanctuary to an isolated kiwi-free island by the New Zealand Department of Conservation. The hybrids subsequently produced chicks, confirming that F1 kiwi hybrids are able to survive and successfully reproduce.

The historical frequency of hybridization among kiwi is unknown, and so it is unclear if current levels of hybridization are elevated due to anthropomorphic disturbance and small population size. The ~5 million years of divergence between the brown (rowi) and spotted (LSK) kiwi clades suggests that hybridization could

result in outbreeding depression (Allendorf et al. 2001; Frankham et al. 2011). Likewise, if hybridization were allowed to continue in Okarito sanctuary, the world's last remnant population of rowi could be lost to genomic extinction (Allendorf et al. 2001). However, kiwi hybrids have conservation value because they are the only extant source of otherwise extinct alleles (Allendorf et al. 2001; Chan et al. 2019; Frankham et al. 2011) and may aid in genetic rescue of highly inbred populations and improve our understanding of hybridization dynamics in kiwi.

Adaptive and Functional Diversity   Long-term persistence of populations relies on their having significant reservoirs of additive genetic variance (Jamieson and Allendorf 2012). This genetic diversity allows populations to survive challenges that impact their survival and reproduction, such as disease. Comparative genomic studies are elucidating signals of adaptive and functional diversity between taxa at various phylogenetic levels (Kober and Pogson 2017) and even within extinct species (Feigin et al. 2018). For example, a full genome sequence was obtained from a preserved specimen of Tasmanian tiger (*Thylacinus cynocephalus*) and used to place the species in a phylogeny of carnivorous marsupials and examine the genomic basis of its phenotypic convergence with other canids (Feigin et al. 2018).

It is not uncommon for populations that cannot be differentiated at neutral genetic loci to show adaptive divergent at functional loci due to local adaptation. For example, cryptic population structure was found in Atlantic cod (*Gadus morhua*; Berg et al. 2016) and sea scallop (*Placopecten magellanicus*; Van Wyngaarden et al. 2017) via genomic analysis of loci under selection. Indeed, significant adaptive genetic variation may not be protected if management units are based on overall or neutral genetic population structure. All that is currently known of potential adaptive genetic diversity in kiwi is outlined in this paper. Robust, hypothesis-driven studies of adaptive variation have the potential to greatly improve our understanding and management of kiwi.

4.2   *Challenges and Opportunities*

Genomics is a young field and there are still numerous issues to be resolved in its application to species conservation. With the large number of markers now being tested simultaneously, it is unclear how best to correct for multiple tests and false positives are a concern (Johnson et al. 2010; Kober and Pogson 2017). It can also be difficult to assess independence among loci unless markers can be mapped to a reference genome. It remains important to detect outlier loci and deal with them separately based on the focal questions. Also, it is often difficult to assign meaning to or understand the effects of genes that show evidence of positive selection. Traditional statistical analyses can be challenging or useless with genomic datasets comprised of SNPs (e.g., tests for genetic bottleneck effects that rely on highly polymorphic loci; Cornuet and Luikart 1996; Luikart et al. 1998). However, new

statistical tests are being developed and the rich body of population genetics theory remains foundational. Effectively conveying the value of these techniques to the public broadly, including relevant findings and limitations, will also be a challenge.

Regardless of the difficulties listed above, genomic techniques are the new gold standard in conservation and should be taken up immediately to improve management of imperiled taxa. Critically small populations that are actively managed or manipulated should have a genomic management plan that it based on proper taxonomy and tracks individuals. Such plans help managers maintain genomic diversity via effective selection of founders for new populations and captive breeding programs, track reproductive success of and relatedness among individuals, monitor levels of inbreeding, and respond to demographic crises (e.g., disease outbreaks). Such a genomic management plan is urgently needed for kiwi. Five kiwi species are currently recognized but there could be as many as 11 (Weir et al. 2016; White et al. 2018). Understanding kiwi social structure will allow managers to maximize chick and sub-adult survival by defining windows for their capture and release. Identifying important functional variation among kiwi help will allow managers to weigh the costs and likelihood of outbreeding versus inbreeding depression and thus assess the conservation value of hybrids. A central repository for kiwi genomic data and samples would vastly improve monitoring, translocation, and captive incubation programs for this ancient avian lineage.

The shift from genetics to genomics will be a challenge for researchers and managers. However, reduced representation techniques, such as Rapture and RADcap, are excellent starting points for wading into the field. These techniques allow for discovery and reliable capture of thousands of SNPs in hundreds of individuals simultaneously and can be applied to numerous conservation questions. They are also relatively fast, straightforward, and cost-effective techniques and are being employed with increasing frequency to non-model species (Ali et al. 2016; Hoffberg et al. 2016). More genomic resources are forthcoming, including whole-genome sequences, transcriptome assemblies, new molecular techniques, and new analytical tools, and will provide a foundation for an unprecedented amount of highly powerful research. The challenge now is to bring the full weight of innovative genomics to the management and conservation of at-risk species broadly.

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