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RESEARCH ARTICLE

Dispersal and speciation in purple swamphens (Rallidae: *Porphyrio*)

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ABSTRACT

Dispersal, when accompanied by reduced gene flow and natural selection, influences speciation rates among groups of organisms. We used molecular phylogenetics, divergence time estimates, and population genetics to reconstruct the mode, pattern, and tempo of diversification within the wide-ranging purple swamphens (genus *Porphyrio*), with emphasis on the "supertramp" *P. porphyrio*. Our results suggest that the *Porphyrio* clade arose during the Middle Miocene in Africa, with a single colonization in the Americas and several other colonizations in Southeast Asia and the Indo-Pacific around 10 mya. We found that the widespread *P. porphyrio* is not monophyletic. Indeed, several subspecies and subspecies groups may represent species-level lineages. The *P. p. melanotus* lineage probably reached Australasia during the Pleistocene (600 kya), although some islands were colonized only in the past few hundred years. New Zealand, and some other islands, had previously been colonized (~2.5 mya) by flying *Porphyrio* that evolved into flightless endemic species. Early and recent lineages are now sympatric. Widespread occupation of oceanic islands implies high dispersal and colonization rates, but gene flow probably occurs episodically and follows varying routes at different times. This pattern of colonization enables populations to differentiate and, ultimately, speciate.

Keywords: biogeography, dispersal, phylogeny, speciation

Dispersión y especiación en las gallinas de agua (Rallidae: *Porphyrio*)

RESUMEN

Dispersión, cuando es acompañada de reducción en el flujo de genes y la selección natural, influye en las tasas de especiación entre los grupos de organismos. Aquí, usamos filogenética molecular, estimaciones de tiempo de divergencia y genética de poblaciones para reconstruir el modo, patrón y tiempo de diversificación en las gallinas de agua (género *Porphyrio*) con énfasis en el super colonizador *Porphyrio porphyrio*. Nuestros resultados sugieren que el clado *Porphyrio* surgió en Africa durante el Mioceno Medio, con una única colonización en las Américas y varias otras colonizaciones en el sudeste de Asia y el Indo-Pacífico alrededor de 10 mya. La ampliamente distribuida *Porphyrio porphyrio* no es monofilética. De hecho, varias subespecies y grupos de subespecies pueden representar linajes a nivel de especie. El linaje *P. p. melanotus* probablemente llegó a Australasia durante el Pleistoceno (600 kya), aunque algunas islas fueron colonizadas sólo en los últimos cientos de años. Nueva Zelanda, y algunas otras islas, previamente habían sido colonizadas (~ 2.5 mya) por un *Porphyrio* volador que evolucionó hasta convertirse en especies endémicas no voladoras. Linajes tempranos y recientes son ahora simpátricas. La amplia presencia en las islas oceánicas implica altas tasas de dispersión y colonización, pero el flujo de genes probablemente se ha producido en forma episódica y siguiendo diferentes rutas en diferentes momentos. Este patrón de colonización permite a las poblaciones llegar a diferenciarse y en última instancia dar lugar a nuevas especies.

Palabras clave: biogeografía, dispersión, especiación, filogenia

INTRODUCTION

The ability to disperse and colonize new habitats provides organisms with ecological opportunities to harvest novel resources and establish new populations. Vagrant species that reach, via long-distance dispersal, new regions or isolated islands that are thousands of kilometers from their traditional breeding range may generate new flocks of dispersers before being totally or partially displaced by more efficient competitors (Diamond 1974, 1975). This "supertramp" strategy can also give rise to numerous, phenotypically distinct variants, which may lead to speciation (Simpson 1953, Diamond 1974, Diamond et al. 1976, Grant 1986, Whittaker 1998, Crisp et al. 2011). However, for a single species to persist, without differentiation, over a large breeding range of fragmented habitat, individuals must move between habitat patches at a rate sufficient to counter the evolutionary effects of isolation. This requirement predicts that such widespread species have a high level of gene flow among populations

distributed across several landscapes. Alternatively, relatively small, isolated populations can segregate and accumulate phenotypic differences if range expansion is not constant through time or if the direction of dispersal changes. Outcomes may vary, depending on the number of independent radiations, differences in diversification rates, rate and pattern of gene flow, and rapidity of species radiation following a wave of dispersal (Mayr and Diamond 2001, Moyle et al. 2009, Cibois et al. 2011).

Inferring the geographic origin and temporal diversification of organisms is an essential part of biogeography and depends on an accurate estimate of evolutionary relationships among species (Rosen 1978, Filardi and Moyle 2005). In the case of birds, enabled by flight to disperse long distances, the spatiotemporal patterns of diversification can be challenging to analyze, especially when shallow radiations on islands generate differential morphological traits that obscure evolutionary affinities (Filardi and Moyle 2005, Irestedt et al. 2013). Dispersal and adaptation together are important drivers of insular diversification of many bird groups (Pratt 2005, Grant and Grant 2008) and account for much of the diversity that we find today in archipelagos (Trewick and Gibb 2010, Trewick 2011). Colonization of islands sometimes involves loss of the capability for further long-distance dispersal, when flight is not integral to foraging, social interaction, or predator avoidance (McNab 1994, McNab and Ellis 2006, Steadman 2006). Reduction, and even loss, of flight capacity can be an adaptive response to island life, and some speciation may occur with adaptation of flightlessness, as a result of altered selective environments (Milá et al. 2010, Sly et al. 2011, Alonso et al. 2012, Runemark et al. 2012).

Family Rallidae (Aves: Gruiformes) is diverse and cosmopolitan. It includes common species that are good dispersers, as well as regional and island endemics. Many oceanic islands that were naturally without terrestrial mammal predators appear to have favored reversion to a terrestrial lifestyle after colonization and speciation by flying ancestors (Ripley 1977, Steadman 2006). This combination of high dispersal and high endemicity associated with the loss of flight makes them interesting subjects for evolutionary analysis. In particular, the large, flamboyant purple swamphens (genus *Porphyrio*) demonstrate extraordinary dispersal capabilities, with evidence of multiple invasions, apparently spaced out in time, that resulted in divergences of size, color, and other traits (Ripley 1977, Remsen and Parker 1990, Trewick 1996). Seven species of purple swamphens are currently recognized, 4 of which are or were present in the Oceania region (Trewick 1996, Taylor 1998). Principal among these is the widespread "supertramp" Purple Swamphen (*Porphyrio porphyrio*), which occurs from Africa and the Mediterranean east to the Pacific (Ripley 1977). This taxon comprises apparently parapatric morphological variants that have, at times, been classified into ~13 subspecies or species (Figure 1A; Ripley 1977, Taylor 1998). Although sometimes considered a reluctant flier (Craig 1977, Craig and Jamieson 1990), this taxon has nevertheless established populations on many oceanic islands, throughout the Indian and western Pacific Ocean (Mayr 1949, Ripley 1977, Trewick 1997, 2011). At least 2 colonizations of New Zealand resulted in the presence of the North Island Takahe (*P. mantelli*) and South Island Takahe (*P. hochstetteri*), endemic flightless herbivores that were sympatric with flying swamphens (Trewick 1997, Trewick and Worthy 2001). Some island populations and subspecies are known only from fossils that reveal the numerous extinctions that followed colonization of those islands by people (Steadman 1995, 2006, Steadman et al. 1999). Insular endemics have been recognized as distinct species on New Caledonia and New Zealand (Balouet and Olson 1989, Trewick and Worthy 2001) and on other Pacific islands (Steadman 1988, Kirchman and Steadman 2006).

We used multilocus DNA sequence data to generate a dated phylogenetic hypothesis of relationships within *Porphyrio* and to explore the pattern of gene flow among populations of *P. porphyrio*. We address the following questions to gain insights into the biogeographic origin and diversification of these birds: (1) What is the phylogenetic structure of the genus? (2) What time of diversification, and pattern of dispersal and colonization, explains current diversity? (3) Is there support for a single or multiple range expansions? (4) Are regional subspecies of *P. porphyrio* monophyletic, or is there a mismatch between clade structure and taxonomy?

METHODS

Sampling

To obtain DNA for analysis, we sampled bones, toe pads, feathers, blood, and muscle tissue from specimens of the 7 known species in the genus *Porphyrio* (Figure 1B), including representatives of *P. porphyrio* subspecies from Africa, Europe, Asia, and Pacific islands (Table 1 and Figure 1A). Additionally, we sampled several populations in New Zealand and Australia separated by ~1,500 km of sea and graded terrestrial landscapes to explore gene flow at different spatial scales.

DNA Extraction

DNA extractions from bones and toe pads were carried out in a dedicated ancient DNA (aDNA) laboratory (Ecology Group, Massey University, Palmerston North, New Zealand; see DNA Toolkit at http://evolves.massey.ac.nz). DNA extractions from toe pad samples were performed using the QiAMP DNA Minikit (Qiagen, Valencia, California, USA), following the manufacturer's

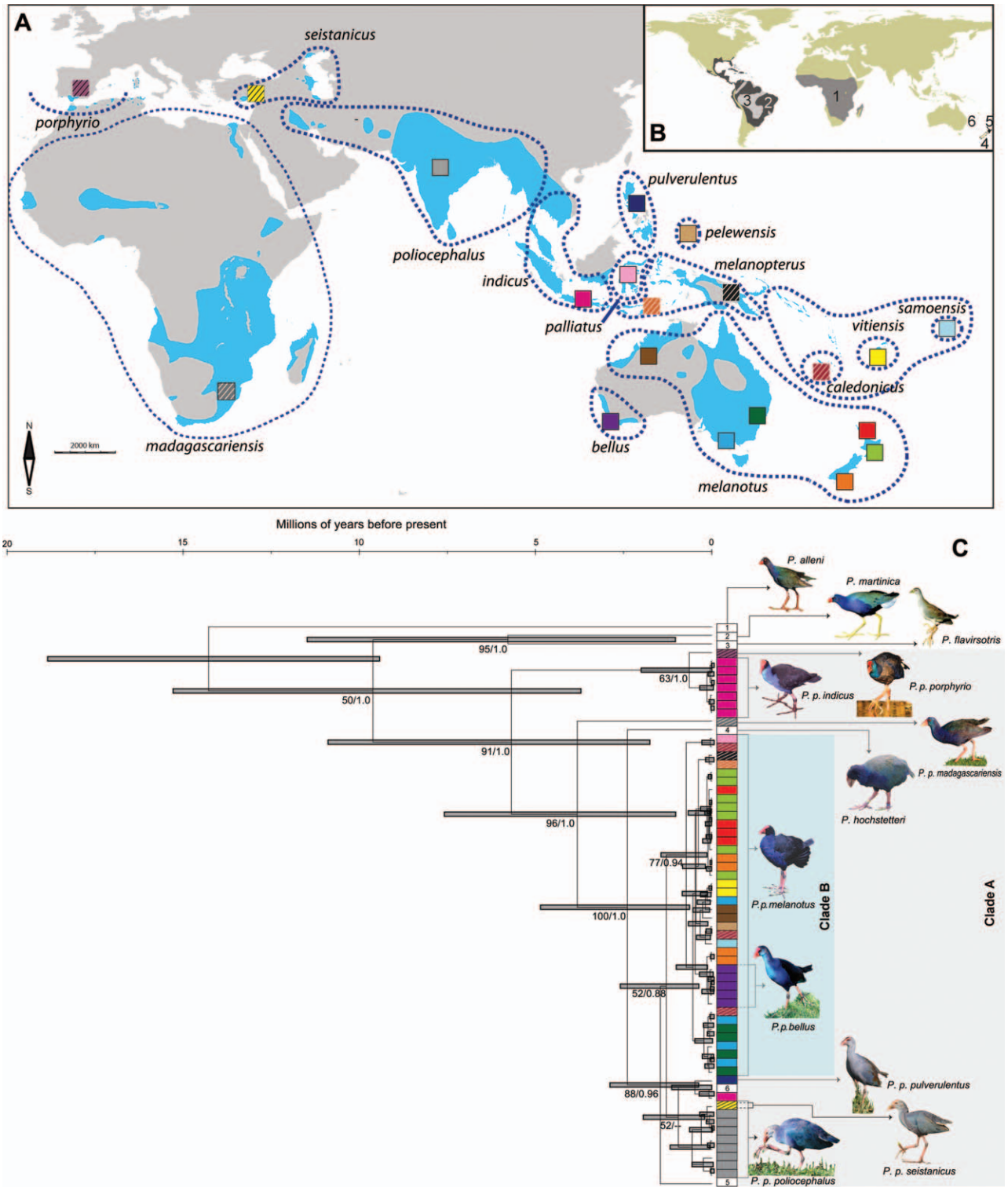

FIGURE 1. Geographic distribution, phylogenetic relationships, and time of divergence of purple swamphens. (**A**) Distributions and sampling localities of the *Porphyrio porphyrio* subspecies included in our study (source: IUCN). Subspecies *caledonicus* and *vitiensis* are included within *samoensis* (following Ripley 1997). (**B**) Geographic distributions of the other 6 *Porphyrio* species around the world: 1 = *P. alleni* (Africa), 2 = *P. martinica* (North and South America), 3 = *P. flavirostris* (South America), 4 = *P. hochstetteri* (South Island, New Zealand), 5 = *P. mantelli* (North Island, New Zealand), and 6 = *P. albus* (Lord Howe Island, Australia). (**C**) Chronogram based on analysis of concatenated sequences of mitochondrial and nuclear genes using a relaxed-clock Bayesian analysis in BEAST. Age constraints were based on estimates by Garcia-R. et al. (2014) of the basal divergence of *Porphyrio* and the split between

instructions and standard procedures for aDNA (Cooper and Poinar 2000, Rohland and Hofreiter 2007). DNA from bones was extracted using decalcification with EDTA and proteinase K digestion in Tris-buffered saline, followed by purification with phenol–chloroform. DNA from fresh tissues was extracted in a laboratory geographically separated from the aDNA laboratory, using either Tissue DNeasy kit (Qiagen; following the manufacturer's instructions) or incubation at 55°C with proteinase K and a CTAB buffer (2% Hexadecyl trimethyl ammonium bromide, 100 mM Tris–HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA), followed by a combined phenol–chloroform–isoamyl alcohol (25:24:1) cleanup.

Mitochondrial and Nuclear DNA Amplification

We sequenced 2 mitochondrial genes and 1 nuclear gene for population genetic analyses of *P. porphyrio* in Australia and New Zealand: mitochondrial control region (CR) and cytochrome oxidase *b* (cyt *b*), plus a fragment of the nuclear beta-fibrinogen intron 7 (BFG-7). For phylogenetic analysis of *Porphyrio*, parts of 2 additional mitochondrial genes (ribosomal RNA 12S and 16S) and 1 nuclear gene (recombination activating gene 1 [RAG-1]) were amplified from representative specimens of the currently recognized species and subspecies of *P. porphyrio*. Additional sequences were downloaded from GenBank (Table 4 in Appendix A). Standard polymerase chain reaction (PCR) methods (using the primers listed in Table 5 in Appendix A) were used for amplification of nuclear and mitochondrial fragments. Amplification products were purified with QIAquick PCR cleanup kit (Qiagen) or ExoI/SAP digest. For each PCR product, both strands were sequenced using Big Dye Terminator version 3.1 reagents and an ABI 3730XL automated DNA sequencer (Applied Biosystems, Foster City, California, USA). Short sequences without GenBank accession numbers are provided in Appendix B.

Phylogeny and Divergence Times

All sequences were edited, assembled, and aligned using Geneious version 6.0.5 (Drummond et al. 2012a) and checked by eye. Alignments of ribosomal genes, CR, and BFG-7 were conducted using Gblocks version 0.91b (Castresana 2000) and evaluated by eye. Cytochrome oxidase *b* and RAG-1 were checked for the presence of indels and stop codons. Prior to concatenated analyses, we performed individual gene tree analysis to detect spurious branch-length patterns and evidence of significant incongruence. We built a supermatrix with a six-way partition by gene: 12S, 16S, CR, cyt *b*, BFG-7, and RAG-1 (Wiens 2006, Holland et al. 2007, Wiens and Moen 2008, Johnson et al. 2012). Maximum likelihood (ML) trees were implemented in RAxML version 8.0.24 via the CIPRES portal (Miller et al. 2010). We used a general time-reversible model with gamma distribution (GTR + Γ), which allowed RAxML to halt bootstrap resampling automatically (bootstopping) once split support values converged (Pattengale et al. 2010). We conducted Bayesian phylogenetic analyses using MrBayes version 3.2.2 as implemented in the CIPRES portal under a GTR + Γ+ I model of evolution. The model was estimated in ModelTest version 3.7, using Akaike's Information Criterion (Posada and Crandall 1998). After performing shorter test runs, we conducted 3 parallel runs of the Metropolis Coupled Markov Chain Monte Carlo (MCMCMC) algorithm for 5 million generations each, sampling 1 tree with associated parameter values per 5,000 generations, and employing 3 heated chains and 1 cold chain. Convergence and diagnostics of the Markov process were visualized using Tracer version 1.6 (see Acknowledgments). The first half million generations (10%) were discarded as burn-in. A burn-in of 10% gave optimal results, and we obtained effective sample sizes (ESS) >200 for 95% of the parameters. The ML and Bayesian trees were viewed using FigTree version 1.4.2 (see Acknowledgments) and SplitsTree version 4.12.8 (Huson and Bryant 2006). *Amaurornis flavirostra* was used as an outgroup to root the tree.

Divergence times among lineages of *Porphyrio* were estimated using a relaxed Bayesian clock implemented in BEAST version 1.7.5 (Drummond et al. 2012b). For calibration constraints, we used the basal divergence estimate of *Porphyrio* with a normal distribution of 11–20 Ma (95% range) and the basal split of *Amaurornis flavirostra* and *Porphyrio* with a normal distribution of 27–35 Ma (95% range), as previously calculated from an analysis using a widely sampled dataset of mitochondrial and nuclear genes (Garcia-R. et al. 2014). We combined the results of 3 independent runs of 30 million generations to ensure ESS scores >200 for 95% of the parameters in each run. Chains were sampled every 4,000 generations, and a burn-in of 10% (3 million generations) was used. The tree, with times of divergence and highest posterior density (HPD) intervals, was visualized using FigTree.

Population Differentiation and Demographic History

Population-level analyses were carried out with 2 datasets: (1) concatenated mitochondrial loci CR and cyt *b*; and (2) the autosomal locus BFG-7. Sequence ambiguities at heterozygous sites in BFG-7 that indicated separate alleles

←
Porphyrio and *Amaurornis* in a normal distribution (see text). Gray bars show estimated time of divergence and 95% HPD intervals of node ages. Support values for key clades are indicated below branches and correspond to bootstrap supports (>50%) and posterior probabilities (>0.80), respectively.

TABLE 1. Taxa, museum voucher numbers, locality, type of tissue, and GenBank accession numbers of data included in our study. An asterisk indicates sequences <200 bp (see Appendix B).

Species [a]	Subspecies [a]	Museum voucher [b, c]	Locality [d]	Tissue type	GenBank accession number					
					Mitochondrial genes				Nuclear genes	
					12S	16S	Cyt *b*	CR	BFG-7	RAG-1
Porphyrio alleni		UWBM 86785	Captive	Muscle	KJ685955			KJ686052		
P. flavirostris		USNM 623070	Guyana	Muscle	KJ685950	KJ685965	KJ686071		KJ685996	KJ686117
P. mantelli		NMNZ DM7930	New Zealand	Bone			*			
P. martinica		AMNH DOT7585	USA	Muscle	KJ685956			KJ686053		
P. albus		NMW 50.761	Lord Howe Island	Toe pad			KJ686084			
P. porphyrio	*bellus*	WAM 36186	WA, Australia	Muscle	KJ685953	KJ685960	KJ686067	KJ686045	KJ685995	KJ686118
		WAM 34492	WA, Australia	Muscle			KJ686082	KJ686031	KJ685968	
		WAM 27444	WA, Australia	Muscle			KJ686064	KJ686030	KJ685987	
		ANWC 50436	WA, Australia	Muscle			KJ686085	KJ686035	KJ686003	
		ANWC 31914	WA, Australia	Muscle			KJ686079	KJ686042	KJ685984	
	caledonicus	None	New Caledonia	Feather	*		KJ686108	KJ686010	KJ685974	KJ686124
		None	New Caledonia	Feather			KJ686106			
		None	New Caledonia	Feather				KJ686049	KJ685982	
	indicus	None	Indonesia	Blood			KJ686110	KJ686020		
		None	Indonesia	Blood			KJ686104	KJ686057	KJ685973	
		None	Indonesia	Blood			KJ686099	KJ686025	KJ685991	KJ686125
		None	Indonesia	Blood			KJ686063	KJ686008	KJ685971	
		None	Indonesia	Blood			KJ686075	KJ686011	KJ685976	
		None	Indonesia	Blood			KJ686078	KJ686023	KJ685986	
		None	Indonesia	Blood			KJ686083	KJ686026	KJ686002	
		None	Indonesia	Blood			KJ686080	KJ686014	KJ685975	
	madagascariensis	TM 61998	South Africa	Muscle		KJ685958	KJ686094	KJ686050	KJ685977	KJ686114
	melanopterus	ANWC 8523	Papua New Guinea	Toe pad	KJ685949					KJ686123
		ANWC 30171	East Timor	Toe pad		KJ685964	KJ686107	KJ686054		KJ686115
	melanotus	None	Palmerston North, NZ	Muscle	KJ685951	KJ685963	KJ686113	KJ686028	KJ686007	
		None	Palmerston North, NZ	Muscle			KJ686076	KJ686038	KJ686006	
		None	Palmerston North, NZ	Muscle			KJ686081	KJ686009	KJ685970	
		None	Palmerston North, NZ	Muscle			KJ686101	KJ686036	KJ685983	
		None	Palmerston North, NZ	Muscle			KJ686097	KJ686015	KJ685988	
		None	Palmerston North, NZ	Muscle			KJ686112	KJ686034	KJ685972	
		None	Palmerston North, NZ	Muscle			KJ686096	KJ686027	KJ685997	
		None	Northland, NZ	Muscle			KJ686088	KJ686032	KJ685993	
		None	Northland, NZ	Muscle			KJ686103	KJ686040	KJ685980	
		None	Northland, NZ	Muscle			KJ686093	KJ686051	KJ686001	
		None	Northland, NZ	Muscle			KJ686066	KJ686017	KJ685967	
		None	Otago, NZ	Muscle			KJ686111	KJ686019	KJ685989	
		None	Otago, NZ	Muscle			KJ686077	KJ686058	KJ685985	
		None	Otago, NZ	Muscle			KJ686059	KJ686022	KJ685981	
		None	Otago, NZ	Muscle			KJ686074	KJ686024	KJ686000	
		ANWC 50696	WA, Australia	Muscle			KJ686065	KJ686012	KJ685994	
		ANWC 50991	WA, Australia	Muscle			KJ686091	KJ686046	KJ685999	
		ANWC 51269	NSW, Australia	Muscle			KJ686086	KJ686016	KJ685990	

TABLE 1. Continued.

Species [a]	Subspecies [a]	Museum voucher [b, c]	Locality [d]	Tissue type	GenBank accession number					
					Mitochondrial genes				Nuclear genes	
					12S	16S	Cyt *b*	CR	BFG-7	RAG-1
		ANWC 34145	NSW, Australia	Muscle			KJ686095	KJ686055	KJ686005	
		EBU 39915	NSW, Australia	Muscle			KJ686098	KJ686048		
		MV 4317	NSW, Australia	Muscle			KJ686061	KJ686044	KJ685992	
		MV 4193	Victoria, Australia	Muscle			KJ686060	KJ686056		
		MV 4191	Victoria, Australia	Muscle			KJ686062	KJ686037		
		MV 4917	Victoria, Australia	Muscle			KJ686102	KJ686047	KJ685978	
		MV 5180	Victoria, Australia	Muscle			KJ686105	KJ686033	KJ685969	
	palliatus	LIV T9048	Sulawesi	Toe pad	*		KJ686090			
	pelewensis	LIV T9774	Palau	Toe pad	*		KJ686089; KJ686092	KJ686029		
	poliocephalus	AMNH DOT17002	Captive	Muscle	*	KJ685961	KJ686109	KJ686013	KJ685979	KJ686119
	porphyrio	BM 93-0242-T	Spain	Muscle	*	KJ685959	KJ686068	KJ686021		KJ686120
	pulverulentus	USNM 578176	Philippines	Toe pad		KJ685962	KJ686087; KJ686100	KJ686039		KJ686121
	samoensis	UWBM 90389	American Samoa	Muscle	KJ685954	KJ685957	KJ686070	KJ686041	KJ685966	KJ686116
	seistanicus	BMNH 1965.M.2494	Turkey	Toe pad			KJ686072			
	vitiensis	None	Fiji	Feather			KJ686069		KJ686004	KJ686122
		None	Fiji	Feather			KJ686073	KJ686043	KJ685998	

[a] We followed Ripley (1977), Trewick (1996, 1997), and Livezey (1998) in our taxonomic treatment of species and subspecies.
[b] Specimens of *P. p. melanotus* without museum voucher information were taken from road-kill animals or hunting-season harvest in New Zealand. Samples of *P. p. indicus* in Indonesia, *P. p. caledonicus* in New Caledonia, and *P. p. vitiensis* in Fiji used blood and/or feathers taken from wild-captured specimens, which were released at the site of capture.
[c] Acronyms for museums: AMNH = American Museum of Natural History, USA; ANWC = Australian National Wildlife Collection, Australia; BM = Barcelona Museum, Spain; BMNH = British Museum of Natural History–Tring Museum, England; EBU = Evolutionary Biology Unit at the Australian Museum, Australia; LIV = Liverpool Museum, England; MV = Museum Victoria, Australia; NMNZ = Te Papa Museum, New Zealand; NMW = Natural History Museum of Vienna, Austria; TM = Ditsong Museum, South Africa; USNM = Smithsonian Institution, USA; UWBM = Burke Museum of Natural History and Culture, USA; WAM = Western Australia Museum, Australia.
[d] Specimens of *P. porphyrio* from New Zealand (NZ) and Australia were used for population genetic analyses.

were resolved using PHASE implemented in DnaSP version 5.0 (Librado and Rozas 2009) with the default parameters. To test for intralocus recombination in BFG-7, we used the PHI test (Bruen et al. 2006) implemented in SplitsTree. This is a robust test that can reliably detect recombination and report few false positives (Martin et al. 2011). We calculated the following summary statistics for genetic variation of each population in DnaSP: number of haplotypes (h), nucleotide diversity per site (π), number of segregating sites (S), Watterson's estimator of per site population mutation rate (θ_W), Tajima's D statistic (D_T), and Ramos and Rozas's R_2-test. For each population, D_T was also analyzed, using 10^3 coalescent simulations conditioned on the sample size and the observed number of segregating sites (Hudson 1990). Demographic expansion was assessed using the R_2-test implemented in DnaSP because it is the most powerful test when dealing with limited sample sizes (Rozas et al. 2003).

Lynch and Crease's pairwise F_{ST} (Lynch and Crease 1990) was calculated in DnaSP, using 5,000 replicates and a significance level of $\leq$0.05 to test the null hypothesis of panmixia (Raymond and Rousset 1995) between pairs of *P. porphyrio* populations in Oceania. The level of population genetic structure was tested using an analysis of molecular variance (AMOVA) implemented in Arlequin version 3.5 (Excoffier et al. 2005). To complement the phylogenetic inferences, haplotype networks were constructed using Network version 4.5.1.0 (Bandelt et al. 1999) with median joining to visualize the relationship between haplotypes and their geographic distribution. A Mantel test was conducted for correlation between uncorrected mitochondrial genetic distances estimated in MEGA version 5.2 (Tamura et al. 2011) and linear geographic distances (Jensen et al. 2005) using the ade4 package (Dray and Dufour 2007) in R (R Development Core Team 2008) with 10,000 permutations.

RESULTS

Phylogenetic Analyses

The present study includes wider taxonomic representation than the first molecular phylogenetic study of *Porphyrio*, which compared 4 taxa using a single gene, mitochondrial 12S rRNA (Trewick 1997). We have included *Porphyrio alleni*, *P. flavirostris*, and *P. albus*, as well as the *P. porphyrio* subspecies *porphyrio*, *indicus*, *poliocephalus* and other Australasian subspecies (note that Trewick [1997] included *P. p. seistanicus* from Turkey). The complete alignment of 6 gene fragments contained 4,304 base pairs [bp], comprising 816 bp of cyt *b*, 699 bp of CR, 728 bp of 16S, 402 bp of 12S, 868 bp of RAG-1, and 791 bp of BFG-7. No premature stop codons were detected in the 2 protein-coding genes. Individual gene trees did not reveal spurious sequences or significant conflict among individual phylogenies. Phylogenetic analyses that included just 1 representative of each species and subspecies that had the most complete gene sets yielded similar topologies (results not shown). Topologies from ML and Bayesian analyses were congruent for the concatenated dataset. The African species *P. alleni* is sister to the New World species pair *P. martinica* and *P. flavirostris* (Figure 1C). *Porphyrio porphyrio* did not form a monophyletic group. Instead, it comprised 6 distinct clades (*porphyrio*, *indicus*, *madagascariensis*, *pulverulentus*, *poliocephalus* [including *seistanicus*], and *melanotus*), and it was paraphyletic with respect to 3 species-level taxa: *P. mantelli* and *P. hochstetteri* from New Zealand, and *P. albus* from Lord Howe Island (Figure 1C, clade A). *Porphyrio p. melanotus* (Figure 1C, clade B) includes the parapatrically distributed subspecies *bellus*, *caledonicus*, *samoensis*, *vitiensis*, *palliatus*, *pelewensis*, *melanopterus*, and *chathamensis* (the latter was previously demonstrated to be invariant at the 12S locus by Trewick [1997]). This phylogenetic and spatial structure corresponds with significant differences in color and size that have been previously described in some detail (Mayr 1949, Ripley 1977, Simmons et al. 1980, Sangster 1998, Taylor 1998). One Indonesian specimen (*P. p. indicus*) did not group with other specimens from this region but instead was more closely related to the clade composed of *P. p. pulverulentus* and *P. albus* (Figure 1C). The close similarity of *P. p. pulverulentus* and *P. albus* sequences indicates a complex history of exchange, because their lineage is not recorded in islands between. Sequence data for *P. albus* came from old and rare museum specimens, and such aDNA sources have to be treated with caution. However, consistent results were obtained from separate samples and replicate PCRs. There is the possibility of mislabeling of museum specimens, but the white plumage characteristic of *P. albus* is uncommon in other populations.

Molecular Dating

Divergence time analysis suggests a Middle Miocene origin of diversification within *Porphyrio*, with the split between the lineages that led to the African species *P. alleni* and to other species occurring earliest, around 14 (19–9) mya. Splitting among *P. porphyrio* "subspecies" was estimated to have occurred about 6 (11–2) mya, with a likely colonization of *P. p. melanotus* in Australasia occurring in the late Pleistocene (600 kya). However, an earlier colonization by a flying *P. porphyrio* at the start of the Pleistocene ($\sim$2.5 mya) resulted in the flightless takahe endemic to New Zealand.

Population Genetic Structure

The mitochondrial data contained 1 to 4 haplotypes (h) in each of the populations sampled (Figure 2A). Nucleotide

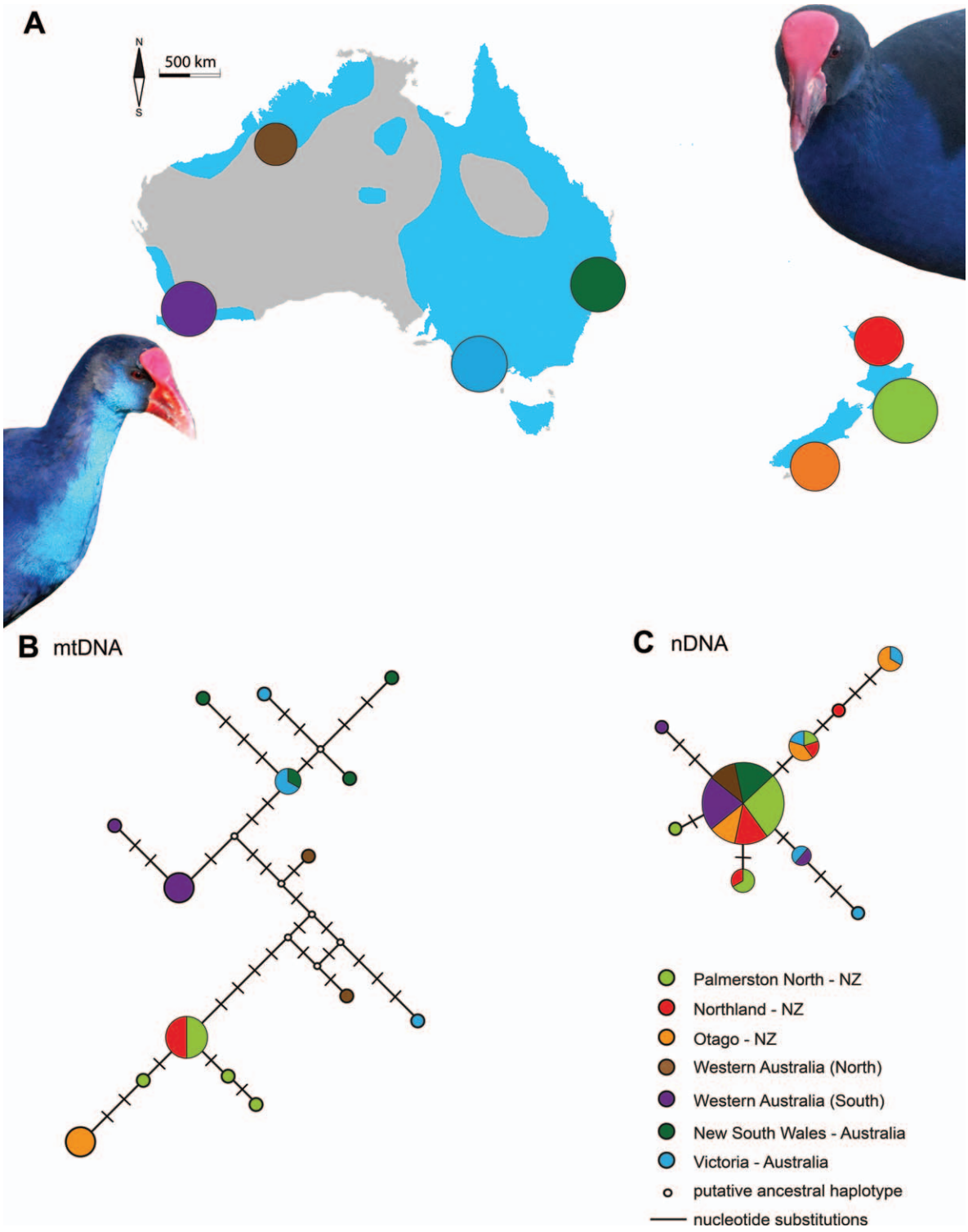

FIGURE 2. (**A**) Localities in Australia and New Zealand where individual *Porphyrio porphyrio* were sampled for population genetic analyses and haplotype networks. Colored circles are proportional to sample size at each locality. Inset images show plumage coloration of *P. p. bellus* (left) and *P. p. melanotus* (right). Median-joining haplotype networks of (**B**) mitochondrial DNA (mtDNA) dataset and (**C**) nuclear gene BFG-7 (nDNA). Circle area is proportional to the number of individuals found of each haplotype. Each line connecting haplotypes indicates a mutational step.

TABLE 2. Summary of descriptive statistics for mtDNA data (cyt *b* and CR) and the nuclear locus (BFG-7) used in population genetic analyses.

Locality	*n*/2*n*	*h*	*S*	θ_W	π	D_T	Probability that $D_T \neq 0$ (simululated coalescence)	R_2
Palmerston North	7/14	4/4	3/3	0.00122/0.00119	0.00104/0.00069	−0.654/−1.278	0.377/0.150	0.171/0.124
Northland	4/8	1/4	0/3	0/0.00146	0/0.00117	NA/−0.812	NA/0.235	NA/0.163
Otago	4/8	1/3	0/4	0/0.00195	0/0.00235	NA/0.899	NA/0.871	NA/0.232
Western Australia (south)	5/10	2/3	2/3	0.00096/0.00134	0.00081/0.00076	−0.972/−1.562	0.466/0.141	0.400/0.213
Western Australia (north)	2/4	2/1	5/0	0.00498/0	0.00498/0	NA/NA	NA/NA	0.501/NA
New South Wales	4/6	4/1	7/0	0.00381/0	0.00360/0	−0.389/NA	0.543/NA	0.164/NA
Victoria	4/4	3/4	12/6	0.00652/0.00414	0.00592/0.00440	−0.840/0.673	0.267/0.802	0.303/0.216

Notes: n = number of sequences, $2n$ = number of sequences inferred for nuclear locus, h = number of haplotypes, S = number of segregating sites, θ_W = population mutation rate per site, π = nucleotide diversity per site, D_T = Tajima's D statistic, R_2 = Ramos and Rozas's statistic, and NA = not applicable.

diversity (π) at sampling localities with $n \geq 2$ was variable, ranging from 0.0 in Northland and Otago, New Zealand, to 0.0059 in Victoria, Australia. Zero to 12 segregating sites (S) were observed in each population, yielding a population mutation rate per site (θ_W) between 0.0 and 0.0065 (Table 2). For BFG-7, two alternative haplotypes were identified for alleles possessed by heterozygous individuals. No statistically significant evidence for recombination ($P = 0.06$) was detected using a phi test. Between 4 and 14 inferred BFG-7 sequences were sampled per population, and 1 to 4 unique haplotypes were found (Table 2). The number of segregating sites in each population varied from 0 to 6. Aside from invariant samples from New South Wales and Western Australia (north), nucleotide diversity ranged from 0.00069 in Palmerston North, New Zealand, to 0.00440 in Victoria. This latter population also had the highest inferred population mutation rate per site (0.00414). No population showed a significantly skewed D_T for mitochondrial DNA (mtDNA) or nuclear data, although power to reject the null hypothesis of neutrality may have been hampered by small sample sizes. The population-size-change (R_2) test did not find evidence of demographic expansion of the populations sampled (Table 2).

Panmixia was evident in 2 localities sampled on the same island and separated by ~500 km: Palmerston North and Northland on North Island, New Zealand. Nevertheless, population genetic structuring was found among other populations more geographically remote from one another, with the exception of some of the comparisons among Western Australia (north) and Victoria, which was probably due to the low sample size (Table 3). Analysis of BFG-7 showed little population genetic structuring (Table 3) among localities at which mtDNA diversity was clearly partitioned. Significantly different values were obtained for most pairwise comparisons among Victoria populations because this location had endemic haplotypes. Consistent with the population pairwise differentiation analysis of the mitochondrial data, most genetic variation was explained by differences among populations (70.5%; $P < 0.0001$). The AMOVA of the BFG-7 data indicated that a small but significant (14%; $P < 0.01$) component of variance was

TABLE 3. Pairwise comparisons between populations using mtDNA data (above diagonal) and the nuclear locus BFG-7 (below diagonal). Negative values represent a program idiosyncrasy due to the small sample size and are effectively zero. Significant values are in bold (*$0.01 < P < 0.05$; **$0.001 < P < 0.01$).

Locality	Palmerston North	Northland	Otago	Western Australia (south)	Western Australia (north)	New South Wales	Victoria
Palmerston North		0.083	**0.840****	**0.883****	**0.572***	**0.740****	**0.605****
Northland	−0.030		**1.0***	**0.945****	0.615	**0.784***	**0.647***
Otago	0.170	0.031		**0.950****	0.736	**0.807***	**0.684***
Western Australia (south)	0.018	0.044	**0.207***		**0.508***	**0.544****	**0.358****
Western Australia (north)	0.038	0.071	0.257	0.0		0.44	0.185
New South Wales	0.038	0.071	0.257	0.0	0.0		−0.040
Victoria	**0.170****	**0.091***	−0.070	**0.163***	**0.222***	**0.222****	

attributable among populations. Population geographic structure was evident in the mitochondrial and nuclear haplotype networks (Figure 2B, 2C), and the Mantel test showed a significant correlation between genetic and geographic distances among populations ($r = 0.508$, $P < 0.05$), even though only ~26% of genetic divergence was explained by geographic distance.

DISCUSSION

Biogeography and Evolution of Swamphens

Our phylogenetic analyses and molecular dating support independent and temporally nonoverlapping colonization events among *Porphyrio* species. This interpretation is, however, based on surviving or recently extinct lineages only; other colonizations are represented by fossils on Oceanic islands (Steadman 1988, 2006, Steadman et al. 1999) or have left no trace at all. The most likely area of origin of *Porphyrio* is Africa, with colonization westward into the Americas and several other colonizations northeastward (Europe, Asia, and Oceania) during the Miocene and Pleistocene. The oldest split among the currently recognized *P. porphyrio* lineage (Figure 1C, clade A) occurred in the Late Miocene (~6 mya), giving rise to *P. p. porphyrio* on the Mediterranean coast of Europe and *P. p. indicus* in Indonesia. Further diversification took place during the Pliocene, giving rise to *P. p. madagascariensis* in Africa and a radiation into Oceania.

The unique sequence obtained from the extinct *P. albus* of Lord Howe Island suggests a close affinity to Philippine *P. p. pulverulentus*, indicating that it was perhaps a white color variant founded from *P. p. pulverulentus* migrants. The flightless status of *P. albus* appears to be equivocal, and the population seems to have been polymorphic for plumage, with a high frequency of white individuals (White 1790, Hindwood 1940, Greenway 1967). Aberrations in color have been found in some insular populations, caused perhaps by an allele fixed through a founder effect (Cunningham 1955, Steadman 2006, Uy et al. 2009). White *Porphyrio* occur intermittently, and recent observations include an individual *P. p. melanotus* in Otago, New Zealand (Trewick and Morgan-Richards 2014). The Lord Howe population may have been established from a small number of colonizing individuals from the Philippines during the late Pleistocene (~500 kya), but this would have involved dispersal from the Philippines to Lord Howe Island over other islands. We remain cautious about the short DNA sequence obtained from *P. albus*.

Despite the appearance that flight is used only infrequently among *P. porphyrio* subspecies, the lineage has dispersed, colonized, and established populations multiple times across open expanses of water. Haplotypes from Indonesia (a specimen from Java is closely related to a specimen from the Philippines) and New Caledonia (specimens from this locality are closely related to specimens from localities as far away as Sulawesi and Palau) support the inference of exchange (Figure 1C). Although rare misplaced haplotypes of this sort might be evidence of ongoing exchange among island populations, they could also be the product of incomplete lineage sorting or past migration events.

Porphyrio p. melanotus (Figure 1C, clade B) appears to have entered Australasia within the past 600,000 yr, but bone deposits show a more recent arrival on some remote islands (Millener 1981, Taylor 1998, Steadman 2006). This includes New Zealand, where deposits indicate colonization ~500 yr ago, after Polynesian settlement (Trewick and Worthy 2001). This dating is much more recent than the estimated divergence of *P. porphyrio* and takahe lineages (*P. hochstetteri* and *P. mantelli*) that must represent an earlier, separate colonization. Within the Australia–New Zealand geographic region, the distribution of genetic variability (phylogenetic analysis, F_{ST}, AMOVA, haplotype networks, and Mantel test) indicates that the genetic structure of *P. p. melanotus* populations is not homogeneous. This lineage may have originated in Wallacea, and eustatic sea-level changes could have aided colonization by reducing overwater dispersal distances. Lowered sea level during glacial phases of the Pleistocene reduced the distance between some land areas, including between Papua New Guinea and Australia (Voris 2000, Hall 2009, Jønsson et al. 2010, Wurster et al. 2010, Lohman et al. 2011, Condamine et al. 2013, Irestedt et al. 2013). They did not, however, significantly alter the overwater distance between Australia and New Zealand (Graham 2008). This colonization pattern has created the allopatric distribution currently shown in Oceania. We note that higher genetic diversity in Australia than across the Tasman Sea reflects a recent arrival in New Zealand and indicates an influence of distribution due to persistence of habitat rather than geographic distance correlated with human settlement and clearance of forest.

Pairwise differences among Australasian populations were higher for the mtDNA data than for the nuclear DNA gene. This difference may be explained by the higher mutation rate and lack of recombination in the mitochondrial genome (Neiman and Taylor 2009). However, different population genetic (e.g., background selection), demographic (e.g., effective sex ratio and/or male-biased migration rates), or natural selection also must be considered for those higher mtDNA F_{ST} estimates (Palumbi and Baker 1994, Charlesworth 1998, 2009, Stinchcombe and Hoekstra 2008, Muir et al. 2012). Despite the indication that the populations studied have not undergone recent demographic changes, with a marked reduction of genetic variation within populations and increasing genetic differentiation, the swamphens within *P.*

p. melanotus of south Western Australia (Figure 1C, clade B) show exceptional and not subtle differentiation in plumage color pattern (Whittell 1934). The current nominate subspecies *P. p. bellus* in south Western Australia has a prominently brighter blue breast and throat color than *P. p. melanotus* (see images in Figure 2A). Differences in color, size, and other traits are evident among other lineages within the *melanotus* clade (Ripley 1977) and in other clades. For instance, within the *poliocephalus* clade, the Middle Eastern "*seistanicus*" population is grayish compared with individuals from India (see color photographs in Figure 1C). The mismatch between plumage patterns and the distribution of neutral population genetic markers suggests that differentiation in color and other traits have arisen rapidly in *Porphyrio* and are subject to selection in local environments (Mayr 1954, Nosil et al. 2009, Feder et al. 2012) or to stochastic genetic drift (Clegg et al. 2002a, 2002b). The lack of sorting at the BFG-7 locus suggests that fixed plumage-color differences among populations are not the result of drift but are perhaps better explained by "purifying" sexual selection.

Selection that results in character divergence among populations can occur without being detectable by neutral genetic markers (Charlesworth and Charlesworth 2009, Nosil et al. 2009). There may be lineages with genomic region(s) involved in adaptive divergence, and these regions may respond independently to environment pressures via selection (Schneider et al. 1999, Clegg et al. 2002a, 2002b, Schluter 2009, Via 2009, Cooke et al. 2012). Appearance is a trait that is important in assortative mating by individuals within a population (Schluter 2009, Maan and Seehausen 2011) and is strongly implicated in the behavior of communally breeding *P. p. melanotus* (Jamieson 1988). As such, it may drive monomorphism in local populations. Selection on mate choice and kin fitness likely maintains local population phenotypes in stable frequencies (Andersson et al. 1998, Eaton 2005, Johnsen et al. 2006, Pryke and Griffith 2006, Murphy 2008).

Although *P. p. melanotus* is not the lineage that gave rise to the flightless insular species of New Zealand (*P. mantelli* and *P. hochstetteri*), the recent success of *P. p. melanotus* in reaching several remote islands in the Pacific is testimony to the high success rate of dispersal and colonization. However, the restriction of gene flow evident in F_{ST} values suggests that range expansion is probably episodic, and this enhances the opportunity for speciation and establishment of reproductive barriers. Sexual selection could help drive locus-specific evolution without being evident in genes that are (with respect to those traits) neutral, and reproductive isolation could evolve as a consequence of local adaptation and selection on characters involved in mate choice and inclusive fitness by way of mating behavior.

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APPENDIX A

TABLE 4. Taxa, GenBank accession numbers, and original source of data for additional DNA sequences included in our study.

		GenBank accession number						
Species	Subspecies	12S	16S	Cyt *b*	CR	BFG-7	RAG-1	Source
Porphyrio alleni			KC614015	KC614100		KC613893	KC613952	Garcia-R. et al. 2014
P. hochstetteri		NC010092	NC010092	NC010092	NC010092	KC613909	KC613974	Morgan-Richards et al. 2008, Garcia-R. et al. 2014
P. mantelli		U77144						Trewick 1997
P. martinica			KC614019	KC614103		KC613897	KC613956	Garcia-R. et al. 2014
P. porphyrio	*madagascariensis*	U77142						Trewick 1997
	melanotus						KC613975	Garcia-R. et al. 2014
	poliocephalus			HQ916674	HQ896255			Pachlore et al. personal communication
				HQ916670	HQ896247			Pachlore et al. personal communication
				HQ916671	HQ896248			Pachlore et al. personal communication
				HQ916672	HQ896249			Pachlore et al. personal communication
				HQ916675	HQ896254			Pachlore et al. personal communication
				HQ916678	HQ896256			Pachlore et al. personal communication
				HQ916676	HQ896252			Pachlore et al. personal communication
	pulverulentus	U77140						Trewick 1997
	seistanicus	U77139						Trewick 1997

TABLE 5. Primers for PCR and DNA sequencing employed in our study. Asterisks denote primers taken from the primer database of the Allan Wilson Centre for Molecular Ecology and Evolution, Massey University.

Loci	Primer name	Sequence	Reference
12S	L1753	CAAACTGGGATTAGATACCCCACTAT	Cooper 1994
	L1873	CCCAACCTAGAGGAGCCTGTTC	Modified from Cooper et al. 1992
	H2171	GAGGGTGACGGGCGGTATGTACGT	Modified from Cooper et al. 1992
16S	Av2672F*	GTGGGATGACTTGTTAGT	
	Av3282R*	TGATTATGCTACCTTTGCACGGTCAGGATACC	
	Av3782R*	CGGTCTGAACTCAGATCACGTA	
Cyt *b*	Av15107F*	CATCCGTTGCCCACACATGYCG	
	Av16065R*	GYGRTCTTCYGTCTTTGGTTTACAAGAC	
	Av15425R*	GATTCTTCGCCCTTCACTTCC	
	L14841	AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA	Kocher et al. 1989, Thomas et al. 1989
	L15134	CAATACGGCTGACTACTCCG	Kirchman 2012
	H15156	AAACTGCAGCCCCTCAGAATGATATTT	Trewick 1997, Chambers and MacAvoy 1999
	TAK2F	CTACTACGGATCATACCTCTAT	Present study
	TAK2R	GGTTTGTAATGACTGTAGC	Present study
	TAK3R	CCTCCTCATGCTCATTCTAC	Present study
	TAK3F	CTTCGTAGGTTATGTCCTACC	Present study
CR	Av438F*	TCACGAGAAATCAGCAACCC	
	Av807R*	CTAGKTGTGGGTCAAAGTGCATCAGTG	
	Av1449R*	GAGTRCCCGTGGGGGTGTGGC	
BFG-7	Fib–BI7U	GGAGAAAACAGGACAATGACAATTCAC	Prychitko and Moore 1997
	Fib–BI7L	TCCCCAGTAGTATCTGCCATTAGGGTTT	Prychitko and Moore 1997
	Fib.8R	CCATCCACCACCATCTTCTT	Kimball et al. 2009
RAG-1	R17	CCCTCCTGCTGGTATCCTTGCTT	Groth and Barrowclough 1999
	R22	GAATGTTCTCAGGATGCCTCCCAT	Groth and Barrowclough 1999
	R52	CAAGCAGATGAAYTGGAGGC	Irestedt et al. 2001
	R53	TCCATGTCCTTTAAGGCACA	Irestedt et al. 2001

APPENDIX B

Short Sequences

These sequences are <200 bp, too short to be submitted to GenBank. Specimen name is followed by museum voucher when available. Acronyms for museums are the same as in Table 1.

12S rRNA

>*Porphyrio porphyrio porphyrio*_BM 93-0242-T
AGTACCCGCCTGAGAACTACGAGCACAAACGCTT
AAAACTCTAAGGACTTGGCGGTGCTCCAAACCCA
CCTAGAGGAGCCTGTTCTGTAATCGATAACCCACG
ATATACCCAACCCCTTCTCGCCCAAAGCAGC

>*Porphyrio porphyrio pelewensis*_LIV T9774
AACTGGGATTAGATACCCCACTATGCTTGGCCCTA
AATCCAGATACTCACCACCACTAGAGTATCCGCCT
GAGAACTACGAGCACAAACGCTTAAAACTCTAAG
GACTTGGCGGTGCCCCAAACCCACCTAGAGGAGC
CTGTTCTGTAATCGATAACCCACGATATACCCAAC
CCCTTCTTGCCCAAAGCAGC

>*Porphyrio porphyrio palliatus*_LIV T9048
AACTGGGATTAGATACCCCACTATGCTTGGCCCTA
AATCCAGATACTCACTACCACTAGAGTATCCGCCT
GAGAACTACGAGCACAAACGCTTAAAACTCTAAG
GACTTGGCGGTGCCCCAAACCCACCTAGAGGAGC
CTGTTCTGTAATCGATAACCCACGATATACCCAAC
CCCTTCTTGCCCAAAGCAGC

>*Porphyrio porphyrio poliocephalus*_AMNH DOT17002
CGATATACCCAACCCCTTCTTGCCCAAAGCAGCCT
ACATACCGCCGTCCCCAGCTCACCTCCCCTGAGAG
CCTAAATAGTGAGCACAACAACACCTCGCTAATAA
GACAGGTCAAGGTATAGCCCATGAAGGGGTAGAA
ATGGGCTACATTTTCTAAAATAGAAA

>*Porphyrio porphyrio caledonicus*
CGATATACCCAACCCCTTCTTGCCCAAAGCAGCCT
ACATACCGCCGTCCCCAGCTCACCTCCCCTGAGAG
CCTAAATAGTGAGCACAACAACACCTCGCTAATAA
GACAGGTCAAGGTATAGCCCATGAAGGGGTAGAA
ATGGGCTACATTTTCTAAAATAGAAA

Cyt *b*

>*Porphyrio mantelli*_NMNZ DM7930
GGATCATACCTCTATAAAGAAACCTGAAACACAGG
AATCATCCTACTACTCACCCTAATAGCCACTGCCT
TCGTAGGCTATGTCCTACCATGAGGACAAATATCC
TTCTGAGGCGCTACAGTCATTACAAACCTATTCTC
AGCCATC