The occurrence of hybridisation between the Pacific Black Duck (*Anas superciliosa*) and other dabbling ducks (Genus: *Anas*) in Australia.

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Abstract

Hybridisation between closely-related species is an ongoing threat to many species that can be exacerbated by anthropogenic practices. The Pacific Black Duck (*Anas superciliosa*) is a dabbling duck native to the southwest Pacific that is currently under threat of hybridisation with introduced Mallard (*A. platyrhynchos*). Mallard are known to hybridise with and threaten many other dabbling duck species throughout the world. To evaluate the threat posed by hybridisation with introduced domestic Mallards to the Australian subspecies of the Pacific Black Duck (*A. s. rogersi*), a set of nine microsatellite markers were selected to genetically differentiate these species and detect hybrids. The use of these microsatellite markers on putative Pacific Black Ducks found that the overall frequency of hybridisation in Australia is currently low (1.5%) and also revealed that the frequency of hybridisation tended to be higher in urban compared to rural Victoria. Behavioural observations suggested that while Mallards are dominant over Pacific Black Ducks, the latter have not been excluded from parks inhabited by Mallards.

In contrast to mainland Australia, most birds on Lord Howe Island appear to be Mallards of New Zealand stock descent. It's unclear whether hybrids resident were bred locally or whether they emigrated from New Zealand. The lack of Pacific Black Duck phenotypes on Lord Howe Island suggests that this species has been outcompeted by Mallards.

In addition to hybridisation with Mallards, anecdotal reports have suggested that Pacific Black Ducks are also hybridising with Chestnut Teals (*A. castanea*). Based on microsatellite analysis, Chestnut Teal - Pacific Black Duck hybrids were detected, albeit at a low frequency (0.5%), among a sample of putative Pacific Black Ducks sampled throughout Australia

Overall, Mallard – Pacific Black Duck hybridisation does not currently seem to be widespread. However, the precautionary principle would advise eradication methods to be put into place to prevent the spread of the Mallard genome throughout Australia's Pacific Black Duck population to protect the genetic integrity of the Australian subspecies.

Doctor of Philosophy Declaration

I, Alice Taysom, declare that the PhD thesis entitled 'The occurrence of hybridisation between the Pacific Black Duck (*Anas superciliosa*) and other dabbling ducks (Genus: *Anas*) in Australia' is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

Signature:

Date:

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List of Publications

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Taysom, A., van Dongen, W., Johnson, J., & Guay, P.-J. (In Preparation) The behaviour and mechanisms behind hybridisation of Mallards (*Anas platyrhynchos*) and Pacific Black Ducks (*Anas superciliosa*) in metropolitan Melbourne.

Taysom, A., van Dongen, W., Johnson, J., & Guay, P.-J. (In Preparation) Are urban ponds acting as reservoirs of Mallard – Pacific Black Duck hybrids in Victoria, Australia?

Taysom, A., van Dongen, W., Johnson, J., & Guay, P.-J. (In Preparation) Cases of hybridisation between the Mallard (*Anas platyrhynchos*) and other dabbling duck species (genus: *Anas*) and their associated management implications.

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Taysom, A. J., Johnson, J. & Guay, P.-J. (In Preparation) The genetic origins of the Mallard (*Anas platyrhynchos*) and occurrence of Mallard - Pacific Black Duck (*A. superciliosa*) hybrids on Lord Howe Island.

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Guay, P.-J., Taysom, A. J., Robinson, R. & Tracey, J. (2014) Hybridisation between the Mallard and native dabbling ducks: causes, consequences and Management. *Pacific Conservation Biology.* **20**, 41-47

Guay, P.-J., McLeod, E. M., Taysom, A. J. & Weston, M. A. (2014) Are vehicles 'mobile bird hides'? A test of the 'cars cause less disturbance' hypothesis. *Victorian Naturalist*. **131**, 150-155

McLeod, E. M., Guay**,** P.-J., Taysom, A. J., Robinson, R. W. & Weston, M. A. (2013) Buses, cars, bicycles and walkers: the influence of the type of human transport on the flight responses of waterbirds. *PLoS One*. **8**, 1-11

Taysom, A. J., Stuart-Fox, D. & Cardoso, G. C. (2011) The contribution of psittacofulvin-, structural- and melanin-based colouration to sexual dichromatism in Australiasian parrots. *Journal of Evolutionary Biology*. **24**, 303-313

List of Awards

2014 Birdlife Australia Conference Travel Award

2012 Holsworth Wildlife Research Endowment

2011 Birdlife Australia Research Award

2011 BOCA Research Award

2011 M. A. Ingram Research Award

2011 Australian Postgraduate Award

2010 Birdlife Australia – Victoria Research Award

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Chapter 1

Introduction

A worldwide loss of biodiversity has occurred in recent years as a result of human alteration to the environment (Chapin et al. 2000). Anthropogenic practices have altered the global distribution of species by homogenising the landscape and enhancing the mobility of some organisms by removing ecological barriers (Chapin et al. 2000; Seehausen et al. 2007). Human-dominated environments have also brought numerous invasive species that can also alter the evolutionary processes of native species by competitive exclusion, niche displacement, introgressive hybridisation and ultimately extinction (Mooney & Cleland 2001). Hybridisation occurs when two genetically distinct species interbreed, whereas, introgressive hybridisation is the complete mixing of two gene pools (Mallet 2005).

While hybridisation can be problematic, it can also be beneficial in some cases (Grant & Grant 1992; Tompkins et al. 2006). For example, hybridisation can benefit species as it can increase survivorship (e.g., Pfennig 2007), hybrid fitness (e.g., Grant & Grant 1992; Veen et al. 2001) and promote a superior immune system (e.g., Tompkins et al. 2006). A study of a two Spadefoot toad species, *Spea bombifrons* and *S. multiplicata*, found that *S. bombifrons* females were more likely to choose heterospecific males when environmental conditions favoured hybridisation by increasing the survivorship of offspring (Pfennig 2007).

Although hybridisation is a natural phenomenon and can even be beneficial in some cases, it becomes a conservation issue when it is influenced by human activities (Rhymer & Simberloff 1996; Allendorf et al. 2001; Rhymer 2006). For example, human activities, such as hunting and deforestation, led to the strong decline European wolf numbers over the last centuries (Boitani 2003). Since the 1980s, the confined wild wolf populaton in Italy has started to re-colonise its former range and has come into contact with an expanding feral dog population (Randi 2008). While wild wolves are currently not thought to be at risk of extinction due to hybridisation, genetic analyses of wild wolves had shown admixture was occurring and was probably occurring at a frequency higher than detected (Verardi et al. 2006; Randi 2008).

Hybridisation is also known to be very common in birds, and in particular among waterbirds (Anseriformes) with overlapping breeding ranges (McCarthy 2006). It is known that growing urbanisation and homogenisation of the landscape has increased contact between historically allopatric species (Chapin et al. 2000; Seehausen et al. 2007). Cases of hybridisation between both wild and domestic varieties of Mallard (*Anas platyrhynchos*) and other closely-related species are among the most well documented (McCarthy 2006).

The Mallard, native to the northern hemisphere, are often larger, more aggressive and dominant over other dabbling duck species (Genus: *Anas*). The domestication of the Mallard has resulted in many behavioural changes including increased testosterone, decreased ability for long distance flights,

increased promiscuity and forced extra-pair copulations (Desforges & Wood-Gush 1975; Hepp et al. 1988). These behavioural changes in domestic Mallard are thought to promote interspecific hybridisation (Desforges & Wood-Gush 1975; Hepp et al. 1988).

In addition to hybridisation, Mallards in particular are known to threaten other dabbling duck species via competition and displacement (Brodsky & Weatherhead 1984; Brodsky et al. 1988; Williams & Basse 2006). Larger and more aggressive Mallards may outcompete other dabbling duck species with which they co-occur (Brodsky & Weatherhead 1984; Brodsky et al. 1988; Kear et al. 2005; Williams & Basse 2006). It is important to differentiate between competition and hybridisation, as the two may have different consequences and therefore different management practices.

The Mallard was introduced to Australia and New Zealand during the 19th and the 20th centuries (Thomson 1922; Marchant & Higgins 1990; McDowall 1994; Dyer & Williams 2010). Mallards initially released in New Zealand were from Britain totaling at least 400 Mallards by 1930 (Dyer & Williams 2010). The popularity of Mallards as a sporting bird led to the introduction of at least another 25,000 Mallards from North America (Dyer & Williams 2010). In comparison, little is known about wild Mallards imported to Australia (Braithwaite & Miller 1975). Mallards were originally introduced widely in the southeast and southwest of Australia, with a record from the Otago Acclimation Society in New Zealand of a pair of birds resembling wild Mallards, which were imported from Melbourne, Victoria (Braithwaite & Miller

1975). However, most of the original introductions throughout Victoria were considered unsuccessful (Marchant & Higgins 1990). Thus, most of the Mallards remaining in Australia today are of domestic origin and were therefore farmstock (Braithwaite & Miller 1975).

Since the release of game Mallards in New Zealand, they have increased in number and have ultimately colonised the entire country, including many neighbouring islands (Thomson 1922; Bailey & Sorensen 1962; McKean & Hindwood 1965; Gillespie 1985; Hermes et al. 1986; Norman 1987; Marchant & Higgins 1990; Tennyson 1998; Miskelly et al. 2001). For example, Mallards arrived on Lord Howe Island, located mid-way between Australia and New Zealand, in 1963 (McKean & Hindwood 1965). The concurrent release of Mallards in New Zealand and their arrival on Lord Howe Island has led to the opinion that Mallards on Lord Howe Island emigrated from New Zealand (McKean & Hindwood 1965; Tracey et al. 2008). This idea has not been genetically tested.

The release and arrival of Mallards in the southwest Pacific soon led to reports of hybridisation with the native Pacific Black Duck (*A. superciliosa*) (Thomson 1922; Bailey & Sorensen 1962; Warham & Keeley 1969; Gillespie 1985; Hermes et al. 1986; Norman 1987; Rhymer et al. 1994; Tracey et al. 2008). In New Zealand, analysis of mitochondrial DNA (mtDNA) and nuclear DNA has confirmed introgressive hybridisation between the two species with few pure Pacific Black Ducks believed to remain (Rhymer et al. 2004; Muller 2009).

In contrast, domestic Mallards, which are more common in urban Australia, tend to be sedentary and have poor dispersal capabilities (Braithwaite & Miller 1975; Marchant & Higgins 1990). They were therefore thought pose no significant threat to native Pacific Black Ducks, which are also common in rural environments (Braithwaite & Miller 1975; Marchant & Higgins 1990). However, phenotypic studies have indicated that Mallard – Pacific Black Duck hybrids are spreading into rural areas of South Australia (Paton et al. 1992) and that putative hybrids are now harvested by hunters in New South Wales, Victoria and Tasmania (Guay & Tracey 2009). In contrast to New Zealand, a systematic investigation into the rates of hybridisation between the introduced Mallard and the Pacific Black Duck throughout Australia has, to date, not been undertaken. Thus the current frequency of hybridisation and the threat posed by introduced Mallards to this species is currently unknown (Guay & Tracey 2009).

Whilst hybridisation with introduced Mallards in Australia is a potential threat, Pacific Black Ducks are also known to hybridise with other dabbling duck species with which they co-occur (McCarthy 2006). While they are known to hybridise with the closely-related Grey Teal (*A. gracilis*), little is known about the potential of hybridisation with the Chestnut Teal (*A. castanea*) (see McCarthy 2006). The confirmation of hybridisation between the Chestnut Teal and the Pacific Black Duck would provide further evidence of natural hybridisation between Pacific Black Ducks and other species and would provide some information on the level of genetic exchange.

Historically, the detection of Mallard – Pacific Black Duck hybrids in Australia and New Zealand was carried out using phenotype scoring keys originally developed in Australia (Braithwaite & Miller 1975; Gillespie 1985; Paton et al. 1992; Rhymer et al. 1994). For example, previous studies suggested that hybridisation occurs in various locations throughout Australia (Braithwaite & Miller 1975; Paton et al. 1992; Sinden et al. 2003; Guay et al. 2014). These studies, however, are based on anecdotal evidence, phenotype and allozyme analyses and are therefore limited in their ability to detect the true frequency of hybridisation. For example, Rhymer et al. (1994) did not find a strong correlation between phenotype and maternally inherited haplotypes of New Zealand Pacific Black Ducks, Mallards and their hybrids. Plumage characteristics of hybrids have never been established with certainty, as species morphological variation is generally greater than expected (Allendorf et al. 2001). For the complete characterisation of all hybrid individuals, Muller (2009) employed biparentally inherited nuclear markers to distinguish between pure species and their hybrids. Muller (2009) found a correlation between genetype and phenotype, however, the study used an insufficient number of loci (see Vaha & Primmer 2006; Hale et al. 2012) and therefore the number of hybrids was probably underestimated.

Nuclear DNA displays higher levels of variation compared to allozymes and are currently considered the most reliable method in determining the frequency of hybridisation (Braithwaite & Miller 1975; Milstein & Osterhoff 1975; Hitchmough et al. 1990; Guay et al. 2014). Therefore, nuclear markers

and were incorporated into this study, except in situations where DNA was of a low quality and quantity (i.e., DNA samples that were obtained from feathers).

The overall aim of this thesis was to identify the occurrence and extent of hybridisation between Pacific Black Duck and other dabbling ducks species with a particular focus on the threat of introduced Mallards. The following specific aims were therefore addressed:

- To review the scientific literature and describe all suspected cases of ongoing hybridisation between the Mallard and other closely-related species that occur throughout the world (Chapter 2). This review summarises each case of hybridisation and suggests potential strategies for Mallard management.
- To assess and validate a set of microsatellite loci to establish a genetic system that distinguishes between domestic Mallard, Australian Pacific Black Duck and their hybrids (Chapter 3).
- To utilise these microsatellite loci to screen for hybrids from various locations throughout Australia (Chapter 4).
- To undertake behavioural observations of Pacific Black Ducks and Mallards in urban areas, where Mallards are more common, to identify whether competition, displacement and/or hybridisation pose a threat. Also, to compare the frequencies of hybrids between these two species in urban areas and in rural areas, to identify whether urban ponds represent a potential source of hybrids that could spread to rural areas (Chapter 5).

- To determine the origin of resident Mallards and Pacific Black Duck - Mallard hybrids on Lord Howe Island, a potential entry point for Mallard hybrids into Australia, using mitochondrial control region haplotypes (Chapter 6).
- In addition to the investigation of Mallards and Pacific Black Ducks, this research also aimed to genetically and phenotypically describe a putative Chestnut Teal – Pacific Black Duck hybrid and screen for evidence of Chestnut Teal cryptic hybrids among an Australia-wide sample of putative Pacific Black Ducks (Chapter 7).

Chapter 2

Cases of hybridisation between the Mallard (*Anas platyrhynchos*) and other dabbling duck species (genus: *Anas*) and their associated management implications.

Unpublished manuscript

Abstract

Biodiversity is decreasing worldwide as a result of diverse processes. One factor which can be overlooked in the conservation of dabbling ducks, and has been throughout Australia, is hybridisation with domestic and wild Mallards. Extensive hybridisation can eventually threaten a species by introgression, which may ultimately lead to extinction. The Mallard has the largest range of all dabbling ducks and its range continues to increase due to introductions into new areas and the removal of extrinsic barriers. Furthermore, the domestication of Mallards has led to behavioural changes, including increased promiscuity, aggression and human tolerance. These changes in the Mallard's range and behaviour have led to an increased rate of hybridisation between Mallards and 11 other closely-related species. Here I review the status and suggest management strategies for all 11 cases of hybridisation with Mallards. In most instances where hybridisation is extensive and widespread (e.g., the American Black Duck, the Mottled Duck, the Mexican Duck, the Eastern Spot-billed Duck and the New Zealand Pacific Black Duck) there are no effective control measures. Therefore, I suggest that captive breeding of genetically-confirmed pure individuals be used to maintain species integrity. In cases where hybridisation with Mallards is

locally restricted, due to the domestic ancestry of some Mallards or their limited range on small islands, I suggest that Mallards and their genetically-confirmed hybrids be eradicated (e.g., the Laysan Duck, the Philippine Duck, the Australian Pacific Black Duck, the Yellow-billed Duck and Meller's Duck). A less desirable strategy is to contain Mallards and their hybrids from pure species, or vice versa, where their movements can be restricted (e.g., Great Barrier Island where pure Brown Teal are still found). Furthermore, Mallards should be prevented from establishing populations in areas where they currently do not occur.

Introduction

Hybridisation is common throughout the animal kingdom (Dowling & Childs 1992; Hubbard et al. 1992; Baumel et al. 2003) and is known to be especially common in birds. In particular, amongst waterfowl (family: Anatidae), more than 500 different inter-specific hybrid crosses have been observed in the wild and captivity (McCarthy 2006). Most hybrid crosses within the Anatidae involve dabbling ducks (genus: *Anas*) (McCarthy 2006). Dabbling ducks are a group of closely-related surface feeding ducks that are widely distributed throughout the world with many migratory species (Kear et al. 2005). Many studies have failed to unambiguously resolve the phylogenetic relationship within the genus (Livezey 1991; Johnson & Sorenson 1999; Lavretsky et al. 2014) and hybrid crosses between species frequently result in fertile offspring (Phillips 1915; Sibley 1957; McCarthy 2006; Lavretsky et al. 2014). While hybridisation can facilitate beneficial horizontal genetic exchange between

some species, increasing genetic diversity, it can also result in the extintion of others, especially where species have been introduced (Kraus et al. 2012).

Within the dabbling ducks, a group of 12 closely-related species are referred to as the mallard complex (Table 1; Livezey 1991; Johnson & Sorenson 1999; Lavretsky et al. 2014). Most members of the mallard complex have similar life history traits, behaviours and a lack of sexual dimorphism (Delacour 1956). The Mallard (*A. platyrhynchos*) is the only member of this group that displays sexual dichromatism, where the male possesses bright nuptial plumage whilst the female has dull coloured plumage suited for camouflage (Delacour & Mayr 1945). While it appears that the different species of the mallard complex have evolved in allopatry, anthropogenic influences have altered the range of mallard species and Mallards have now come into secondary contact with most of the other species within the complex (Delacour & Mayr 1945; Lavretsky et al. 2014).

Increased contact of the Mallard with other closely-related species may not only result in an increased frequency of hybridisation, but also an increased threat of competition and exclusion (Williams & Basse 2006; Petrie et al. 2012). Due to the different management techniques that may be required for the different threats, only hybridisation is discussed here.

Table 1. The *Anas* species that make up the mallard complex as identified by Livezey (1991), Johnsson & Sorenson (1999) and Lavretsky et al. (2014).

	Species
1	Mallard *A. platyrhynchos*
2	American Black Duck *A. rubripes*
3	Mottled Duck *A. fulvigula*
4	Mexican Duck *A. diazi*
5	Hawaiian Duck *A. wyvilliana*
6	Laysan Duck *A. laysanensis*
7	Spot-billed Duck *A. poecilorhyncha*
8	Philippine Duck *A. luzonica*
9	Pacific Black Duck *A. superciliosa*
10	Yellow-billed Duck *A. undulata*
11	Meller's Duck *A. melleri*
12	African Black Duck *A. sparsa*

The Mallard is native to the Northern Hemisphere, tends to be slightly larger than other dabbling duck species and also tends to be dominant over other species (Brodsky & Weatherhead 1984; Brodsky et al. 1988; Kear et al. 2005). Their dominance over other species may be due a combination of their relatively aggressive nature and larger size. Furthermore, humans have domesticated Mallards and today most domestic varieties of ducks are descendants of wild Mallards (Kear et al. 2005). Domestication has resulted in behavioural changes, including an increased tolerance of humans, decreased flightiness, a shift from a monogamous mating system to promiscuity, longer breeding seasons and higher levels of testosterone (Desforges & Wood-Gush 1975; Hepp et al. 1988). Their aesthetic and game value has led to introductions in many geographical locations around the world (e.g., Heusmann 1974; Milstein & Osterhoff 1975; Marchant & Higgins

1990). These factors have led to increased contact of Mallards with other dabbling duck species and an increased rate of hybridisation.

The Mallard is thought to be able to hybridise and produce at least partially fertile offspring with most dabbling duck species with which it co-occurs (McCarthy 2006). McCarthy (2006) generated a list of 40 species that have been recorded to hybridise with Mallard in the wild and an additional 20 species that undergo hybridisation with Mallard in captivity. Of the 11 other species in the mallard complex, nine undergo hybridisation with Mallard. The only two mallard species not currently threatened by hybridisation with the Mallard is the African Black Duck (*A. sparsa*) and the Laysan Duck (*A. laysanensis*). The African Black Duck is a species that is not characteristic of the genus *Anas* and is confined to mountainous regions and fast-moving streams, habitat which is unsuitable for Mallards (Phillips 1923; Delacour 1956; McKinney et al. 1978). The Laysan Duck is a species that inhabits a remote island in the Hawaiian archipelago where Mallards have not been recorded and appears to be more at risk of inbreeding, habitat loss and predation (Moulton & Weller 1984; Browne et al. 1993; Kear et al. 2005). In total, the Mallard is thought to threaten 10 duck species in the wild via ongoing hybridisation. These species include the American Black Duck (*A. rubripes*) of eastern North America, the Mottled Duck (*A. fulvigula*) of Florida, the Mexican Duck (*A. diazi*) of Mexico, the Hawaiian Duck (*A. wyviliana*) of the Hawaiian Islands, the Eastern Spot-billed Duck (*A. poecilorhyncha zonorhyncha*) of eastern Asia, the Philippine Duck (*A. luzonica*) of the Philippine Islands, the Yellow-billed Duck (*A. undulata*) of southern and

eastern Africa, Meller's Duck (*A. melleri*) of Madagascar, the Brown Teal (*A. chlorotis*) of New Zealand and the Pacific Black Duck (*A. superciliosa*) of the south western Pacific (Figure 1) (Gemmell & Flint 2000; Kennedy & Spencer 2000; McCarthy 2006; Banks et al. 2008). The Brown Teal is the only species affected by Mallard hybridisation that is not of the mallard complex and thus was not listed in Table 1. While all of these listed species are under threat due to hybridisation with Mallard, this review has a particular focus on the Pacific Black Duck, as it's the subject of this thesis.

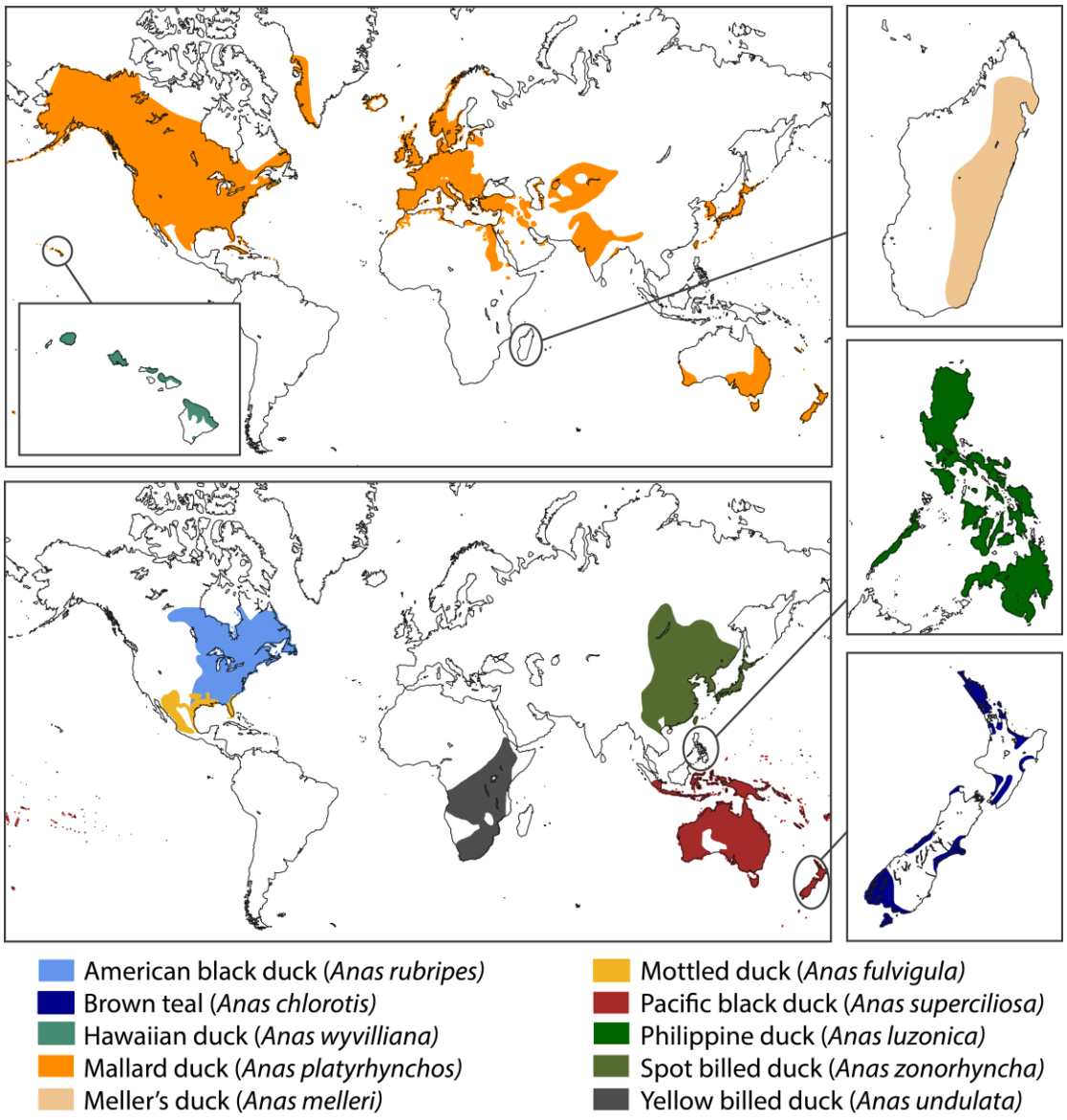

Figure 1. The distribution map of the Mallard and 10 other species with which it hybridises. The distribution of the Mexican Duck (*Anas diazi*) is incorporated into the distribution for the Mottled Duck (*A. fulvigula*). Shapefiles were provided by Birdlife International and NatureServe (2014).

Guay et al. (2014) have suggested five management strategies, based on available hybridisation data, that should be considered in reference to Mallard hybridisation: prevention, eradication, containment, no control and captive breeding. Prevention involves stopping Mallards from establishing a foothold

in a Mallard-free area and it is considered the most cost-effective management strategy. Once Mallards are established, active management is required. Eradication should be considered where Mallards have recently colonised or where hybridisation is localised. Containment of Mallards and their hybrids away from pure native species may be an option where their movements are localised by a limited distribution (i.e., islands) or where the movements of domestic Mallards and their hybrids is minimal due to their limited capacity for flight. If hybridisation is widespread or near complete, 'no control', the establishment of captive breeding programs or translocation of pure individuals to islands may be the only practical options. The benefits of captive breeding programs, such as species preservation, knowledge and education, only sometimes outweigh the challenges of captive breeding programs, which include locating pure individuals and maintaining a viable breeding population, and often may do very little to reduce the risk of extinction (Lynch & O'Hely 2001; Kostow 2004; Fraser 2008).

Determination of the appropriate management strategy to apply to each situation is dependent on knowledge of Mallard distribution, the extent of hybridisation with native species, the conservation status of the native species and feasibility of monitoring and management options. In this review, the current level of hybridisation of each species under threat due to hybridisation with the Mallard is assessed, where possible, potential management strategies are suggested and further work that is required to achieve an informed management decision are recommended for each case study.

American Black Duck (*Anas rubripes*)

The American Black Duck is one of three monomorphic mallard species that inhabits North America. It is endemic to eastern North America (Delacour 1956; Kear et al. 2005) and was once largely nonoverlapping distributions to the Mallard (Brodsky & Weatherhead 1984). Since European settlement, the Mallard's range in North America has been extending further eastwards (Howe & Allen 1901; Knight 1901; Allen 1909; Johnsgard 1967; Heusmann 1974). The Mallard's increasing range has been attributed to anthropogenic releases, as well as land use changes, including deforestation and the construction of ponds, providing more favourable habitat for Mallards (Foley et al. 1961; Johnsgard 1967; Heusmann 1974; Brodsky & Weatherhead 1984; Hepp et al. 1988; Rush et al. 1989; Plattner et al. 2010). Throughout the 1900s the status of the Mallard in the north east of the USA has changed from rare migrant to major game bird (Heusmann 1974). In 1969, for the first time, more Mallards than American Black Ducks were shot in the Atlantic Flyway (Heusmann 1974). This shift in ratios is partly due to a decline in American Black Ducks, but mostly due to an increase in Mallard numbers (Heusmann 1974).

The separated geographical range and habitat differences of the American Black Duck and Mallards had, until recently, constrained hybridisation between the two species (Brodsky & Weatherhead 1984). Phillips (1915; 1921) bred and described fertile captive hybrid offspring in the early 1900s. Wild hybrids were first described in Toronto, Canada, where Mallard numbers appeared to be increasing compared to the number of American Black Ducks

(Goodwin 1956). Since then the incidence of hybridisation has been increasing and is now frequent throughout the eastern states and provinces of North America due to changes in Mallard distribution (Johnsgard 1967; Kaufman 2001).

Ankney et al. (1987) suggested that introgressive hybridisation and/or competitive exclusion of American Black Ducks by Mallards was responsible for the decline in the numbers of American Black Ducks in Ontario and Quebec from 1971-85. However, debate remained over whether overhunting and habitat loss, as opposed to hybridisation, were the main contributing factors to the overall decline in American Black Duck numbers (Ankney et al. 1987; Conroy et al. 1989; Longcore et al. 2000).

Hybridisation between the Mallard and American Black Duck has largely been attributed to the removal of allopatric barriers and Mallard introductions (Foley et al. 1961; Johnsgard 1967; Heusmann 1974; Brodsky & Weatherhead 1984; Hepp et al. 1988; Rush et al. 1989; Plattner et al. 2010). Nonetheless, ecological factors, including male-biased sex ratios in breeding grounds, artificial feeding, reduction in breeding habitat, earlier pair formation and Mallard drake dominance over male American Black Ducks are also thought to be contributing factors to Mallard - American Black Duck hybridisation (Brodsky & Weatherhead 1984; Brodsky et al. 1988; Hepp et al. 1988; Petrie et al. 2012).

A number of studies using different genetic markers have determined a close evolutionary relationship and widespread introgressive hybridisation between American Black Ducks and Mallards (Patton & Avise 1985; Ankney et al. 1986; Avise et al. 1990; Mank et al. 2004). For example, Avise et al. (1990) also found a close evolutionary relationship between the Mallard and the American Black Duck using mtDNA. In addition, Ankney et al. (1986) found greater genetic diffferentiation between eastern populations of Mallards/American Black Ducks and western populations of Mallards than between species using allozymes. This result is consistent with the idea of introgressive hybridisation along the east coast where the American Black Duck is predominantly found. Finally, Mank et al. (2004) used microsatellite markers to determine that a significant reduction in genetic differentiation between Mallards and American Black Ducks occurred more recently and thus, is not due to a close evolutionary relationship. This evidence supports theory for interspecific hybridisation between the Mallard and the American Black Duck.

While the American Black Duck is not listed as a species of international concern, the population trend is recognised to be decreasing (IUCN Red List 2015). Mallard introductions into the American Black Duck's range have largely increased the number of Mallards and hybrids and attempting to control their numbers may be an ineffective approach. Captive breeding is the only available management strategy to preserve the American Black Duck (Table 2).

Mottled Duck (*Anas fulvigula*)

The Mottled Duck is a monogamous, non-migratory dabbling duck that inhabits the Gulf Coast of North America (Delacour 1956; Moorman & Gray 1994; Kear et al. 2005). The species closely resembles the Mexican Duck and can be divided into two subspecies; *A. f. fulvigula* from Florida and *A. f. maculosa* from the Western Gulf Coast, comprising of Texas, Louisiana and coastal Mexican states of Tamaulipas and Veracruz (Delacour 1956; Moorman & Gray 1994; McCracken et al. 2001; Delany & Scott 2006; Bielefeld et al. 2010; Lavretsky et al. 2014). The Mottled Duck and the Mallard have similar courtship displays (Mazourek & Gray 1994) and have been known to produce fertile hybrid offspring for almost a century (Phillips 1915; Phillips 1921).

Both populations of Mottled Ducks are known to undergo extensive hybridisation with Mallards in the wild (Stutzenbaker 1988; Mazourek & Gray 1994; McCracken et al. 2001; Williams et al. 2005). Mallard releases in the state of Florida have amplified the frequency of hybridisation with the Mottled Duck, which along with habitat loss, is associated with their decline (Mazourek & Gray 1994; Florida Fish and Wildlife Conservation Commission 2011). Released Mallards were generally of domestic origin and therefore the birds did not migrate north to the Mallards normal breeding grounds (Mazourek & Gray 1994). Texas and Louisiana populations of *A. f. maculosa* have declined since the arrival of European settlers (Stutzenbaker 1988). The decline in numbers of *A. f. maculosa* is associated with habitat loss and hunting pressure (Stutzenbaker 1988). Furthermore, 100 out of the 10,000

ducks observed in Texas and Louisiana were phenotypically identified as hybrids between the Mottled Duck and Mallard (Stutzenbaker 1988).

Studies of the mitochondrial control region and nuclear DNA of *A. f. maculosa* and *A. f. fulvigula* have shown evidence of introgression between both subspecies and the Mallard (McCracken et al. 2001; Peters et al. 2014). A genetic study that incorporated microsatellite makers to detect hybridisation between *A. f. fulvigula* and Mallards in Florida and South Carolina, where the Mottled Duck was introduced, found that there was no clear genetic distinction between Mallard and Mottled Ducks in South Carolina (Williams et al. 2005). In comparison, hybridisation was detected in Florida, but to a lesser degree with rates ranging from 0 to 24% depending on the population sampled (Williams et al. 2005).

There are currently no measures in place to control hybridisation between Mallards and Mottled Ducks. Both subspecies of Mottled Duck are already under threat due to habitat destruction due to urbanisation and agriculture practices (McCracken et al. 2001; Williams et al. 2005). Human induced habitat modification has created more favourable habitat for Mallards and therefore increased contact between the species and the possibility for hybridisation (Florida Fish and Wildlife Conservation Commission 2011). While the Mottled Duck is not listed as a species of international concern, the population trend is recognised to be decreasing (IUCN Red List 2015) and introgressive hybridisation with feral Mallards is thought to be one of the biggest threats to the Florida Mottled Duck (Bielefeld et al. 2010). Further

degradation of Mottled Duck habitat is expected to reduce the ecological barrier between the two species and this is enhanced by Mallard introductions (Florida Fish and Wildlife Conservation Commission 2011). Prevention, eradication and containment are therefore not an option. Controlling Mallard numbers alone would probably be unfeasible, therefore, captive breeding after genetic testing for the prevalence of pure individuals is probably the only management strategy that will successfully preserve the genetic integrity of the Mottled Duck (Table 2).

Mexican Duck (*Anas diazi*)

The Mexican Duck is a non-migratory dabbling duck that has a similar courtship display to Mallards and closely resembles female Mallards (Delacour 1956; Kear et al. 2005). The Mexican Duck inhabits Mexico and southern states of the USA (Delacour 1956; Kear et al. 2005) and is closely related to the American Black Duck and the Mottled Duck, which also occur in North America (McCracken et al. 2001).

Even though the Mexican Duck was removed from the USFWS Endangered species list in 1978, it still appears to be threatened by habitat destruction and ongoing hybridisation with wild Mallards, whose migratory range overlaps with that of the Mexican Duck (Aldrich & Baer 1970; Scott & Reynolds 1984). The number of Mexican Duck – Mallard hybrids have increased and hybrids are thought to outnumber pure Mexican Ducks in some areas (Aldrich & Baer 1970). In addition, plumage indices have shown a gradual increase in

Mallard-like phenotypes from north to south of the Mexican Duck's range (Scott & Reynolds 1984).

While these historical phenotype studies suggests that hybridisation between the two species is nearing completion, only a comprehensive genetic study will reveal the full extent of hybridisation. Similar to the case of the American Black Duck and the Mottled Duck, anthropogenic practices are creating more suitable habitat for Mallards. If further research, that includes both genetic and phenotype studies, indicates that is indeed widespread, than the only practical measure available to preserve the species are captive breeding programs (Table 2).

Hawaiian Duck (*Anas wyviliana*)

The Hawaiian Duck, endemic to the Hawaiian Islands, is a non-migratory, monomorphic dabbling duck that is closely related to the Mallard, but is somewhat smaller (Delacour 1956; Kear et al. 2005). The Hawaiian Duck formerly inhabited all of the larger Hawaiian Islands with the exception of Lanai and Kahoolawe (Delacour 1956; Browne et al. 1993; Engilis & Pratt 1993). The numbers of Hawaiian Ducks started to decline at the start of the 20th century and by 1960 the numbers were reduced to 3,000 and were restricted to Kauai (Scott et al. 1988; Browne et al. 1993). The declines in Hawaiian Duck numbers were attributed to the draining of wetlands, hunting and predators (Delacour 1956). However, hybridisation with domestic Mallards is currently considered to be the major threat posed to the Hawaiian

Duck and the species has been listed as Endangered since 1967 by federal and state departments (USFWS 2012; 2013) and the IUCN Red List (2015).

Domestic Mallards were first brought to the Hawaiian Islands in the 1800s and their numbers were supplemented in the 1950s and 1960s with large stocks from North America, many of which are now feral (Fowler et al. 2009). Mallards have since hybridised with Hawaiian Ducks in the wild and while evidence suggested that pure Hawaiian Ducks remained on the islands of Kaua‘i, Ni‘ihau, and highlands of Hawaii, it is now believed that hybridisation exists in these pure populations (Browne et al. 1993; Engilis & Pratt 1993; Rhymer 2001; Engilis et al. 2002).

Various genetic studies, using allozymes, mtDNA and nuclear DNA markers, have all obtained similar results, concluding that hybridisation is extensive, possibly asymmetric, and occurs throughout the range of the Hawaiian Ducks (Browne et al. 1993; Rhymer 2001; Fowler et al. 2009). For example, using allozymes, Browne et al. (1993) found evidence of Mallard – Hawaiian Duck introgression on Kauai. In addition, Rhymer (2001), used mitochondrial and nuclear DNA analyses and found a close genetic relationship between the Mallard and the Hawaiian Duck and evidence of hybridisation on O’ahu and Hawaii. Finally, Fowler et al. (2009) used two sets of nuclear markers to successfully distinguish between Mallards, Hawaiian Ducks and their hybrids and also found that hybridisation on the Hawaiian Islands may be more common between Mallard females and Hawaiian Duck drakes. The long-term viability of the Hawaiian Duck species is therefore threatened by ongoing

extensive hybridisation with feral Mallards. Therefore, whilst prevention is not an option, eradication and containment may be the most viable options if further genetic testing on the islands of Kauaʻi, Niʻihau, and highlands of Hawaii reveals populations of pure Hawaiian Ducks (Table 2).

Spot-billed Duck (*Anas poecilorhyncha*)

The Spot-billed Duck is a monomorphic dabbling duck native to eastern Asia (Delacour 1956; Kear et al. 2005). The two subspecies of Spot-billed Duck, Eastern Spot-billed Duck (*Anas p. zonorhyncha*) and the Western Spot-billed Duck (*Anas p. poecilorhyncha*) have recently been elevated to full species status (e.g., Lavretsky et al. 2014) and are now treated separately in all major taxonomic works (e.g., BirdLife International 2015). Due to the split of Spot-billed Duck into two species being recent, historical records usually refer to *Anas poecilorhyncha* in the classical sense and thus both species of Spot-billed Duck are considered together here.

The Spot-billed Duck and the Mallard were historically allopatric and were rarely observed together (Phillips 1923), but recently the Spot-billed Duck has been increasing its range further northwest where it is coming into contact with native Mallards (Kulikova et al. 2004). It has been suggested that climate change, habitat loss in the form of fragmentation and wetland destruction are factors contributing to the increase in range of the Spot-billed Duck (Kulikova et al. 2004). The breeding range of Spot-billed Ducks and Mallards now overlaps in south-eastern Siberia, the southern Russian Far East, northern Japan and north-eastern China (Kulikova et al. 2004). The Spot-billed Duck

has a similar courtship behaviour to Mallards (Delacour 1956), increasing the likelihood of mistaken identity and thus hybridisation. And while hybridisation between the two species has predominantly been detected within the Mallards range, hybrids have been observed within the Spot-billed Duck's range (Kulikova et al. 2004), threatening the genetic integrity of a species with a comparatively limited range.

Studies of the mtDNA control region have found that extensive hybridisation is ongoing between the Spot-billed Duck and the Mallard in the Russian Far East (Kulikova et al. 2003; Kulikova et al. 2004; Kulikova et al. 2005). Furthermore, the mitochondrial control region of Spot-billed Ducks appears indistinguishable from that of the Asian Mallards, also indicating extensive hybridisation (Lavretsky et al. 2014). Unsurprisingly, Spot-billed Duck – Mallard hybrids are reportedly moving further south into the Spot-billed Ducks range (Kulikova et al. 2004).

The Spot-billed Duck is not listed as a species of international concern, however, the population trend is recognised to be decreasing (IUCN Red List 2015). Similarly to the case of a number of North American mallard species, hybridisation between Spot-billed Ducks and Mallards cannot be effectively controlled. Anthropogenic influences have altered the environment so that no control method is adequate to control hybridisation. Therefore, captive breeding programs may be the only effective measure to preserve the Spot-billed Duck (Table 2).

Philippine Duck (*Anas luzonica*)

The Philippine Duck is a monomorphic dabbling duck that is endemic to the Philippine archipelago and has a similar display and call as the Mallard (Phillips 1923; Delacour 1956; Kear et al. 2005). The Philippine Duck is listed as Vulnerable due to over hunting and habitat modification (IUCN Red List 2015). There are few records of hybridisation between the Philippine Duck and the Mallard (see McCarthy 2006). However, considering the close genetic relationship between the Philippine Duck and the Pacific Black Duck (Lavretsky et al. 2014), a species which can undergo introgressive hybridisation with the Mallard, it is likely that the Philippine Duck can hybridise with vagrant or domestic Mallards. Genetic studies investigating the level of hybridisation are urgently required to determine the risk posed to the already Vulnerable Philippine Duck. If genetic studies reveal that hybridisation between the two species does persist, management should focus on the prevention of feral Mallards establishing a stronghold in the Philippines (Table 2).

Yellow-billed Duck (*Anas undulata*)

The Yellow-billed Duck is a dabbling duck native to southern and eastern Africa that is common on waters in open country (Phillips 1923; Delacour 1956). The Yellow-billed Duck is slightly more slender than the Mallard and is similar to the Mallard in the way of habits, voice and behaviour (Phillips 1923; Delacour 1956).

Yellow-billed Duck – Mallard hybridisation was first recorded in captivity, however, the most recent anecdotal evidence suggests that hybridisation in the wild is now widespread (Phillips 1923; Milstein & Osterhoff 1975; Lavretsky et al. 2014). Mallards in South Africa are predominantly of domestic origin and for that reason are typically sedentary and appear to be restricted to urban areas (Berruti 1991). However, extensive hybridisation with native Yellow-billed Ducks has led to Mallards being included in eradication campaigns (Berruti 1991).

The Yellow-billed Duck is not listed as a species of international concern (IUCN Red List 2015). Further genetic studies are required to confirm the presence of hybrids before the correct management strategy can be determined. The containment of Mallards and their hybrids is currently not a viable option because of the high dispersal ability of Yellow-billed Ducks and potentially their hybrids (Table 2).

Meller's Duck (*Anas melleri*)

Meller's Duck is a monomorphic dabbling duck native to Madagascar that has similar habits and display to the Mallard (Delacour 1956; Kear et al. 2005). Meller's Duck is listed as Endangered (IUCN Red List 2015) with overhunting and agricultural practices believed to be their primary threat (Young & Rhymer 1998). In addition to these threats, Mallards are now thought to be present and breeding in Madagascar (Banks et al. 2008), however there have been no reports of hybrids to date. Genetic studies are required to determine the

conservation risk to the Meller's Duck posed by hybridisation with introduced Mallards.

In addition, both the Mallard and Meller's Duck have been introduced to Mauritius and are known to readily hybridise (Young & Rhymer 1998). While not native to Mauritius, eradicating Mallards and Mallard hybrids from Mauritius will help to ensure the long-term survival of Meller's Duck (Young & Rhymer 1998). Genetic testing will be required to determine the full extent of hybridisation on Mauritius to verify whether pure Meller's Ducks are still present in the population and warrant protection. Following genetic confirmation of hybridisation, eradication and containment of Mallards and their hybrids in both Madagascar and on Mauritius Islands will help to preserve these two Meller's Duck populations, where Meller's Duck face other anthropogenic threats (Table 2).

Brown Teal (*Anas chlorotis*)

The Brown Teal is a sexually dichromatic, relatively large teal species that has a similar call and display to the Mallard (Delacour 1956; Dumbell 1986). It is the only non-mallard species known to be threatened by hybridisation with the Mallard. The Brown Teal is endemic to New Zealand, where it was once widespread, but its range has now been reduced due to anthropogenic influences, such as introduced mammalian predators (Kear et al. 2005; O'Connor et al. 2007). A dramatic decline in numbers has caused the species to become globally threatened (IUCN Endangered)(Gemmell & Flint 2000; Hitchmough 2002; IUCN Red List 2015).

In addition to other anthropogenic pressures, hybridisation between the Brown Teal and the Mallard was detected along the maternal line in Brown Teal (Gemmell & Flint 2000; Kennedy & Spencer 2000). However, extensive levels of hybridisation between the Mallard and New Zealand Pacific Black Duck have made it difficult to ascertain which species or hybrid has hybridised with the Brown Teal (Gemmell & Flint 2000). Investigations of mtDNA have shown that either Mallards or Pacific Black Ducks are the female parent, and thus, the smaller, Brown Teal was the male parent (Gemmell & Flint 2000).

Currently, hybridisation with Mallard has been detected on both the North and the South Island of New Zealand (Gemmell & Flint 2000; O'Connor et al. 2007). Mallards and their hybrids with Pacific Black Duck are already widespread throughout the New Zealand and hybridisation with the Brown Teal appears only to be a threat where the population is small and declining (O'Connor et al. 2007). Furthermore, there is no evidence that the frequency of hybrids has increased in larger populations (O'Connor et al. 2007). Prevention and eradication measures for hybridisation is no longer an option, therefore containment of pure Brown Teal on offshore islands, such as the Great Barrier Island is the only realistic management option to maintain populations of pure Brown Teal (Table 2).

Pacific Black Duck (*Anas superciliosa*)

The Pacific Black Duck is a monomorphic dabbling duck native to the southwest Pacific (Marchant & Higgins 1990; Kear et al. 2005). Like other

species from the mallard complex, the Pacific Black Duck is similar in display, call and habit to the Mallard (Delacour 1956). The Pacific Black Duck is not listed as a species of international concern (IUCN Red List 2015). It is typically divided into three subspecies; *A. s. rogersi* from Australia, New Guinea and Indonesia, *A. s. superciliosa* from New Zealand and outlying islands and *A. s. pelewensis* from Pelew and other islands in the West Pacific (Marchant & Higgins 1990; Rhymer et al. 2004). While *A. s. pelewensis* is slightly smaller, both of the other sub-species cannot be easily differentiated from one another (Frith 1982). mtDNA has revealed that there is greater genetic differentiation between *A. s. superciliosa* on the North and South Island of New Zealand compared to that between sub-species, indicating that the designated sub-species are not supported genetically (Rhymer et al. 2004). The Mallard, which is physically dominant over the Pacific Black Duck, has been introduced to Australia and New Zealand and many of their offshore islands where it has subsequently hybridised with native Pacific Black Ducks (Phillips 1923; Norman 1987; Marchant & Higgins 1990; Rhymer et al. 1994; Williams & Basse 2006; Tracey et al. 2008; Guay & Tracey 2009).

New Zealand

In New Zealand, wild Mallards were released primarily for game purposes (Williams & Basse 2006; Dyer & Williams 2010; Guay et al. 2015). Mallards were first introduced to New Zealand in the 1800s and stocks were further supplemented with introductions from North America and Europe during the 19th and 20th centuries (Williams 1982; McDowall 1994). The first wild hybrid was reported in 1917 and their presence notably increased throughout the

20th century, whilst the numbers of Pacific Black Duck declined (Thomson 1922; Gillespie 1985; Marchant & Higgins 1990). Morphometrics and phenotypes were initially used to differentiate New Zealand Pacific Black Ducks, Mallards and their hybrids (Gillespie 1985). Introgressive hybridisation was later confirmed by analysis of mtDNA (Rhymer et al. 2004) and nuclear DNA (Muller 2009). Pacific Black Ducks in New Zealand are now considered Nationally critical due to hybridisation with introduced Mallards and genetic studies have revealed that there may be few pure Pacific Black Ducks remaining (Rhymer et al. 1994; Rhymer et al. 2004; Muller 2009; Robertson et al. 2012).

Mallards introduced to New Zealand rapidly colonised many offshore islands including the Snares Islands (48°01'S 166°36'E), the Chatham Islands (44°15'S 176°12'W), Campbell Island (52°33'S 169°09'E), Auckland Island (50°29'S 165°52'E), Norfolk Island (29°02'S 167°57'E), Macquarie Island (54°30'S, 158°57'E), and Lord Howe Island (31°33'S, 159°05'E) (Bailey & Sorensen 1962; McKean & Hindwood 1965; Hermes et al. 1986; Norman 1987; Marchant & Higgins 1990; Tennyson 1998; Miskelly et al. 2001). The colonisation of most of these islands were followed by reports of Pacific Black Duck – Mallard hybrids (Bailey & Sorensen 1962; Warham & Keeley 1969; Hermes et al. 1986; Norman 1987; Tracey et al. 2008).

Snares Island

Mallards and the first Pacific Black Duck – Mallard hybrid were originally observed on the Snares Islands in 1968-69 (Warham & Keeley 1969) and

were repeatedly observed throughout the 1970s and 80s (Horning & Horning 1974; Robertson et al. 1981; Miskelly et al. 2001). Breeding Mallards were first observed in 1984-85 and the proportion of Mallards to Pacific Black Ducks remained consistent at around 50% during the 1980s and 1990s (Miskelly et al. 2001). Observations made in 2000 indicate a dramatic decline in the numbers of Pacific Black Duck on Snares Island with 24 observations of Mallard and none of Pacific Black Duck (Miskelly et al. 2001).

Chatham Islands

Mallards have been observed on the Chatham Islands since the 1950s and were continually observed through the 1960s and in relatively large flocks in the 1980s (Freeman 1994). Conversely, Pacific Black Duck on the Chatham Islands were originally observed in large flocks in the 1950s, whilst their numbers appeared to have decreased by the 1980s (Freeman 1994). Tennyson (1998) confirmed that breeding Mallards existed on the Chatham Islands, in greater numbers than Pacific Black Ducks.

Campbell Island

Pacific Black Ducks were observed on Campbell Island in 1840 and were observed throughout the 1940-50s (Bailey & Sorensen 1962). The first observation of Mallard on Campbell Island was made in 1960 and specimens with mixed phenotypes were also observed (Bailey & Sorensen 1962). The status of hybridisation on Campbell Island is currently unknown.

Norfolk Island

Mallards were first reported on Norfolk Island in 1976 (McKean et al. 1976). 100 wild adult and juvenile Mallards were banded on Norfolk Island during 1982-83, and four of the juveniles were recorded in New Caledonia, Vanuatu and New Zealand the following year (Hermes et al. 1986), indicating high levels of dispersal. A high rate of hybridisation has also been reported amongst feral domestic Mallards and Pacific Black Duck (Hermes et al. 1986).

Macquarie Island

Pacific Black Ducks have been observed on Macquarie Island since it's discovery in 1810 (Norman 1987). The first Mallard reported on the island in 1949 was probably a vagrant (Gwynn 1953). Breeding populations of Pacific Black Ducks were first described in the 1950s, however, Mallard populations did not appear to be established until 1975 (Norman 1987). The first hybrid was observed on the island in 1973, although it was unclear as to whether the hybrid was bred locally or whether it had immigrated to the island (Norman 1987). Mallards and their hybrids have since become more frequent on the island, and most ducks now observed are hybrids (Norman 1987; 1990).

Lord Howe Island

Breeding populations of Pacific Black Duck were first observed on Lord Howe Island in 1852 and have since been infrequently observed (MacDonald 1853; Hindwood & Cunningham 1950; Rogers 1972). Mallards colonised the island in 1963, probably migrating from New Zealand (McKean & Hindwood 1965; Tracey et al. 2008). Since 1975, reports of Pacific Black Ducks on the island

were thought to have been Mallard hybrids (Rogers 1976). Survey efforts have since indicated that pure Pacific Black Ducks may now be extinct on Lord Howe Island (Hutton 1991; Tracey et al. 2008).

Australia

In contrast to Mallards released in New Zealand, Mallards in Australia are typically of domestic origin (Guay & Tracey 2009). This has led to the hypothesis that domestic Mallards and their hybrids are restricted to urban areas due to their poor dispersal capabilities, where they pose no threat to native Pacific Black Ducks (Braithwaite & Norman 1974; Guay & Tracey 2009). Phenotype studies have suggested that hybridisation is spreading in rural areas (Paton et al. 1992; Guay & Tracey 2009). The occurrence of hybrids in Australia was genetically investigated in this study (Chapter 4).

Pacific Black Duck management strategies

In the case of New Zealand where hybridisation is extensive and eradication is not feasible translocation management plans should be implemented. Rhymer et al. (2004) suggested that if genetically pure Pacific Black Ducks still exist, they should be translocated to offshore islands where Mallard numbers can be controlled (Table 2). On offshore islands (i.e., Macquarie Island and Lord Howe Island) where Mallards and their hybrids can be managed more easily, eradication efforts should be in place, with an overall aim for Pacific Black Ducks to re-colonise the islands from Australia.

In contrast, because the overall level of hybridisation in Australia is unknown, genetic testing of putative Pacific Black Ducks is required to determine the overall threat and to make the correct management decision (Guay & Tracey 2009; Guay et al. 2014). Meanwhile, we suggest that Mallards and hybrid outbreaks should be eradicated and not contained as Pacific Black Duck hybrids may have inherited the Pacific Black Ducks ability of long-distance flight (Table 2).

Conclusion

Overall, the threat of hybridisation with Mallards is predominant where hybridisation cannot be isolated and culling is impractical. For example, species on large continents where Mallards also naturally occur are under the most threat (e.g., the American Black Duck, the Mottled Duck, the Mexican Duck and the Spot-billed Duck). On the other hand, the New Zealand Pacific Black Duck also faces extinction due to extensive and widespread hybridisation with introduced Mallard. In such cases, captive breeding programs of genetically pure individuals is often the only option left to preserve the species under threat. In other circumstances where hybridisation can be isolated (e.g., islands) and where dispersal is low (e.g., domestic Mallards) Mallards and their hybrids should be eradicated or potentially contained (e.g., the Hawaiian Duck, the Australian Pacific Black Duck, the Yellow-billed Duck, Meller's Duck and the Brown Teal). In contrast, where hybridisation has not been recorded (e.g., the Philippine Islands), but where Mallards are known to be present, preventative measures, such as ongoing monitoring and eradication of feral Mallard populations should be in

place. While the vast majority of the species mentioned face a bleak future, ongoing genetic monitoring will help to confirm that some species that have not already undergone extensive hybridisation uphold their genetic integrity. The Mallard appears to have an expanding range in most parts of the world due to Mallard releases for hunting and releases of unwanted domestic Mallards. Misinformed management may lead to the protection of hybrid swarms. For example, the Marianas Mallard (*A. oustaleti*) was later discovered to be a Pacific Black Duck – Mallard hybrid population and the Pacific Black Duck from the Marianas Islands is now extinct (Yamashina 1948). While ongoing hybridisation with Mallards can lead to the local extinction of a species, it also adds to a greater loss of biodiversity triggered by human alterations to the natural environment.

Table 2. The list of species that Mallard threaten due to the potential of ongoing hybridisation together with their management recommendations. N/A is listed in columns where the management recommendation is not applicable, because an earlier management technique has been recommended.

Species	More genetic testing required	Prevention	Eradication	Containment	No control	Captive breeding
Brown Teal	✓	× Too late	× Mallard too widespread	✓ Achievable	N/A	N/A
Mexican Duck	✓	× Too late	× Mallard too widespread	× Not viable	Last option	Last option
Mottled Duck		× Too late	× Mallard too widespread	× Not viable	Last option	Last option
Philipppine Duck	✓	✓ Achievable	N/A	N/A	N/A	N/A
Meller's Duck	✓	× Too late	✓ Achievable	✓ Achievable	N/A	N/A
Spot-billed Duck		× Too late	× Not an option	× Not viable	Last option	Last option
American Black Duck		× Too late	× Mallard too widespread	× Not viable	Last option	Last option
New Zealand Pacific Black Duck		× Too late	× Mallard too widespread	× Not viable	Last option	Last option
Australian Pacific Black Duck	✓	× Too late	✓ Achievable	N/A	N/A	N/A
Yellow-billed Duck	✓	× Too late	✓ Achievable	× Not viable	N/A	N/A
Hawaiian Duck	✓	× Too late	✓ Achievable	✓ Achievable	N/A	N/A

Chapter 3

Establishing a genetic system to distinguish between domestic Mallards, Pacific Black Ducks and their hybrids.

Taysom, A. J., Johnson, J. & Guay, P.-J. (2014). *Conservation Genetics Resources*. **6**, 197-199. (Appendix 1).

Abstract

Dabbling ducks are subject to many threatening processes. Hybridisation with introduced Mallards (*Anas platyrhynchos*) is a threat that is commonly overlooked. Domestic Mallards have been introduced to Australia and are known to hybridise with native Pacific Black Ducks (*A. superciliosa*). While Mallards are known to threaten other dabbling duck species across the world due to ongoing hybridisation, the degree of hybridisation between Mallards and Pacific Black Ducks in Australia is currently unknown. The majority of studies conducted in Australia to date have identified hybrids using intermediate phenotype characteristics. However, phenotype-based studies generally underestimate the frequency of hybridisation. A more robust method to reliably identify hybrids using genetic analysis, such as microsatellite markers, was therefore needed. In total, 27 cross-amplifying waterfowl microsatellite markers were screened and a set of 9 markers was identified that could be used for genotyping and assignment tests to identify cryptic hybrids. Using a model-based clustering method, specimens of a known species (Mallard or Pacific Black Duck) were assigned to the correct group, with a probability of $q < 0.10$ for Mallards and $q > 0.90$ for Pacific Black Ducks. The system was used to confirm the hybrid status of two phenotypically-

identified putative hybrids. The successful application of this system demonstrates its potential to effectively determine the frequency of hybridisation between introduced Mallards and Pacific Black Ducks throughout Australia and hence the level of threat posed by Mallards towards Pacific Black Ducks.

Introduction

Species throughout the world are subject to many threatening processes (e.g., habitat destruction, climate change, introduction of exotic species) (Chapin et al. 2000). While the introduction of exotic species may add an additional competitive pressure, many species are also subject to the threat of hybridisation with introduced species (e.g., Dowling & Childs 1992; Perry et al. 2001; Munoz-Fuentes et al. 2007). The Mallard (*Anas platyrhynchos*), native to the northern hemisphere, is known to hybridise with and threaten many other dabbling duck species (genus: *Anas*) with which it co-occurs (Chapter 2). The Mallard has been introduced, as both game and ornamental birds, to many parts of the world, including Australia, where they encounter, hybridise and consequently threaten many native dabbling duck species (Chapter 2). Before the widespread use of genetic technology, Mallard hybrids were identified using intermediate phenotype characteristics (e.g., Braithwaite & Miller 1975; Stutzenbaker 1988; Paton et al. 1992; Rhymer et al. 1994; Kirby et al. 2000). While this method can be useful in identifying first generation hybrids, it can underestimate the overall frequency of hybrids as later generation hybrids, or cryptic hybrids, are often overlooked (Green et al. 2000; Fowler et al. 2009).

In contrast, genetic studies have proven vital in more accurately determining the extent of hybridisation (e.g., Browne et al. 1993; Kulikova et al. 2004; Mank et al. 2004; Williams et al. 2005; Fowler et al. 2009; Muller 2009). Various molecular techniques can be utilised to examine hybridisation, including the use of allozymes, mitochondrial DNA (mtDNA) markers and nuclear DNA markers (reviewed in Guay et al. 2014). Nuclear DNA markers, such as single nucleotide polymorphisms (SNP) and microsatellite markers, are the most reliable method to identify hybridisation because they are not reliant on the bird's phenotype and they do not only measure hybridisation along the maternal line, as is the case for mtDNA markers (reviewed in Guay et al. 2014). Nonetheless, mtDNA markers can also be useful to determine the directionality of hybridisation (Watanabe et al. 1985) and the level of introgression in cases where little genetic material is available (e.g. small feathers or historic specimens).

The Pacific Black Duck (*A. superciliosa*), which is native to Australia, co-occurs and hybridises with introduced domestic Mallard, which are now common in Australia (Marchant & Higgins 1990). Until now, the majority of the work identifying Pacific Black Duck - Mallard hybrids in Australia has been conducted using phenotype-scoring keys (Braithwaite & Miller 1975; Paton et al. 1992). While identifying individual cases of hybrids using phenotype can be useful, larger-scale studies identifying the Australia-wide rate of hybridisation require more robust methods that can be used to identify hybrids from a large number of individuals. The aim of this study was to establish a

robust genetic system that could accurately discriminate between domestic Mallards, Pacific Black Ducks and their hybrids using DNA microsatellite markers.

Methods

Genetic samples (blood or tissue) from 10 putatively-pure Pacific Black Ducks, 10 domestic Mallards and two putative hybrids that were identified based on phenotype were collected throughout Australia from 2006 through 2012 (Table 1). DNA was extracted using a salting-out procedure (Bruford et al. 1992).

Table 1. Specimens used in the study, including 10 putative domestic Mallard, 10 putative Pacific Black Duck and two putative hybrids.

ID number	Species	Date	Locality
AT157	*Anas platyrhynchos*	6 Jan 2012	Luv-A-Duck retailers, Frankston, Victoria
AT158	*Anas platyrhynchos*	6 Jan 2012	Luv-A-Duck retailers, Frankston, Victoria
AT159	*Anas platyrhynchos*	8 Jan 2012	Luv-A-Duck retailers, Frankston, Victoria
PJG035	*Anas platyrhynchos*	Unknown	Ballarat, Victoria
PJG036	*Anas platyrhynchos*	Unknown	Ballarat region, Victoria
PJG037	*Anas platyrhynchos*	Unknown	Ballarat region, Victoria
PJG039	*Anas platyrhynchos*	Unknown	Ballarat, Victoria
PJG390	*Anas platyrhynchos*	20 Feb 2006	Ballarat, Victoria
PJG392	*Anas platyrhynchos*	20 Feb 2006	Ballarat, Victoria
PJG503	*Anas platyrhynchos*	14 May 2007	Ballarat, Victoria
PJG844	*Anas superciliosa*	21 Mar 2009	Reedy Lake, Leopold, Victoria
PJG848	*Anas superciliosa*	21 Mar 2009	Reedy Lake, Leopold, Victoria
T184	*Anas superciliosa*	2 Mar 2006	Waterhouse Lake, Tasmania
T186	*Anas superciliosa*	2 Mar 2006	Waterhouse Lake, Tasmania
T187	*Anas superciliosa*	2 Mar 2006	Waterhouse Lake, Tasmania
T192	*Anas superciliosa*	2 Mar 2006	Waterhouse Lake, Tasmania

ID number	Species	Date	Locality
T194	*Anas superciliosa*	2 Mar 2006	Waterhouse Lake, Tasmania
T195	*Anas superciliosa*	2 Mar 2006	Waterhouse Lake, Tasmania
T196	*Anas superciliosa*	2 Mar 2006	Waterhouse Lake, Tasmania
T197	*Anas superciliosa*	2 Mar 2006	Waterhouse Lake, Tasmania
AT031	*Anas platyrhynchos* / *Anas superciliosa* putative hybrid	Mar 2011	Reedy Lake, Leopold, Victoria
AT156	*Anas platyrhynchos* / *Anas superciliosa* putative hybrid	2012	Shepparton, Victoria

A total of 27 waterfowl microsatellites were screened for cross-amplification in both Pacific Black Ducks and Mallards (Table 2). Primer pairs that consistently amplified a product were screened for polymorphism. The forward primer of each primer pair was synthesized with a 5'-M13 Tag (5'CACGACGTTGTAAAACGAC) for use in the universal dye labeling method (Boutin-Ganache et al. 2001). PCR reactions (10μL) contained 0.25 units Taq polymerase (Promega), $MgCl_2$ (Table 3), 1X reaction buffer, 200μM dNTPs, 300nM of an M13 primer 5'-labelled with an ABI dye (NED, FAM, VIC and PET), the locus-specific tailed (20nM) and untailed (300nM) primers and approximately 100ng of genomic DNA. The reactions were heated to 95°C for 60 seconds, followed by 40 cycles of 94°C for 20 seconds, 55°C for 30 seconds, 73°C for 90 seconds, with a final extension step of 73°C for 5 min on a MyCycler Personal Thermal Cycler (Biorad). PCR product sizes were scored commercially (Australian Genomic Resource Facility, AGRF). AGRF used AB3730 DNA analyser and GeneMapper software for DNA sizing and allele calls for electrophoresis (Applied Biosystems). PCR and genotyping for all specimens was completed twice to reduce the possibility of PCR errors.

Polymorphic markers were tested for linkage disequilibrium using GENEPOP 4.2 (Raymond & Rousset 1995; Rousset 2008) and for deviation from the Hardy-Weinberg equilibrium using FSTAT 2.9.3 (Goudet 1995). Finally, each locus was tested for allele frequency differences to test of species differences by calculating loci-specific pairwise F_{ST} using FSTAT (Table 3) and MICROCHECKER (van Oosterhout et al. 2004) was used to detect null alleles.

Table 2. Published microsatellite loci are listed below. Each forward primer had an additional M13 tag added to its 5' end, with the exception of Blm5, Blm10 and Blm12 that had the M13 tag added to the reverse primer. Those in bold font amplified successfully and consistently and were incorporated into the genotyping system.

Locus	Primer sequence	References	Focal species from original study	GenBank accession numbers
Aph12	F: M13-TTAGTAGCATGTCAGGTTTATT R: GCTTGTAGACTTCAGAGTTC	Maak et al. 2000	*Anas platyrhynchos*	AJ515888
Aph13	F: M13-CAACGAGTGACAATGATAAAA R: CAATGATCTCACTCCCAATAG	Maak et al. 2000	*Anas platyrhynchos*	AJ515889
Aph15	F: M13-TGAATATGCGTGGCTGAA R: CAGTGAGGAATGTGTTTGAGTT	Maak et al. 2000	*Anas platyrhynchos*	AJ515890
Aph16	**F: M13-CCTTCTGAACCTTCGTAG R: AAATATAGACTTTTGTCCTGAA**	**Maak et al. 2000**	***Anas platyrhynchos***	**AJ515891**
Aph17	F: M13-GGACATTTTCAACCATAAACTC R: CATCCATGACAGACAGAAGA	Maak et al. 2000	*Anas platyrhynchos*	AJ515892
Aph19	F: M13-CATGGAGCAAGCAATCGTCTG R: ACCACGTCCATCCTGAAGAAA	Maak et al. 2000	*Anas platyrhynchos*	AJ515894
Aph20	F: M13-ACCAGCCTAGCAAGCACTGT R: GAGGCTTTAGGAGAGATTGAAAAA	Maak et al. 2000	*Anas platyrhynchos*	AJ515895
Aph21	F: M13-CTTAAAGCAAAGCGCACGTC R: AGATGCCCAAAGTCTGTGCT	Maak et al. 2000	*Anas platyrhynchos*	AJ515896
Aph23	F: M13-TCCTCTGCTCTAGTTGTGATGG R: CCTCAGCAGTCTTCCTCAGTG	Maak et al. 2000	*Anas platyrhynchos*	AJ515898
Aph24	F: M13-TCAACCAGTGGTCAGAGAAAAA R: AGGTCAGCCCCCATTTTAGT	Maak et al. 2000	*Anas platyrhynchos*	AJ515899

Locus	Primer sequence	References	Focal species from original study	GenBank accession numbers
Aph25	**F: M13-CCGTCAGACTGTAGGGAAGG R: AAAGCTCCACAGAGGCAAAG**	**Maak et al. 2000**	***Anas platyrhynchos***	**AJ515900**
Apl11	**F: M13-AACTACAGGGCACCTTATTTCC R: TTGCATCAGGGTCTGTATTTTC**	**Denk et al. 2004**	***Anas platyrhynchos***	**AY498541**
Bcau4	**F: M13-ACAACCTTCAAAGTCAATCCAAT R: TCCTGACGCTCTCGGACGAGT**	**Buchholz et al. 1998**	***Anas platyrhynchos***	**AF025892**
Bcau6	F: M13-TTTAACCCAGTAGCCTATCATGTCA R: GTCTGAAGATAATGCTGCATGGTT	Buchholz et al. 1998	*Anas platyrhynchos*	AF025894
Bcau11	F: M13-TAGAAAAGGCTGAAGGAGTGGC R: TGAGGAAGCAACTGTAAATAGGAGA	Buchholz et al. 1998	*Anas platyrhynchos*	AF025899
Blm5	F: GCCACTTCTTTTGAAGTCACC R: M13-GAAGCATCTTGTATGGCTTGC	Guay & Mulder 2005	*Biziura lobate*	AY766439
Blm10	F: CAAAGTATATCTTCTCAGGGACACG R: M13-TGCATTGCTGTGAAGAGACC	Guay & Mulder 2005	*Biziura lobate*	AY766443
Blm12	F: TTCTGTGGGAGAAGACAAAGG R: M13-ACTTGCCTGCTTCACTCC	Guay & Mulder 2005	*Biziura lobate*	AY766445
CM09	**F: M13-GGATGTTGCCCCACATATTT R: TTGCCTTGTTTATGAGCCAT**	**Maak et al. 2000**	***Anas platyrhynchos***	**AJ271212**
Cam2	F: M13-TCCACAAGGACACCATTAGG R: GGTATTTCTTTTGC	Carew et al. 2003	*Cygnus atratus*	AY130973
Cam3	**F: M13-AACATCTACTTTGGCCTCTCC R: TCTGTGCCCTGTTCTACTGC**	**Carew et al. 2003**	***Cygnus atratus***	**AY130974**
Cam9	F: M13-AATTGCAGCACTAATGAGC R: GCTCATCAATCAAAACATTCC	Carew et al. 2003	*Cygnus atratus*	AY130978
CmAAT 16	F: M13-TCCCAAGGGTACCAGTGAA R: TGTTGGCTCCCTGCTTAAAT	Stai & Hughes 2003	*Cairina moschata*	AF509882
Sfiu2	**F: M13-ATAAACGGCTAATATGAAGTCT R: AGGCTAGATATTGCTCTTATCCT**	**Fields & Scribner 1997**	***Somateria fischeri***	**U63682 U63683**
Sfiu4	F: M13-TGAGGGGGAAGAGAATAAGAGA R: CAGGGCAGTATTTTCAGGACATT	Fields & Scribner 1997	*Somateria fischeri*	U63685
Smo7	**F: M13-TTTTCACCCAGTTCACTTCAGCC R: GATTCAAATTTGCCGCAGGATTA**	**Paulus & Tiedemann 2003**	***Somateria mollissima***	**AJ427847**
JCC1	**F: M13-GGATTGGAGATTTTCAGGAGC R: AGGGAACTGATGCCCCA**	**Kim et al. 1991; Wistow et al. 1988**	***Anas platyrhynchos***	**M55132**

Assignment tests were performed using the admixture model with no prior population information on STRUCTURE 2.3.4 (Pritchard et al. 2000) with a burn-in period of 10,000, followed by 1,000,000 replicates, within the burnin range recommended by STRUCTURE and longer run length to obtain a more accurate estimate of the likelihood, as described by Williams et al. (2005). Following Williams et al. (2005), individuals were assigned to the following groups based on their *q* value: Mallard, $q < 0.10$; Pacific Black Duck – Mallard hybrid, $0.10 < q < 0.90$; Pacific Black Duck, $q > 0.90$. Genetic cluster assignment was performed using the method described by Steeves et al. (2010), where 10 independent replicates, using the same burn-in period and number of replicates as above, with different random seeds were run for $K = 1$ and $K = 2$. Estimates of the mean posterior probability were compared using a likelihood ratio test. As recommended by Evanno et al. (2005), *ad hoc* criteria was applied to best estimate whether the number of genetic clusters was one or two. For example, the widely accepted method by Evanno et al. (2005) (see Merrill et al. 2015; Tong et al. 2015; Rato et al. 2016; Kurokochi et al. In press). This method estimates the most likely number of clusters based no the change in log-likelihood probabilities values for each *K* value. Because this method cannot calculate the change in log-likelihood probabilities for $K = 1$, we also incorporated the less accepted method for estimating genetic clusters by Pritchard et al. (2000), which estimates the number of putative clusters based on the lowest value of estimated log probability of the data was applied.

Table 3. Microsatellite loci used in this study with magnesium chloride concentrations, allele size, number of alleles, expected and observed heterozygosity and pairwise F_{ST}.

Locus	$MgCl_2$ (mM)	Pacific Black Duck				Mallard				F_{ST}
		No. of alleles	Allele size range (bp)	H_e	H_o	No. of alleles	Allele size range (bp)	H_e	H_o	
Aph16	5	2	162-164	0.267	0.300	3	162-166	0.194	0.200	0.469
Aph25	2.5	1	185	0.000	0.000	2	185-187	0.478	0.500	0.505
Apl11	2.5	4	111-131	0.578	0.100	5	109-139	0.833	0.778	0.336
Bcaμ4	5	4	207-213	0.783	0.200	1	211	0.000	0.000	0.397
Cam3	2.5	4	167-177	0.561	0.500	3	168-175	0.361	0.200	0.769
CM09	5	5	120-140	0.761	0.600	5	120-128	0.811	0.500	0.143
Sfiμ2	5	2	391-393	0.500	0.100	1	383	0.100	0.000	0.750
Smo7	5	4	202-224	0.694	0.400	2	202-206	0.522	0.500	0.359
JCC1	5	7	167-181	0.833	0.800	6	165-180	0.772	0.600	0.231

Results

Of the 27 polymorphic loci screened 15 failed to amplify in Mallard or Pacific Black Duck and three amplified, but were monomorphic between species. A total of nine loci that consistently amplified and produced significant allele frequency differences between the two species were selected for further analyses (Table 3). No null alleles were detected and following Benjamini-Yekutieli correction for multiple tests ($\sigma = 0.05$; Narum 2006), no significant linkage disequilibrium or deviation from the Hardy-Weinberg equilibrium was detected (Table 3).

Assignment tests confirmed that all individuals were allotted to their expected species (Figure 1). The most likely number of genetic clusters, as determined by STRUCTURE, was two. Pure Pacific Black Ducks and domestic Mallards were assigned to their species cluster at a probability of $q < 0.10$ for Mallards

and q > 0.90 for Pacific Black Ducks. The two hybrids had assignment probability to the Pacific Black Duck cluster of 0.56 and 0.77 respectively (Figure 1).

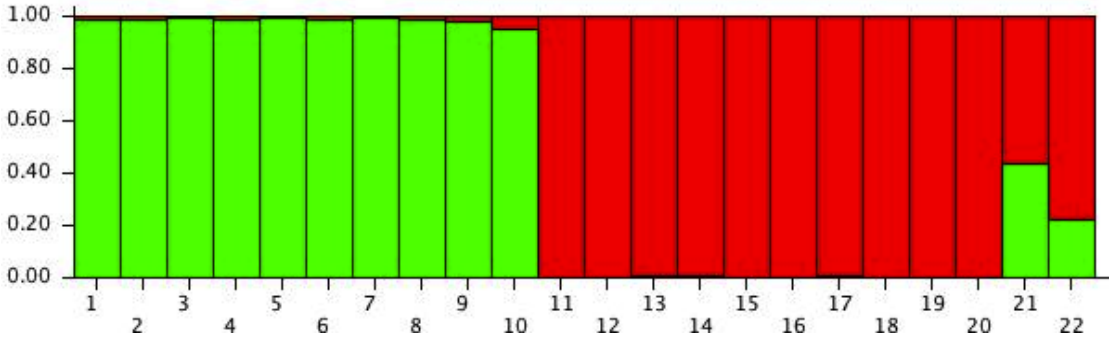

Figure 1. STRUCTURE 2.3.4 (Pritchard et al. 2000) output displaying the proportion of membership based on genetic information for 2 groups, where individuals 1-10 are domestic Mallards and 11-20 are putatively pure Pacific Black Ducks. Specimens 21 and 22 are putative hybrids, where the proportion of membership to the putatively pure Pacific black Duck group is 0.56 and 0.77 respectively.

Discussion

The selected microsatellite markers allowed successful assignment of ducks to either of the two species studied. Furthermore, this set of markers, was able to confirm the hybrid ancestry of ducks putatively identified as hybrids based on phenotype. These results demonstrate that the set of markers described here will allow the unambiguous identification of first generation hybrids, thereby permitting the evaluation of the hybrid frequency of at least first generation hybrids in Australia (Chapters 4 & 5).

This set of markers may also have a wide applicability in determining levels of hybridisation with domestic Mallards, which is recognised as a worldwide

problem (Chapter 2), and between other native dabbling duck species (Chapter 7). While Fowler et al. (2009) adapted mircosatellite and AFLP markers to identify domestic Mallard and Hawaiian Duck (*Anas wyviliana*) hybrids, most other microsatellite marker sets have been developed to determine the frequency of hybridisation in other cases of hybridisation involving wild Mallards (e.g., Williams et al. 2005; Muller 2009). Even though hybridisation with wild Mallards appears to be a larger threat to species decline compared to hybridisation with domestic Mallard (Chapter 2), the former is more intensively studied (e.g., Goodwin 1956; Johnsgard 1967; Ankney et al. 1987; Rhymer et al. 1994; Kulikova et al. 2004; Mank et al. 2004; Williams et al. 2005; Muller 2009) and thus the latter warrants further research.

Vaha and Primmer (2006) suggested that 12 loci with pairwise F_{ST} = 0.21 is sufficient to identify between first generation hybrids and pure species. While this study incorporated slightly less loci than Fowler et al. (2009) and Muller (2009), the pairwise F_{ST} value for each loci was still generally higher than 0.21. Overall, this microsatellite system will allow for the identification of domestic Mallards, Pacific Black Ducks (and potentially other dabbling ducks) and their hybrids, but could be improved by incorporating more microsatellite and other nuclear markers, such as single nucleotide polymorphisms (SNPs). The high polymorphism rate of nuclear markers allows for allele frequency differences to be detected between species, as well as the recognition of hybrids (Vali et al. 2010). A larger number of markers with species-specific allele frequencies are required if species have only recently diverged, if

introgressive hybridisation has occurred over a long period and if backcrosses and later-generation hybrids are to be distinguished (Vaha & Primmer 2006; Vali et al. 2010).

Chapter 4

Hybridisation between introduced Mallards (*Anas platyrhynchos*) and Pacific Black Ducks (*Anas superciliosa*) throughout Australia.

Unpublished manuscript

Abstract

The Mallard (*Anas platyrhynchos*) has been introduced throughout the world and is known to threaten many other species with which it co-occurs. In the south-western Pacific the Mallard is known to threaten the Pacific Black Duck (*A. superciliosa*) via hybridisation throughout their overlapping range. In New Zealand, for example, a Pacific Black Duck subspecies, *A. s. superciliosa*, hybridises widely with introduced Mallards. In contrast, the frequency of hybridisation between the introduced Mallard and the Australian Pacific Black Duck (*A. s. rogersi*) is currently unknown. Unlike Mallards released in New Zealand, Mallards in Australia are typically of a domestic origin and occur mostly in and around cities and thus were thought to pose no threat to rural Pacific Black Ducks. Historically, Pacific Black Duck – Mallard hybrids in Australia have been identified using either phenotype scoring keys based on parental Mallards with wild phenotypes or genetically using allozymes. Both of these methods have limitations. While phenotypes are thought to underestimate the true percentage of hybrids, allozymes are not as informative as other genetic markers due to their relatively low variability. This study evaluated the rate of hybridisation between Mallards and Pacific Black Ducks using an established set of nine microsatellite markers known to allow reliable hybrid identification. This is the first comprehensive study from

Australia to investigate the frequency of hybridisation. The study incorporated a large sample of 390 putative Pacific Black Ducks from six Australian states and King Island (Vic [100], NSW [53], Tas [100], QLD [21], SA [43], WA [55] and KI [18]). The results show that only 1.5% of ducks sampled possessed genotypes indicative of hybrid origins. The Australian Pacific Black Duck, therefore, does not appear to be at imminent risk of extensive hybridisation with introduced Mallards. Continual monitoring will best address the risk of hybridisation in the future.

Introduction

Introduced species threaten native species via competitive exclusion, niche displacement, predation and hybridisation, all of which can ultimately lead to extinction (Mooney & Cleland 2001). Hybridisation, the interbreeding of two distinct species, is known to threaten many plant and animal species (Mallet 2005). It is especially threatening, when compounded by human activities, such as habitat modification and fragmentation, which can remove barriers and thus promote interspecific hybridisation (Allendorf et al. 2001; Seehausen et al. 2007). For example, the Ethiopian Wolf (*Canis simensis*), which is known to be in decline due to habitat loss and extermination by humans, is also known to be threatened by hybridisation with sympatric domestic dogs (Gottelli et al. 1994). The influence of anthropogenic practices in this region has increased in the incidence of hybridisation between these two species.

Hybridisation can eventually lead to the extinction of a species via either outbreeding depression or introgression. Outbreeding depression can lead to

reduced fitness and survivorship in hybrid offspring, through embryonic lethality, hybrid sterility or an inability of young to cope with environmental conditions (e.g., Edmands 2007). On the other hand, introgression is the invasion of foreign genetic material into a genome via hybridisation (Mallet 2005). For example, introgressive hybridisation was detected between populations of Rock Partridge (*Alectoris graeca*) and domestic Chukars (*Alectoris chukar*) using microsatellites loci (Barilani et al. 2007). Widespread hybridisation between the two species suggests that the genetic integrity of the Rock Partridge is threatened by the infiltration of Chukar genes into their gene pool (Barilani et al. 2007).

Hybridisation is known to be common amongst waterfowl and in particular with Mallard (*Anas platyrhynchos*) (McCarthy 2006). The Mallard is native to the northern hemisphere (Kear et al. 2005) and is known to hybridise with most other dabbling ducks with which they co-occur (reviewed in Chapter 2). The Mallard is the only sexually dichromatic species within the mallard complex and is also often larger and dominant over other closely-related species (Delacour 1956; Brodsky & Weatherhead 1984; Brodsky et al. 1988; Marchant & Higgins 1990). It is thought that Mallards may hybridise with other dabbling duck species through forced extra-pair copulation or mispairing (Guay et al. 2014). Furthermore, the popularity of the Mallard amongst humans for aesthetic and game purposes has led to many introductions throughout the world (e.g., Milstein & Osterhoff 1975; Marchant & Higgins 1990). The outcome of these human introductions is that Mallards now co-

occur with and consequently threaten many species of dabbling ducks (reviewed in Chapter 2).

The Pacific Black Duck (*Anas superciliosa*) is a sexually monomorphic dabbling duck that is native to the southwest Pacific (Marchant & Higgins 1990) and consists of three putative subspecies. *A. s. rogersi* occurs in Australia, New Guinea and Indonesia, *A. s. superciliosa* is distributed throughout New Zealand and outlying islands, while *A. s. pelewensis* occurs in Palau and other islands in the west Pacific (Marchant & Higgins 1990; Rhymer et al. 2004). Introduced Mallards are known to hybridise with all three sub-species of Pacific Black Ducks and produce fertile hybrids (Guay & Tracey 2009). For example, Mallards, released in New Zealand during the 19th and the 20th centuries, have colonised the entire country, including many offshore islands, leading to ongoing hybridisation and a corresponding decline in the number of Pacific Black Ducks (Gillespie 1985; Marchant & Higgins 1990; McDowall 1994; Dyer & Williams 2010). A genetic study incorporating microsatellite markers confirmed that few pure New Zealand Pacific Black Ducks remain (Muller 2009).

In contrast to the situation in New Zealand, Mallards in Australia are typically of domestic origin and are thought to be restricted to urban areas (Braithwaite & Norman 1974; Marchant & Higgins 1990; Guay & Tracey 2009). The Mallard was first introduced to mainland Australia in the 1860s, but unlike New Zealand, Mallards in Australia have not yet colonised the entire country (Marchant & Higgins 1990). The Pacific Black Duck, however, has a much

larger distribution than sedentary Mallards, being found throughout Australia (Marchant & Higgins 1990; Barrett et al. 2003). Hybridisation between the two species was originally thought to be restricted to urban areas as most reported cases of hybridisation were from urban habitats (Braithwaite & Miller 1975; Whatmough 1978; Smith & Smith 1990; Paton et al. 1992; Bielewicz & Bielewicz 1996; Guay 2010) and hybrids had not been recorded in hunters bag surveys from rural areas (Braithwaite & Norman 1974; 1976). It was therefore believed that Mallard hybridisation posed no threat to rural Pacific Black Duck populations (Braithwaite & Miller 1975). However, phenotypic studies have suggested that hybridisation is spreading in rural areas of South Australia (Paton et al. 1992) and hybrids are now harvested by hunters in New South Wales, Victoria and Tasmania (Guay & Tracey 2009). Despite these indications that hybridisation between the two species may be increasing, a systematic survey of hybrids has not been undertaken to assess the risk posed by the Mallard to the Pacific Black Duck.

This chapter describes an investigation into the frequency of hybridisation in an Australia-wide sample of Pacific Black Ducks using a microsatellite genotyping system developed for distinguishing between Pacific Black Ducks, Mallards and their hybrids (Chapter 3). The information that this study will contribute a pivotal assessment of the threat posed by Mallards to native Pacific Black Ducks, and thus the development of appropriate management strategies.

Methods

Sampling

A total of 390 putative Pacific Black Ducks and 10 domestic Mallards were sampled throughout Australia from 2005 to 2012. The putative Pacific Black Ducks were sampled from urban and rural settings in six Australian states and King Island (Victoria [100], New South Wales [53], Tasmania [100], Queensland [21], South Australia [43], Western Australia [55] and King Island [18]) and were not used in Chapter 3.

Birds were either caught by hand, using bread to lure ducks closer before lunging at the bird to trap the bird between the catcher and the ground, or shot by hunters. Birds caught by hand had 50μl of blood sample taken from the tarsal vein using a 26-gauge needle. Hunters provided tissue from the neck or webbing from the foot of shot birds. Samples were stored in 70% ethanol to prevent deterioration of the tissue/blood. DNA was extracted using a salting-out procedure (Bruford et al. 1992).

Genotyping

Microsatellite markers were chosen in this study because they can reliably determine the hybridisation status of individuals without relying on the phenotype of the bird. Nine microsatellite loci that have been shown to distinguish between Mallards, Pacific Black Ducks and their hybrids (Chapter 3) were used to genotype all individuals. PCR reactions were conducted as described in Chapter 3. Fragment analysis was conducted commercially (Australian Genomic Resource Facility, AGRF).

Analysis of genotypic data

Assignment tests were performed using the admixture model with no prior population information on STRUCTURE 2.3.4 (Pritchard et al. 2000) with a burn-in period of 10,000, followed by 1,000,000 replicates, as described by Williams et al. (2005). Genetic cluster assignment was performed using the method described by Steeves et al. (2010), where 10 independent replicates, using the same burn-in period and number of replicates as above, with different random seeds were run for $K = 1$ and $K = 2$ (see Chapter 3).

Hybrid detection performance

To further assess the microsatellite assay, the assignment power of the microsatellite loci was tested. In Chapter 3, a hybrid status was assigned to any specimen with $0.10 < q < 0.90$, following Williams et al. (2005). The relatively larger sample size obtained in this dataset comparative to Chapter 3 has allowed further characterisation of the microsatellite set. Here, STRUCTURE was used to identify five categories of species affiliation. Following Vali et al. (2010), individuals were assigned to the following groups based on their q value: Mallard, $q < 0.10$; backcross F_1 x Mallard, $0.15 < q < 0.35$; F_1, $0.40 < q < 0.60$; backcross F_1 x Pacific Black Duck, $0.65 < q < 0.85$; Pacific Black Duck, $q > 0.90$. Other hybrid combinations (e.g., F_2 hybrids) were not tested for because it was unlikely that they would be able to be detected given the number of microsatellite markers used (Vaha & Primmer 2006). HYBRIDLAB 1.0 (Nielsen et al. 2006), a program widely used to statistically validate the distribution of hybrids (e.g., Oliveira et al. 2008; Cullingham et al. 2011; van Dongen et al. 2014), was used to simulate 50

hybrids for each of the three hybrid categories. Simulations were performed on genetic data obtained from pure Mallards identified in Chapter 3 and 390 putative Pacific Black Ducks used in this chapter to obtain maximum genetic variability.

Following the method of Vaha and Primmer (2006), the hybrid detection power of our set of loci was investigated using detection efficiency, accuracy and overall performance. Efficiency is defined as the proportion of individuals in a group that were correctly identified (i.e. the number of individuals correctly identified within a group divided by the true number of individuals within that group). Accuracy is defined as the proportion of individuals assigned to a particular group that actually belong to that group (i.e. number of individuals assigned to a group divided by number of individuals that actually belong to that group). The overall performance of the loci was then calculated by multiplying 'efficiency' by 'accuracy' for a particular group.

Results

Hybrid detection performance

The detection of pure Pacific Black Ducks and F_1 hybrids was the most reliable (efficiency = 100%), followed by pure Mallards (efficiency = 90%), while Pacific Black Duck backcrosses were the most difficult to accurately categorise (efficiency = 36%; Table 1). Thus, hybrid types (e.g., F_1 hybrid or hybrid backcross) could not be confidently distinguished between in this survey.

Table 1. Characteristics of the hybrid detection power of the nine microsatellite loci.

Class	ASS	EFF	ACC	PERF
Mallard	1.0	0.9	0.9	0.81
Pacific Black Duck	1.0	1.0	1.0	1.0
F_1	1.0	1.0	0.38	0.38
Mallard backcross	1.0	0.62	0.42	0.26
Pacific Black Duck backcross	1.0	0.36	0.22	0.08
Hybrid	1.0	0.66	0.34	0.24

Variables are: percentage of individuals assigned to a group regardless of whether this assignment was correct (ASS), detection efficiency (EFF), detection accuracy (ACC) and overall performance of the loci (PERF). Refer to main text for a detailed explanation of each variable. The 'hybrid' class represents all hybrid individuals grouped together (i.e. F_1 plus backcrosses).

Assignment testing

The number of putative clusters was estimated to be two. Assignment tests on a total of 390 putative Pacific Black Ducks and 10 domestic Mallard from throughout Australia detected 6 hybrid ducks based on the criterion of $0.90 > q > 0.10$ (Table 2; Figure 1).

Table 2. Hybrid specimens detected using assignment tests, where a hybrid is defined as $0.10 < q < 0.90$

Hybrid specimen	Locality	Proportion of membership (*q*)	Class
PJG597	Perth, Western Australia	0.86	Either a Pacific Black Duck backcross or a pure Pacific Black Duck ($0.85 < q < 0.90$)
PJG654	Perth, Western Australia	0.79	Pacific Black Duck backcross ($0.65 < q < 0.85$)
PJG682	Perth, Western Australia	0.63	Pacific Black Duck backcross ($0.65 < q < 0.85$)
PJG687	Perth, Western Australia	0.78	Pacific Black Duck backcross ($0.65 < q < 0.85$)
AT206	Innamincka, South Australia	0.89	Either a Pacific Black Duck backcross or a pure Pacific Black Duck ($0.85 < q < 0.90$)
T138	King Island	0.89	Either a Pacific Black Duck backcross or a pure Pacific Black Duck ($0.85 < q < 0.90$)

Western Australia had the highest frequency of hybrids with four ducks out of 55 being classified as hybrids. Three of these hybrids were assigned *q*-values that suggested they were Pacific Black Duck backcrosses (Table 2). The three other identified hybrids collected from Western Australia, South Australia and King Island had *q*-values that were intermediate in values for Pacific Black Duck backcrosses and pure Pacific Black Ducks (Vali et al. 2010). No hybrids were detected in Victoria, New South Wales, Queensland or Tasmania within our samples.

Overall, a low frequency of hybrids was detected throughout Australia, with 1.5% of the sample consisting of hybrids. However, a chi-squared analysis revealed that there was a significant difference in the number of hybrids between the states (X^2 = 18.31, df = 6, p = 0.005; Figure 1).

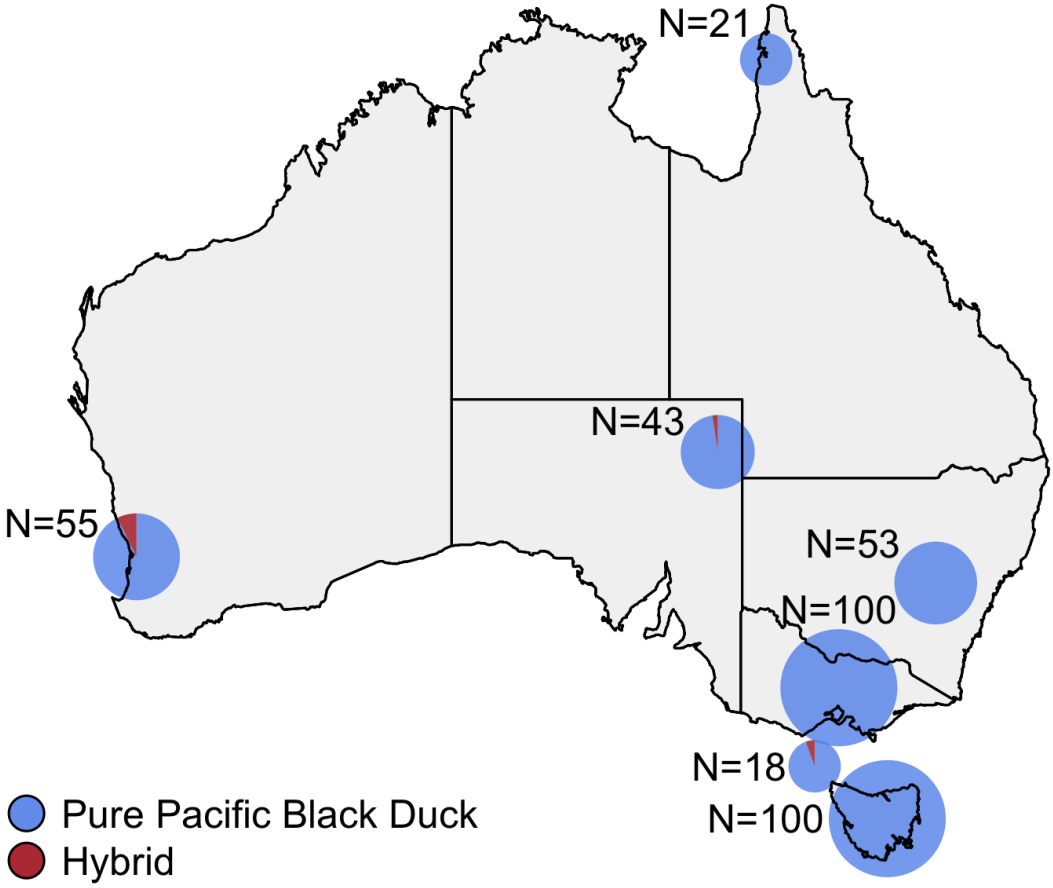

Figure 1. Percentage of pure Pacific Black Ducks and hybrids, as determined by assignment tests, present at each locality across Australia.

Discussion

The hybrid detection performance of the loci set has shown that the detection of pure Pacific Black Ducks was the most reliable and that the detection of

Pacific Black Duck backcrosses the least reliable. The high performance of the loci at detecting pure Pacific Black Ducks, provides solid support for three identified hybrid specimens being Pacific Black Duck backcrosses or potentially F_1 hybrids with an additional three that are likely to be hybrids. van Dongen et al. (2012) also had similar results with the detection of pure species being the most reliable, while backcrosses were the most difficult. The accuracy in detecting between hybrid classes in this study could be improved by incorporating more microsatellite loci (Vaha & Primmer 2006) and/or other informative genetic methods, such as single nucleotide polymorphisms (SNPs) (Vali et al. 2010; Hohenlohe et al. 2011).

Overall, this study suggests that hybridisation between Mallards and Pacific Black Ducks is occurring in Australia, albeit at a low rate. In comparison, the frequency of hybrids in New Zealand was 90% with only 3.4% considered as pure New Zealand Pacific Black Ducks according to the aforementioned criteria (Muller 2009). While the overall frequency detected in Australia was low, this is an important finding because it supports increasing evidence of ongoing hybrdisation (e.g., Braithwaite & Miller 1975; Paton et al. 1992; Sinden et al. 2003; Guay & Tracey 2009). Previous studies in Australia have been limited in their ability to reliably detect the true frequency of hybridisation by not incorporating highly variable nuclear markers (Braithwaite & Miller 1975; Paton et al. 1992; Sinden et al. 2003; Guay & Tracey 2009). This is also the first study to examine the frequency of hybridisation Australia-wide. The incorporation of more microsatellite markers as well as other nuclear markers, such as SNPs, would improve the ability of the genetic system to

detect cryptic hybrids within the sample (Vaha & Primmer 2006; Vali et al. 2010).

While this result is encouraging and suggests that hybridisation is not currently a widespread problem in Australia, it does reveal its existence and its potential to become more frequent if the number of domestic Mallards in contact with wild Pacific Black Ducks increases. For example, the number of American Black Ducks (*A. rubripes*) in eastern Canada has drastically decreased in comparison to an increasing Mallard population through extensive hybridisation and/or competition for resources (Goodwin 1956; Brodsky & Weatherhead 1984; Ankney et al. 1987; Merendino et al. 1993; Petrie et al. 2012). While the effects of competition between domestic Mallards and Pacific Black Ducks in Australia are currently unknown, monitoring of the size of Mallard and hybrid populations in urban areas should occur over time in order to select an appropriate management strategy.

Guay et al. (2014) suggests five management strategies that should be considered when controlling for Mallard outbreaks, including prevention, eradication, containment, no control and captive breeding. Considering the low rate of hybridisation throughout Australia, the employment of eradication methods to control for Mallard releases should be considered. Ongoing genetic monitoring will detect a change in the frequency of hybridisation, which may alter the overall management strategy.

Furthermore, there is the potential for wild Mallards and wild Mallard hybrids with a greater capacity for flight than domestic Mallards in Australia to colonise Australia from Lord Howe Island, located approximately halfway between Australia and New Zealand (Guay & Tracey 2009). Mallards from New Zealand have already been reported along the Australian east coast and as far as South Australia (Paton 1991; Anonymous 2013; ABBBS unpublished data). While it's unlikely that a large number of wild Mallards and their hybrids will emigrate from Lord Howe Island, a random immigration event could potentially alter the frequency of hybridisation on the Australian east coast. Increases in the number of wild Mallards should be noted and if necessary, management plans should be implemented.

Hybridisation between Mallards and Pacific Black Ducks has traditionally been thought to be restricted to urban areas in Australia, where domestic Mallards are most common. Accordingly, most reported cases of hybridisation between the two species have been from urban areas (Braithwaite & Miller 1975; Whatmough 1978; Smith & Smith 1990; Paton et al. 1992; Bielewicz & Bielewicz 1996; Guay & Tracey 2009; Guay 2010). In the current study, the difference in the frequency of hybridisation between urban and rural areas was not tested. Characterising the genetic status of urban putative Pacific Black Ducks and the behaviour of Mallards and Pacific Black Ducks in urban areas is of interest, as it would allow an assessment of whether urban ponds act as genetic reservoirs of Mallard genes. It would also permit the determination of whether Mallards may outcompete Pacific Black Ducks in urban areas (see Chapter 5). Further genetic work along with behavioural

studies will provide further understanding into Mallard – Pacific Black Duck hybridisation in Australia and will assist in making further management recommendations.

Chapter 5

Dominance, competition and the incidence of hybridisation between domestic Mallards (*Anas platyrhynchos*) and Pacific Black Ducks (*Anas superciliosa*) in urban Victoria.

Unpublished manuscript

Abstract

The introduction of alien species to new landscapes has long been recognised as a major conservation problem. Wild and domestic varieties of Mallard (*Anas platyrhynchos*) have been introduced worldwide and are known to threaten native dabbling duck species by competitive exclusion and hybridisation. In Australia, introduced domestic Mallard are believed to hybridise with the native Pacific Black Duck (*A. superciliosa*). Mallard are more common in urban parks than in rural areas yet, to date, there has been no formal study comparing hybridisation between urban and rural areas. Furthermore, there have been no studies in Australia examining the behaviours that may contribute to hybridisation, such as female mate preferences and forced extra-pair copulations. Therefore, the aim of this study was to first investigate whether Mallards are dominant over Pacific Black Ducks, which may permit forced heterospecific copulations by male Mallards or heterospecific female mate choice for dominant males by female Pacific Black Duck. It was also tested whether the frequency of hybridisation was greater in urban areas, where there is a greater abundance of Mallard. Surveys and observations of dominance behaviours were used to assess whether Mallards were dominant over Pacific Black Duck in metropolitan

Melbourne. Next, putative Pacific Black Ducks from urban and rural Victoria were genotyped at nine microsatellite loci to determine whether urban ponds act as a reservoir of Mallard hybrids. While observations of dominance behaviours revealed that Mallards were dominant over Pacific Black Ducks, the latter occurred in greater numbers and in more parks than Mallards and thus did not appear to be outcompeted by Mallards. In addition, the genetic data revealed that hybrids were only detected in urban populations, albeit at low frequency. Eight percent of individuals were classified as hybrids in urban areas, compared to none in rural areas. This difference was marginally non-significant. The higher dominance of Mallards suggests that forced copulations or interspecific mate choice may be occurring. Mallards should therefore be removed from urban settings to lower the chance of interspecific hybridisation.

Introduction

Human-induced changes to the landscape, including land transformation, introductions of exotic animals, overhunting and climate change are known to be detrimental to natural ecosystems by causing a loss in biodiversity (Vitousek et al. 1997). The introduction of exotic species is thought to directly relate to species extinctions via predation, disease, hybridisation, competitive exclusion and displacement (Holway & Suarez 1999; Mooney & Cleland 2001; Gurevitch & Padilla 2004). Competitive exclusion, for example, can be detrimental to native species when they are dominated and outcompeted by an introduced species (Hardin 1960).

The Mallard (*Anas platyrhynchos*) is native to the northern hemisphere and has been introduced throughout the world (reviewed in Chapter 2). Behaviourally, Mallards tend to be more aggressive and outcompete many other dabbling duck (Genus: *Anas*) species (Brodsky & Weatherhead 1984; Brodsky et al. 1988; Williams & Basse 2006). Such behaviour is thought to promote hybridisation via forced-extra pair copulations by the dominant Mallards or a female heterospecific preference for a dominant mate (Bossema & Kruijt 1982; Brodsky & Weatherhead 1984; Brodsky et al. 1988; Williams & Basse 2006). Several other factors are thought to promote hybridisation between Mallards and other dabbling duck species. For example, natural male-biased sex ratios may reduce the number of available female mates (Bowler 2005). In addition, behavioural traits associated with the domestication of Mallards may also promote hybridisation. This includes a longer breeding season that increases the overall period of time for which a Mallard can reproduce, greater promiscuity that increases the likelihood for extra-pair matings and higher levels of testosterone that increases aggression and potentially forced extra-pair copulations (Desforges & Wood-Gush 1975; Hepp et al. 1988).

The Mallard was introduced to Australia and New Zealand in the 19th and the 20th century and while wild Mallards were predominantly released throughout New Zealand, domestic Mallards are more prevalent in Australia today (Williams 1969; Braithwaite & Miller 1975; Marchant & Higgins 1990; Guay et al. 2015). In New Zealand, wild Mallards, with a greater capacity for long distance flights compared to their domestic relatives, have colonised the

entire country, including some offshore islands, and have consequently hybridised with New Zealand Pacific Black Duck (*A. superciliosa superciliosa*) to the point that the former is now considered Nationally critical (reviewed in Chapter 2).

In contrast, domestic Mallards in Australia have not colonised the entire country and appear restricted to urban areas (Braithwaite & Miller 1975; Marchant & Higgins 1990). In Chapter 4, the Australia-wide frequency of hybridisation between domestic Mallard and the Australian Pacific Black Duck (*A. s. rogersi*) was examined and whilst the results revealed that hybridisation is occurring, it is not occurring at a rate threatening the genetic integrity of the Pacific Black Duck. However, the majority of the hybrids detected were from urban parks within Perth, Western Australia (see Chapter 4). While that study examined the frequency of hybrids throughout Australia, it is yet to be determined whether hybridisation is more common in urban wetlands, where Mallards are most common. Previous studies based on phenotypic identification of hybrids have suggested that hybridisation is common in many urban areas within Australia (Braithwaite & Miller 1975; Paton 1976; Whatmough 1978; Bielewicz & Bielewicz 1996; Paton & Pedler 1999; Sinden et al. 2003).

In addition, wild Mallards have been observed to be dominant over New Zealand Pacific Black Ducks and have a tendency to exploit human-dominated environments (Williams & Basse 2006). Such behaviour in wild New Zealand Mallards has led to competitive exclusion or it may create

conditions that favour hybridisation (Rhymer et al. 1994; Rhymer et al. 2004; Williams & Basse 2006). This is also true in non-duck species, for example hybridising Townsend's Warblers (*Dendroica townsend*) are thought to have a selective advantage over Hermit Warblers (*D. occidentalis*) as the former is more aggressive and the hybrid zone is moving into the range of the Hermit Warbler (Rohwer & Wood 1998; Pearson & Rohwer 2000). Therefore, the dominance of one species over another closely-related species may be a confounding factor contributing to interspecific hybridisation. While there have been no investigation of domestic Mallard dominance in Australia, behaviour changes observed in domestic Mallards would suggest that they would be dominant over smaller Pacific Black Ducks (e.g., Hepp et al. 1988). Dominant behaviour in Australian Mallards may favour hybridisation with and/or competitive exclusion of Australian Pacific Black Ducks.

The overall aim of the study was to investigate whether urban areas in Victoria are acting as reservoirs of Mallards and Mallard genes and whether Mallards are dominant over and outcompete native Pacific Black Ducks. To achieve this, surveys and behavioural observations were conducted in metropolitan Melbourne to (1) estimate the relative abundance of the two species, (2) determine the sex ratios of Mallards and to (3) determine whether Mallards are dominant over and potentially outcompete native Pacific Black Ducks in metropolitan Melbourne. Furthermore, (4) the frequency of hybridisation in urban Victoria was compared to rural Victoria using a microsatellite genotyping system established for distinguishing between Pacific Black Ducks, Mallards and their hybrids (Chapter 3).

Methods

Surveys

Surveys of urban wetlands in Melbourne were conducted within a 20 kilometre radius of the central business district, which included 58 different parks and public places containing a wetland identifiable from a Melbourne street directory (Melway 2014 ed.; Appendix 2). Parks containing rivers or creeks were not surveyed due to difficulties in surveying the whole water stream. Surveys were conducted in dry weather conditions during the spring (October – November) of 2013 between 0900 and 1700. The surveyor spent approximately 30 minutes at each park and walked the perimeter of all ponds located within the park. For each urban pond the number of Pacific Black Ducks and Mallards were recorded, including ducklings. The direction in which the ducks were swimming was mentally noted to avoid double counting. Where possible, ducks were sexed using plumage characteristics and calls.

Measuring dominance

Dominance of Pacific Black Ducks and Mallards was measured using feeding trials (e.g., Ewald & Rohwer 1980; Crowhurst et al. 2012). Seventeen feeding trials were conducted at 17 different parks with mixed flocks following completion of the survey. Bread was thrown directly in the water no further than one meter in front of the observer, at a constant rate (1 small piece every 3 secondss), at a site where Mallards and Pacific Black Ducks were both visible. Observations took place over one minute, as the feeding behaviour of the ducks did not appear to change over a longer timeframe. During these

observations it was recorded whether Pacific Black Ducks would feed in the presence of Mallards. Feeding trials were not conducted in the presence of larger, more dominant species (i.e., swans). Other dominance behaviour (i.e., chasing) was also recorded as well as the number of each species.

Sampling of genetic data

A total of 87 putative Pacific Black Ducks were sampled from Victoria, Australia from 2010 to 2014. Forty-eight of the 87 were hunter shot specimens from rural locations (i.e., wetlands outside of cities or regional cities; Appendix 3) and represent a subset of the sample used in Chapter 4. The remaining 39 were hand-caught (see Chapter 4) from urban locations (i.e., parks in cities or regional cities; see Appendix 3) and have not been previously analysed. Birds caught by hand had 50µl of blood taken from the tarsal vein using a 26-gauge needle. Hunters provided tissue from the neck or webbing from the foot of shot birds. Samples were stored in 70% ethanol to prevent deterioration of the tissue/blood and DNA was extracted using a salting-out procedure (Bruford et al. 1992).

Genotyping

All individuals were genotyped at nine microsatellite loci to distinguish between Mallards, Pacific Black Ducks and their hybrids (Chapter 3). PCR reactions were conducted as outlined in Chapter 3. Fragment analysis was conducted commercially (Australian Genomic Resource Facility, AGRF).

Analysis of genotypic data

Assignment tests were performed using the admixture model with no prior population information using STRUCTURE 2.3.4 (Pritchard et al. 2000) with a burn-in period of 10,000, followed by 1,000,000 replicates, as described by Williams et al. (2005). Assignment testing was completed with 10 domestic Mallard samples to test for admixture between the two species (i.e., domestic Mallards and putative Pacific Black Ducks; see Chapter 3). Genetic cluster assignment was performed using the method described by Steeves et al. (2010), where estimates of the posterior probability for $K = 1$ and $K = 2$ are compared using a likelihood ratio test (see Chapter 3).

Following Williams et al. (2005), a hybrid status was assigned to any specimen with $0.10 < q < 0.90$, where $q < 0.10$ was defined as a pure Mallard and $q > 0.90$ was defined as a pure Pacific Black Duck. Due to poor hybrid detection accuracy of specific hybrid classes (i.e. F1 hybrids and backcrosses) (Chapter 4), this study only focused on distinguishing between hybrids and pure species.

Statistical analyses

Due to non-normal data, a Mann-Whitney U-test was used to determine whether ratios of Mallards to Pacific Black Ducks altered feeding patterns in Pacific Black Ducks. A linear regression analysis was used to evaluate the distribution of Mallards and Pacific Black Ducks across parks. To compare the differences in the number of genetically confirmed hybrids between urban and rural Victoria, Fisher's exact test was used. A post-hoc analysis was

used to determine the overall power when comparing the number of genetically confirmed hybrids in urban and rural Victoria. Analyses were completed using SPSS 22.0.

Results

Census

Of the 58 parks containing a water body within a 20 kilometre radius of Melbourne, 113 Mallards resided in 22 parks with 1.9±3.4 SD Mallards per park and 367 Pacific Black Ducks were counted in 43 parks with 7.2±9.0 SD ducks per park (Appendix 3). A paired T-test revealed that there were significantly more Pacific Black Ducks residing in Melbourne parks than Mallards ($t = 4.788$, $df = 57$, $p < 0.001$). Fifty Pacific Black Duck ducklings were observed in 6 different parks (Appendix 3) and no Mallard ducklings were observed.

Coburg Lake, Coburg and Botanic Boulevard, Bundoora had the greatest number of Pacific Black Ducks and Mallards with approximately 30 Pacific Black Ducks and five Mallards counted at both parks (Appendix 3). Jack Roper Reserve, Broadmeadows had the largest number of Mallards observed at one park with a total of 13 Mallards counted (Appendix 3). The number of Pacific Black Ducks was positively correlated to the number of Mallards, which explained approximately 20% of the variation in Pacific Black Duck abundance ($R^2 = 0.195$, $F_{1, 56} = 13.525$, $p < 0.001$; Figure 1).

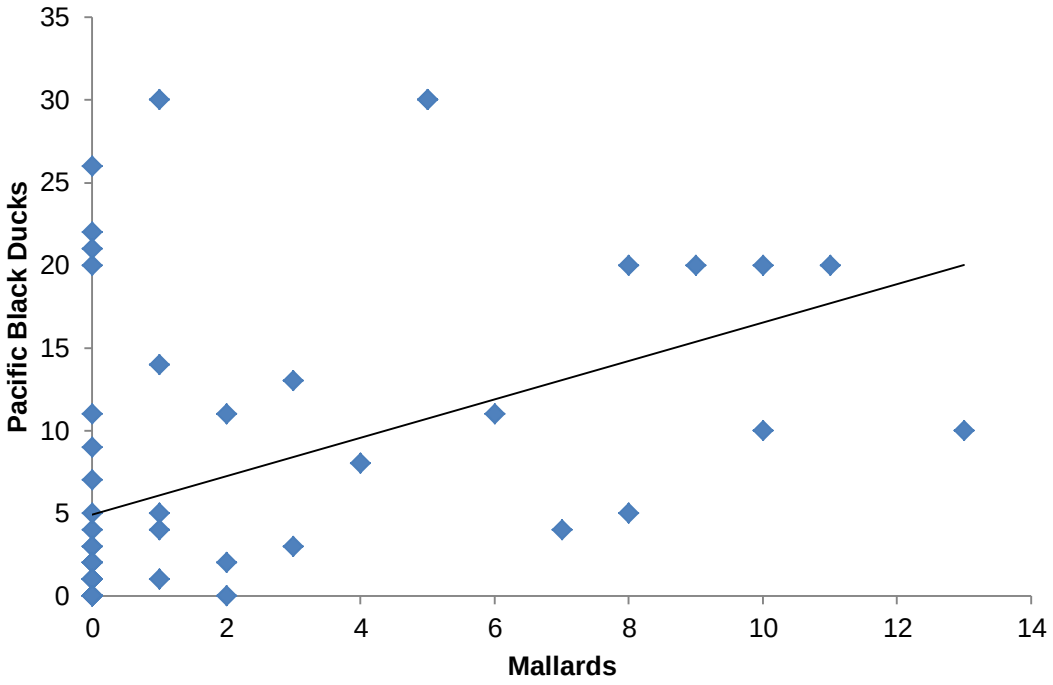

Figure 1. The number of Pacific Black Ducks plotted against the number of Mallards at each of the 58 parks surveyed.

Of the 113 Mallards observed, 79 were male and 23 were female. The remaining 11 Mallards were too far away to be visually sexed (Appendix 3). Pearson's chi-squared analysis determined that there were significantly more male Mallards compared to female Mallards ($X^2 = 30.745$, df = 1, $p < 0.001$).

Dominance experiments

A Mann-Whitney U-test confirmed that Pacific Black Ducks would only feed in the presence of Mallards if Pacific Black Ducks outnumbered Mallards ($F_{1, 15} = 11.570$, $p = 0.003$; Figure 2).

Additionally, at the 18 parks where Mallards and Pacific Black Ducks were observed together, Mallards were observed chasing Pacific Black Ducks at 12

of these parks. However, Pacific Black Ducks were never observed chasing Mallards. Pearson's chi-squared analysis determined that there was a significant difference between the number of parks where chases by Mallards and Pacific Black Ducks were observed ($X^2 = 12$, df = 1, $p < 0.001$).

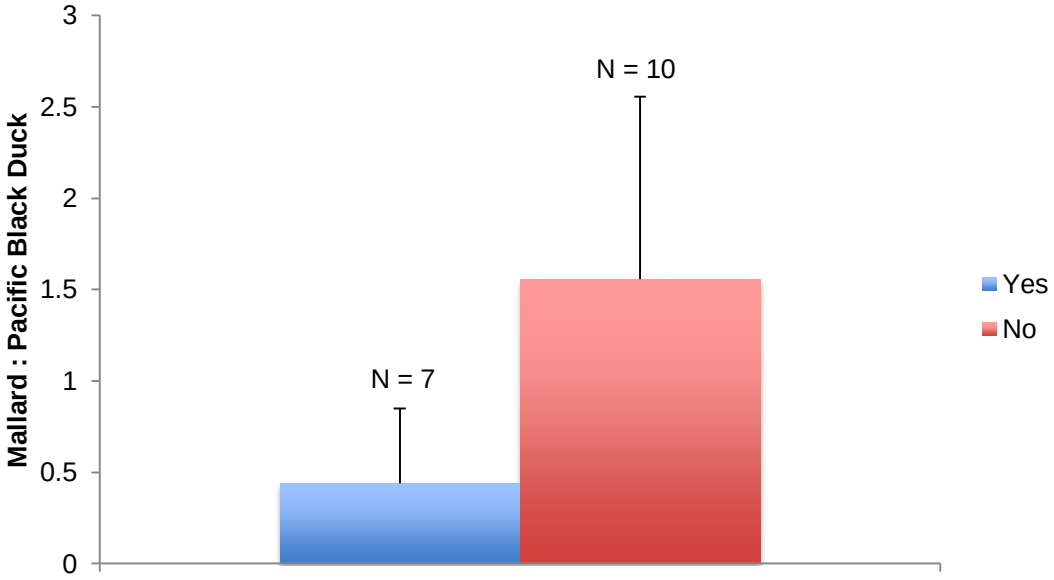

Figure 2. Average mean and standard deviation of the number of Mallard to Pacific Black Duck ratios where Pacific Black Ducks would ("Yes") and would not ("No") feed in the presence of Mallards.

Genotypes

As per Chapter 4, the number of putative clusters was estimated to be two. Three hybrid ducks were detected from two different parks based on the criterion of $0.90 > q > 0.10$ (Figure 3; Appendix 3). Overall, the percentage of hybrids in rural and urban environments was 0% and 8% respectively. Fisher's exact test of the number of hybrids in each area (rural or urban) revealed that hybrids tended to be more common in urban environments, (p =

0.086), but a post hoc analysis revealed that the power for this test was low (power = 0.54).

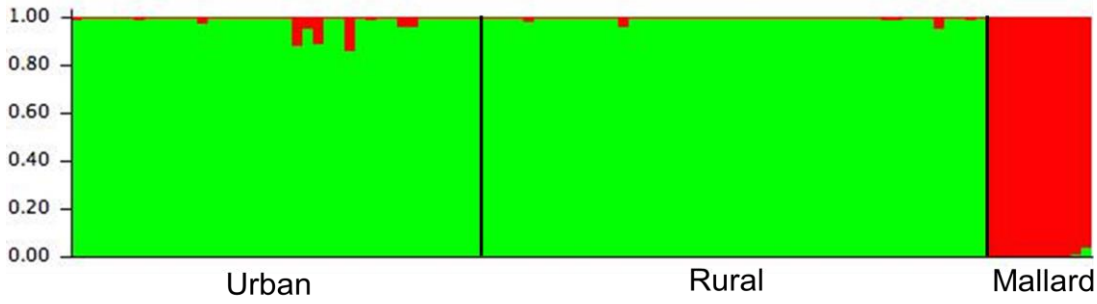

Figure 3. STRUCTURE 2.3.4 (Pritchard et al. 2000) output shows the proportion of membership based on genetic information of 36 Pacific Black Ducks (predominantly green) and three Pacific Black Duck – Mallard hybrid (red/green) from urban locations, 48 Pacific Black Duck (predominantly green) from rural locations and 10 domestic Mallards (predominantly red).

Discussion

Dominance behaviours in Mallards were documented to determine whether they could contribute to hybridisation or competitive exclusion of native Pacific Black Ducks. Mallards appeared to be physically dominant over Pacific Black Ducks, as Pacific Black Ducks were reluctant to feed when they were outnumbered by Mallards. Genetic evidence suggests that hybridisation is also common in urban areas and therefore it may be possible that hybridisation is promoted by dominance behaviours of Mallards that could lead to forced interspecies copulations or interspecific mate choice for dominant males.

In addition, the co-occurrence of large numbers of Pacific Black Ducks compared to Mallards in metropolitain Melbourne suggests that domestic Mallards have not outcompeted Pacific Black Ducks. Wild Mallards in New

Zealand and North America have been found to be dominant over New Zealand Pacific Black Ducks and American Black Ducks (*A. rubripes*), which is believed to be linked to the native species' decline via hybridisation and/or competitive exclusion (Ankney et al. 1987; Brodsky et al. 1988; Merendino et al. 1993; Williams & Basse 2006). However, there is currently no strong evidence that dominance will lead to the exclusion and overall decline of Pacific Black Duck throughout Australia (Chapter 4).

Domestic Mallards in Australia are known to be sedentary and therefore their survival, and also the occurrence of Pacific Black Ducks, may be greater in areas where there are more resources. For example, artificial feeding appears to support populations of domestic Mallards in the Greater Brisbane region (Chapman & Jones 2010) and is also thought to contribute to hybridisation in North America by encouraging flocks to mix (Brodsky & Weatherhead 1984). While there is no information in regards to this in metropolitan Melbourne, it may be possible that artificial feeding by humans may support populations of domestic Mallards and promote the co-existence of the two species that may also contribute to hybridisation. Indeed, anthropogenic feeding of waterbirds is a common practice in the parks of Melbourne (Taysom, personal observation). The effect of artificial feeding on domestic Mallard numbers in urban areas warrants further investigation.

At least in the case of metropolitan Melbourne, the co-habituation of Mallards and Pacific Black Ducks in urban ponds, the dominance of Mallards, a significantly large Mallard drake to female ratio and the identification of

hybrids only in urban areas suggests that hybridisation in urban areas is likely to persist. Male-biased sex ratios occur naturally in wild populations of ducks, which is thought to contribute to interspecific hybridisation between American Black Ducks and Mallards in North America (Brodsky & Weatherhead 1984; Ankney et al. 1987). While these results only identified hybrids in ubran areas, other reports, based on phenotype, suggest that hybrids are dispersing to more rural locations (Paton et al. 1992; Guay & Tracey 2009). The limitations of the current set of microsatellite loci means that backcross hybrids could not be reliably detected (Chapter 4). Thus, a larger set of microsatellite loci may have detected more hybrids in both urban and rural locations.

In this study, all hybrids were detected in urban wetlands where domestic Mallards have been released. Therefore, urbanisation and anthropogenic influences may affect the frequency of hybridisation. For example, the Ethiopian wolf (*Canis simensis*) is known to undergo threatening hybridisation with domestic dogs where wolf populations are sympatric to humans (Gottelli et al. 1994). The effects of urbanisation on the frequency of hybridisation between many domestic and wild varieities of animals should be further examined to determine whether urban areas increase the frequency of hybridisation.

Hybridisation is not extensive throughout Australia (Chapter 4). In this study, relatively few hybrids were detected. However, comparatively more hybrids were detected in urban compared to rural areas. While Mallards are known to

be dominant over and may outcompete other dabbling ducks (e.g., Brodsky et al. 1988; Williams & Basse 2006; Petrie et al. 2012), feral Mallard have not outcompeted the Pacific Black Ducks resident in Melbourne. While these results suggest that hybridisation is not currently a major problem, Mallard behaviour, male-biased sex ratios, proximity between the two species and the existence of hybrids in urban Victoria is a risk to the genetic integrity of the Pacific Black Duck, based on experience in New Zealand. Despite hybrids not being detected in rural Victoria, hybrids have been detected in other rural locations throughout Australia (Chapter 4; Paton et al. 1992; Guay & Tracey 2009). As discussed previously, the limitations of the current set of microsatellite loci means that backcross hybrids could not be reliably detected (Chapter 4). Therefore, the most effective conservation approach, due to their relatively low abundance, would be to eradicate feral Mallards and their hybrids from all areas where they are known to exist (e.g., Lake Torrens, South Australia; see Chapter 2).

Chapter 6

The genetic origins of the Mallard (*Anas platyrhynchos*) and occurrence of Mallard - Pacific Black Duck (*A. superciliosa*) hybrids on Lord Howe Island

Unpublished manuscript

Abstract

The introduction of foreign species can be detrimental to the ongoing survival of native species. Hybridisation, which can lead to extinction, is one known consequence that can arise from the introduction of new species into a landscape. The Pacific Black Duck (*Anas superciliosa*) is a dabbling duck (genus: *Anas*) native to the western Pacific, including Lord Howe Island, that is under threat due to hybridisation with introduced Mallards (*A. platyrhynchos*). Mallards colonised Lord Howe Island in 1963 and while the colonisation event and subsequent hybridisation are well documented, the source population for the Mallard self-introduction has not been established. The risk of colonisation of Australia by wild Mallards from the island has also not been assessed. The aim of this study was to test whether Mallards on Lord Howe Island were from New Zealand and to test for the occurrence of Pacific Black Duck - Mallard hybrids on Lord Howe Island using mitochondrial DNA. A fragment of the mitochondrial DNA control region of Lord Howe ducks was sequenced and compared to the sequences of putative Pacific Black Ducks from Australia and putative Mallards from New Zealand. The results revealed that *Anas* ducks on Lord Howe Island were not significantly different from New Zealand Mallards (Φ_{ST} = -0.010; P = 0.586). This result

suggests that Mallards on Lord Howe Island may have emigrated from New Zealand. This is consistent with banding records, where Mallards banded in New Zealand were later observed on Lord Howe Island. Although no Mallards were sampled from Australia, the colonisation of Lord Howe Island by Australian Mallards is unlikely given their sedentary nature and inability to fly long distances. A lack of Pacific Black Duck phenotypes and haplotypes suggests that hybridisation is directional and that Mallards have outcompeted pure Pacific Black Ducks on Lord Howe Island. Considering that Mallards appeared to have colonised Lord Howe Island from New Zealand, there is the potential for Mallards to colonise Australia from Lord Howe Island. Proactive management and monitoring of Mallards is required to allow the re-colonisation of Pacific Black Ducks on Lord Howe Island and to protect other areas from potential Mallard colonisation (i.e., mainland Australia).

Introduction

Invasive species can have many negative impacts that threaten the survival of native species including competitive exclusion, niche displacement, hybridisation, and predation (Mooney & Cleland 2001). Together, species introductions and habitat modifications can break down reproductive isolating barriers between closely-related species resulting in hybridisation, or interspecies introgression, the admixture of genetically-distinct species (Rhymer & Simberloff 1996; Allendorf et al. 2001; Rhymer 2006; Seehausen et al. 2007). For example, molecular genetic analysis suggests that introgressive hybridisation of native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) with introduced rainbow trout (*Oncorhynchus mykiss*) has

resulted in hybrid offspring with a reduced fitness, with the reproductive success declining by approximately 50% (Muhlfeld et al. 2009).

While hybridisation is known to occur across many animal taxa, it is most common amongst birds, and in particular among many dabbling duck (genus: *Anas*) species (McCarthy 2006). The Mallard is a sexually dimorphic dabbling duck that tends to be larger, more aggressive and dominant over other dabbling duck species (Chapter 5; Brodsky & Weatherhead 1984; Brodsky et al. 1988; Kear et al. 2005). The Mallard, a species native to the northern hemisphere, is known to have an increasing global range, with a concurrent increased rate of hybridisation due to anthropogenic influences, such as foreign introductions, landscape modification and domestication (Johnsgard 1967; Heusmann 1974; Marchant & Higgins 1990; Berruti 1991; Kear et al. 2005). Mallards, thus, now hybridise and threaten many dabbling duck species with which they co-occur (reviewed in Chapter 2).

Mallards have been introduced to both Australia and New Zealand where they are known to readily hybridise with the native Pacific Black Duck (*A. superciliosa*) (Chapter 2 & 4). The Pacific Black Duck is a sexually monomorphic dabbling duck native to the western Pacific (Marchant & Higgins 1990). It can be divided into three subspecies; *A. s. rogersi* from Australia, New Guinea and Indonesia, *A. s. superciliosa* from New Zealand and outlying islands and *A. s. pelewensis* from Palau and other islands in the West Pacific (Amadon 1943; Marchant & Higgins 1990; Rhymer et al. 1994). However, subsequent genetic studies do not support this taxonomy, but rather support

closer a haplotype similarity between Australian and North Island New Zealand birds than between the North and South Island (Rhymer et al. 2004). Despite the limited genetic support for the subspecies classification they are still maintained by Birdlife International (2015).

In Australia, Mallards are typically of domestic origin and are unable to fly long distances (Marchant & Higgins 1990; Guay & Tracey 2009). They were therefore believed to pose no overall threat to rural populations of Pacific Black Ducks (Braithwaite & Miller 1975). However, genetic and phenotype studies have since shown that while hybridisation is not occurring at an alarming rate, hybrids do exist in urban and rural locales throughout Australia (Chaper 4 & 5; Paton et al. 1992; Guay & Tracey 2009).

In contrast to Australia, genetic and phenotypic studies have shown that introduced Mallards and Pacific Black Ducks in New Zealand have hybridised to an extent that few pure Pacific Black Ducks appear to remain (Gillespie 1985; Rhymer et al. 1994; Muller 2009). Moreover, the New Zealand Pacific Black Duck is now considered Nationally critical due to hybridisation (Robertson et al. 2012). In New Zealand, Mallards were originally released during the 19th and the 20th century, primarily as a game species (Williams 1982; McDowall 1994; Williams & Basse 2006; Dyer & Williams 2010; Guay et al. 2015). The high mobility of the wild Mallards introduced in New Zealand is believed to be responsible for the self-colonisation and consequential hybridisation of Mallards with Pacific Black Ducks on many offshore islands surrounding New Zealand including, the Snares Islands (48°01'S 166°36'E),

the Chatham Islands (44°15'S 176°12'W), Campbell Island (52°33'S 169°09'E), Auckland Island (50°29'S 165°52'E), Norfolk Island (29°02'S 167°57'E), Macquarie Island (54°30'S, 158°57'E), and Lord Howe Island (159°05'E, 31°33'S; (Bailey & Sorensen 1962; McKean & Hindwood 1965; Hermes et al. 1986; Norman 1987; Marchant & Higgins 1990; Tennyson 1998; Miskelly et al. 2001).

Lord Howe Island is located 600 kilometres east off the Australian east coast at the intersection of the geographic ranges of the three putative *A. superciliosa* subspecies (Amadon 1943; Rhymer et al. 2004; Tracey et al. 2008). While, Pacific Black Ducks have been observed on Lord Howe Island since the 1850s, Mallards were first recorded in 1963 and records of hybridisation based on phenotype between the two species has occurred since (MacDonald 1853; McKean & Hindwood 1965; Rogers 1976; Tracey et al. 2008). A recent phenotypic study has indicated that most birds on Lord Howe Island are Mallards or Mallard-like hybrids (Tracey et al. 2008). Mallards tend to be larger and dominant over other dabbling ducks, which can lead to competitive exclusion and forced-extra pair copulations by Mallards (Chapter 5; Brodsky & Weatherhead 1984; Brodsky et al. 1988; Williams & Basse 2006; Petrie et al. 2012). While the numbers of Pacific Black Duck have declined since the arrival of Mallards on Lord Howe Island, without a genetic study, it is unclear as to whether introgressive hybridisation of the two populations has occurred.

The initial sightings of Mallards on Lord Howe Island coincided with a major release of Mallards in New Zealand, which has led to speculation that they emigrated from New Zealand (McKean & Hindwood 1965; Tracey et al. 2008). Nonetheless, the hypothesis that Mallards self-colonised from New Zealand has never been tested. This is important to assess the risk of colonisation of wild New Zealand Mallards in Australia, using the Pacific islands as stepping stones.

Genetic monitoring is a reliable method for determining hybridisation rates, and thus, the threat posed to native species by introduced species. For example, mitochondrial DNA (mtDNA) has been used to detect the legacy of North American Mallards in wild Mallards in New Zealand, as well as introgression of New Zealand Pacific Black Duck genes into the Mallard population (Guay et al. 2015). The study by Guay et al. (2015) took advantage of the existence of two distinct Mallard haplotype lineages, type-A and type-B. Old world Mallards possess type-A haplotypes, whereas new world Mallards have both types (Avise et al. 1990; Johnson & Sorenson 1999; McCracken et al. 2001; Kulikova et al. 2004; Fowler et al. 2009). The presence of both types in the North American Mallard is thought to be the result of historical or more recent hybridisation with other native dabbling duck species, with which Mallards were historically allopatric (Palmer 1976; Avise et al. 1990; Johnson & Sorenson 1999; McCracken et al. 2001).

The aims of this study were to test whether (1) the current population on Lord Howe Island consists of only Mallards or Pacific Black Duck - Mallard hybrids

using phenotype and mtDNA sequencing data, and whether (2) haplotypes common in New Zealand hybrids are present on Lord Howe Island. This information will be an important basis for species management efforts on Lord Howe Island and establishment of the risk of Mallard self-colonisation of Australia.

Methods

Sample collection

Twenty-eight duck feather samples were collected from Lord Howe Island in 2007 (representing approximately 30% of the total population) as described by Tracey et al. (2008) (Appendix 4). These were compared to 37 Mallard tissue samples that were collected from New Zealand during the 1991 and 1998 shooting seasons as described by Guay et al. (2015) and 27 tissue and blood samples collected from putative Pacific Black Ducks shot by duck shooters in eastern Australia (NSW [9], VIC [10], Tasmania [8]) during the 2006-2011 duck shooting seasons (Appendix 4). Twenty of the birds on Lord Howe Island that were genetically tested in this study were also scored for phenotype according to the method of Gillespie (1985), which produces low scores (minimum 0) for ducks more similar to Pacific Black Ducks and higher scores (maximum 35) for ducks more similar to Mallards. The Mallard samples from New Zealand were identified as Mallards based on their high (hybrid to Mallard) phenotypic scores (see Guay et al. 2015).

Sequencing

While nuclear DNA is generally preferred to identify hybridisation (see Chapter 3), mtDNA sequencing was used to identify hybridisation in this study because it is more reliable when using small amounts of degraded DNA that are typically extracted from feather samples. Although mtDNA cannot explicitly identify hybrids, the presence of Pacific Black Duck and/or Mallard haplotypes in conjunction with phenotype was used as evidence of hybridisation. The use of mtDNA is also useful in determining the directionality of hybridisation (Watanabe et al. 1985).

DNA was extracted from tissue samples using a salting out procedure (Bruford et al. 1992) and from feather samples using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). Primers L78 (Sorenson & Fleischer 1996) and H774 (Sorenson et al. 1999) were used to amplify the 5' variable region of domains I and II of the avian control region and conserved sequence boxes known in birds were identified (e.g., Marshall & Baker 1997; Randi & Lucchini 1998; McCracken et al. 2001; McCracken & Sorenson 2005). Polymerase chain reactions (PCR) were set up as 50µL reactions containing 2 µL of DNA, 300 pmoles of each primer (L78 and H774), dNTPs (200 nM), $MgCl_2$ (2.5 mM), Colorless GoTaq Flexi Buffer (1X) and 2.5 units of Taq DNA Polymerase (Promega). Betaine (1.0M) was added to the PCR reaction to increase yield (Johnson & Dunn 2006). PCR amplifications were performed at 1 cycle for 7 minutes at 94°C, 45 cycles for 20 seconds at 94°C, 20 seconds at 52°C, 1 minute at 72°C and 1 cycle for 7 minutes at 72°C using a MyCycler thermal cycler (Biorad).

PCR products were separated by agarose gel electrophoresis. The bands were excised and gel purified using a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA), and sequenced commercially (Australian Genome Research Facility Pty Ltd, Melbourne, Australia or Macrogen, Seoul, Korea). Both strands of mtDNA were sequenced, representing 666-667bp from the 5' end of the mtDNA control region. Complementary sequences of mtDNA were reconciled and discrepancies were resolved using SEQUENCHER 3.1 (Gene Codes, Ann Arbor, USA).

Unrooted networks using all haplotypes were calculated using the software NETWORK 4.6.1.1 (Fluxus Technology). Haplotypes and genetic origins of the birds were identified using nucleotide Basic Local Alignment Search Tool (nBLAST) on the National Center for Biotechnology Information website.

Haplotype (H) and nucleotide (π) diversity were calculated to determine the genetic diversity of each sample location using ARLEQUIN 3.5 (Excoffier et al. 2005). The HKY (Hasegawa et al. 1985) was identified as the best-fit model of nucleotide substitution for mtDNA in our dataset using MEGA 5.2 (Tamura et al. 2011). The genetic structure of birds on Lord Howe Island, Australia and New Zealand was investigated using calculated pairwise Φ_{ST} values and closely related K80 (Kimura 1980) nucleotide substitution model in ARLEQUIN 3.5, as the HKY model is not available for this software.

Results

The majority of specimens sampled from New Zealand carried type-A haplotypes (33/37 individuals), only two individuals had type-B haplotypes. The remaining two specimens from New Zealand possessed Pacific Black Duck haplotypes. The majority of specimens from Lord Howe Island were also of type-A haplotype (26/28 individuals), while the remaining two individuals had Pacific Black Duck haplotypes. All individuals from Australia (27 individuals) possessed Pacific Black Duck haplotypes (Figure 1).

Across the three geographical locations, 26 distinct haplotypes were found. Six of these haplotypes were identified as Mallard type-A haplotype (U-Z; Figure 1), one haplotype was identified as Mallard type-B haplotype (T; Figure 1.), and the remaining 19 haplotypes were identified as Pacific Black Duck group II haplotype as denoted by Rhymer et al. (2004; A-S; Figure 1).

Lord Howe Island specimens had both the lowest haplotype and nucleotide diversities, while New Zealand had the highest nucleotide diversity and Australia the highest haplotype diversity (Table 1). 8.5% of the sequence nucleotides were variable and two conserved sequence boxes reported for birds were identified; F box at positions 307 to 334 and D box at positions 411 to 436 (e.g., Marshall & Baker 1997; Randi & Lucchini 1998; McCracken et al. 2001; McCracken & Sorenson 2005).

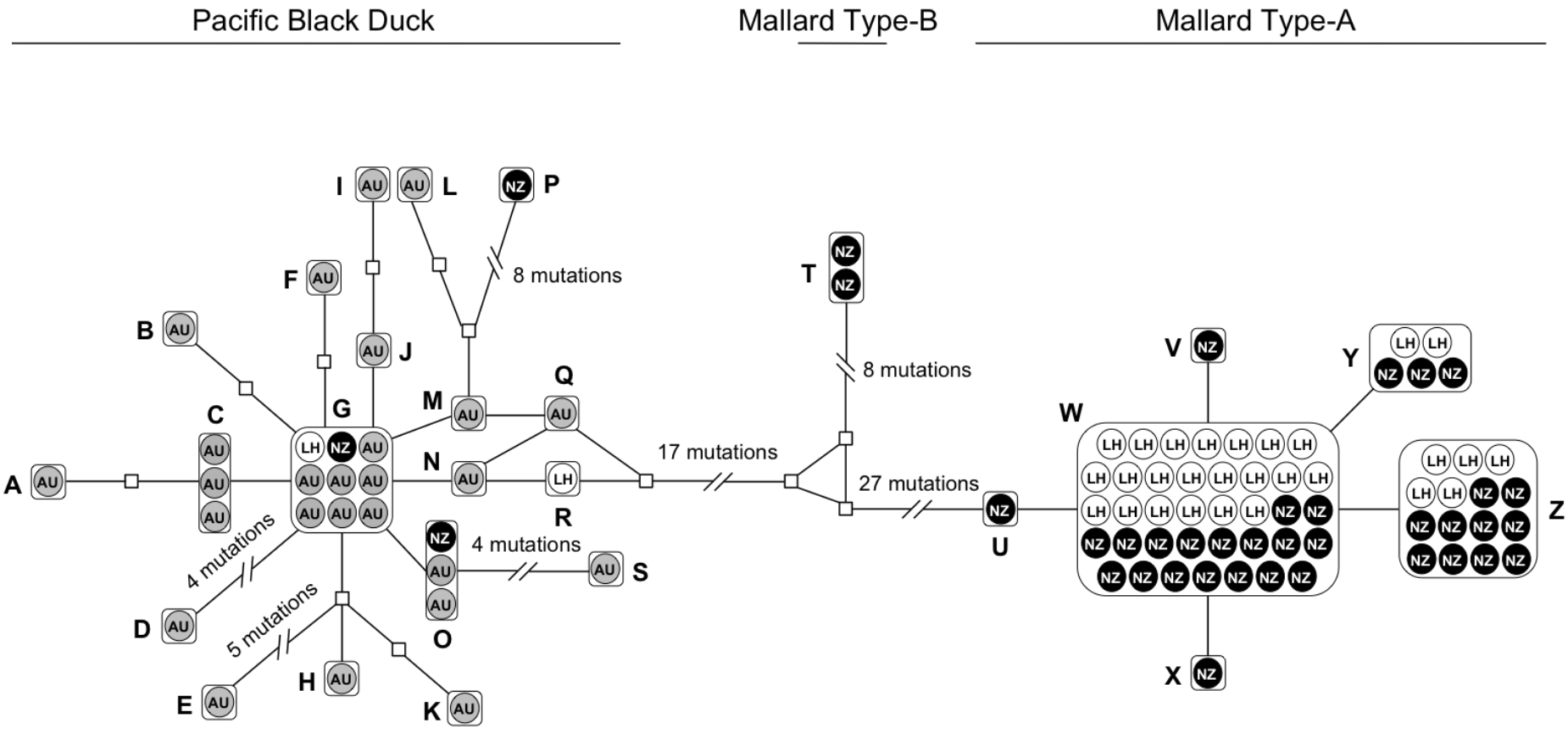

Figure 1. Unrooted haplotype network for the mitochondrial control region for the 27 haplotypes detected from 92 ducks. Sequences are marked 'AU' are from Australia, 'LH' are from Lord Howe Island and 'NZ' are from New Zealand.

Table 1. Haplotype (H) and nucleotide diversities (π) for Pacific Black Ducks from New Zealand, Lord Howe Island and Australia.

Population	***n***	**H**	**π**
New Zealand	37	0.722 ± 0.058	0.007 ± 0.004
Lord Howe Island	28	0.429 ± 0.108	0.005 ± 0.003
Australia	27	0.923 ± 0.039	0.005 ± 0.003

No population genetic structure was detected between birds from Lord Howe Island and New Zealand (Φ_{ST} = -0.010; P = 0.586). However, there was significant population genetic structure between Australian Pacific Black Ducks and Lord Howe Island birds (Φ_{ST} = 0.840; P < 0.001). Furthermore, the 20 birds phenotyped from Lord Howe Island had scores indicative of hybrids or pure Mallards (≥ 10; Table 2).

Table 2. Specimens from Lord Howe Island with their haplotype (see Figure 1) and hybridisation score, where 0-9 is a Pacific Black Duck; 10-24 is a hybrid and; 25-35 is a Mallard according to Gillespie (1985).

Specimen	Haplotype	Haplotype classification	Phenotype score	Phenotype classification according to Gillespie (1985)
CG1	G	Pacific Black Duck	16	Hybrid
CG4	W	Mallard	17	Hybrid
CG9	W	Mallard	32	Mallard
CG40	W	Mallard	19	Hybrid
CG41	W	Mallard	32	Mallard
CG85	Z	Mallard	26	Mallard
CG86	Z	Mallard	33	Mallard
CG87	R	Pacific Black Duck	19	Hybrid
CH85	W	Mallard	31	Mallard
CH88	W	Mallard	21	Hybrid
CH90	W	Mallard	28	Mallard
CH91	W	Mallard	21	Hybrid
CH93	W	Mallard	25	Mallard
CI2	W	Mallard	26	Mallard
CI6	W	Mallard	26	Mallard
CI39	W	Mallard	29	Mallard
CI67	Y	Mallard	33	Mallard
CI83	W	Mallard	34	Mallard
CI84	W	Mallard	26	Mallard
CI89	W	Mallard	33	Mallard

Discussion

Hybridisation and introgression

The majority of ducks on Lord Howe Island possessed Mallard haplotypes and had Mallard or hybrid phenotypes, including the two specimens that were found to have Pacific Black Duck haplotypes. This result extends a phenotype study conducted in 2007 that represented approximately 30% of the population on Lord Howe Island and demonstrated that at least some of the birds with Mallard plumage were hybrids (Tracey et al. 2008).

On Lord Howe Island, the haplotypes of the *Anas* ducks were most similar to New Zealand Mallards. While these haplotypes were not compared to Australian Mallards, only putative Australian Pacific Black Ducks, Mallards in Australia are typically of domestic ancestry and therefore have a limited capacity for long distance flights (Braithwaite & Miller 1975; Marchant & Higgins 1990; Guay & Tracey 2009). Thus, it's unlikely that an Australian Mallard would immigrate to Lord Howe Island.

Most birds on Lord Howe Island had Mallard haplotypes and phenotypes, suggesting that Mallards on Lord Howe Island had outcompleted resident Pacific Black Ducks. This is because phenotypes of both parental species typically exist where introgressive hybridisation occurs (e.g., Yamashina 1948; Muller 2009). While no birds on Lord Howe Island had Pacific Black Duck phenotypes, two birds had Pacific Black Duck haplotypes. This suggests that hybridisation between the two species has occurred, but whether the Paciifc Black Duck haplotype originated from Lord Howe Island ducks or emigrated

with New Zealand hybrids can not be differentiated. Further measures, using more refined methods, such as the use of nuclear markers, will give a better understanding of the rate of hybridisation on Lord Howe Island. Nuclear markers were not utilised in this study, as such studies require relatively high quantities of undegraded nuclear DNA, which is difficult to obtain from from feather samples used here.

Although not an explicit aim of this chapter, these results also revealed no evidence for hybridisation between Pacific Black Duck and Mallard in Australia along the maternal line. This result is in agreement with the low frequency of hybrids detected throughout Australia (Chapter 4 & 5).

Due to the low quality of DNA that is typically extracted from feathers, the use of mtDNA, in conjunction with phenotype, was the most suitable method for this study for identifying hybrids and the direction of hybridisation. The amplification of mtDNA, however, can often result in the co-amplification of numts, nuclear sequences of mitochondrial origin (Sorenson & Quinn 1998). The low levels of variation of the mtDNA sequences within species and identification of matching sequences from tissue, blood and feather specimens supports the conclusion that these sequences are not nuclear copies (McCracken & Sorenson 2005).

Genetic origins

In Asia and North America, much greater haplotype diversities have been detected in Mallard than in New Zealand and on Lord Howe Island (Table 1;

Kulikova et al. 2005; Kraus et al. 2011; Guay et al. 2015). The low haplotype diversity of New Zealand Mallards had been attributed to the small number of founding individuals (Guay et al. 2015). Hybrids on Lord Howe Island possess type-A haplotypes that are common amongst New Zealand birds. No birds from Lord Howe Island were found to possess a type-B haplotype and only two birds out of the 37 sampled from New Zealand had a type-B haplotype. The lack of type-B haplotype on Lord Howe is likely to be a chance event whereby none of the founders of the Mallard population on the island carried type-B haplotypes (Guay et al. 2015).

Haplotype similarity and the lack of structure between Lord Howe Island and New Zealand Mallards supports the hypothesis that Mallards resident on Lord Howe Island originated from New Zealand (McKean & Hindwood 1965; Tracey et al. 2008). This is plausible as Mallards banded in New Zealand have been recorded on Lord Howe Island as well as on Norfolk Island and the Australian mainland (Paton 1991; Anonymous 2013; ABBBS unpublished data; Tracey & Haselden unpublished data). This evidence is also supported by the domestic Mallards limited capacity for flight in Australia (Marchant & Higgins 1990; ABBBS unpublished data). In addition, Mallards on Lord Howe Island had phenotypes similar to wild Mallards rather than domestic strains of Mallard typical in Australia.

Management implications

The results of this study demonstrate a low frequency of Pacific Black Duck haplotypes and phenotypes on Lord Howe Island, which is uncommon where

introgression has occurred (e.g., Yamashina 1948; Muller 2009). It would appear that Mallards and their hybrids have outcompeted Pacific Black Ducks on Lord Howe Island and have undergone hybridisation. It therefore appears that Pacific Black Ducks resident on smaller islands are particularly vulnerable to Mallards via both competition and hybridisation (see Chapter 2). Measures to control Mallards and their hybrids have already been initiated on Lord Howe Island. Local authorities have instigated an ongoing education, monitoring and a Mallard removal program to remove Mallards and their hybrids and prevent re-establishment (Tracey et al. 2008). Guay and Tracey (2009) suggested that monitoring phenotypes and genotypes is essential to gauge the full extent of hybridisation. Cryptic hybrids that arise from the backcrossing of hybrids with both parental phenotypes can eventually be mistaken for pure parental species (Green et al. 2000; Rhymer et al. 2004). Whilst introgressive hybridisation has taken place in New Zealand, hybridisation does not appear to be at the same stage in Australia (Chapter 4; Guay & Tracey 2009). To prevent self-introductions to Australia from Lord Howe Island ongoing visual and genetic monitoring, as well as eradication and management efforts on Lord Howe Island are vital.

Chapter 7

Hybridisation between the Chestnut Teal (*Anas castanea*) and the Pacific Black Duck (*Anas superciliosa*): empirical and genetic evidence.

Unpublished manuscript

Abstract

Hybridisation is a process that occurs between two or more closely-related species when natural isolating mechanisms are removed. Hybridisation can provide an influx of new alleles and genes, increasing genetic diversity or, alternatively, may lead to introgression of the parental genotypes and ultimately extinction. Hybridisation is common amongst waterfowl (Anseriformes) and particularly so amongst dabbling ducks (genus: *Anas*). Despite the exceedingly large list of hybrid combinations amongst the dabbling ducks, hybridisation between the Australian Chestnut Teal (*A. castanea*) and the Pacific Black Duck (*A. superciliosa*) has yet to be formally described. In this chapter empirical and genetic evidence of hybridisation between Chestnut Teals and Pacific Black Ducks are presented. The phenotype of a putative hybrid drake collected by hunters in Tasmania in May 2012 is described and, using microsatellite genotyping and mitochondrial DNA sequencing, it is demonstrated that the putative hybrid is most likely a first generation hybrid between a male Pacific Black Duck and a female Chestnut Teal. Further genetic analyses of putative Pacific Black Ducks from throughout Australia demonstrated that hybridisation between Chestnut Teals and Pacific Black Ducks occurs at a low frequency (0.5%) and therefore neither species is at risk of extinction via hybridisation with the other. This

study is the first to genetically and phenotypically describe natural hybridisation between the Chestnut Teal and the Pacific Black Duck.

Introduction

Hybridisation has been well documented amongst birds, particularly in waterfowl (Anseriformes; McCarthy 2006). Waterfowl have long been kept in captivity and are known to hybridise extensively. Within Anseriformes most hybrid combinations are reported between dabbling duck species (genus: *Anas*) (McCarthy 2006). In particular, two major lineages within *Anas,* the mallards and the teals, have diverged relatively recently and thus have a high incidence of hybridisation, both in the wild and in captivity (McCarthy 2006).

In Australia, one mallard species; the Pacific Black Duck (*A. superciliosa*) and two teal species; the Grey Teal (*A. gracilis*) and the Chestnut Teal (*A. castanea*) exist in sympatry (Phillips 1923; Delacour 1956; Marchant & Higgins 1990). The Pacific Black Duck is a relatively large monomorphic dabbling duck, while the two teal species are both smaller (Marchant & Higgins 1990). Similar to the Pacific Black Duck, the Grey Teal is monomorphic. In contrast, the Chestnut Teal is sexually dimorphic, with the male bearing a distinctive green head and a chestnut-coloured body, while the female has mottled brown plumage similar to that of the Grey Teal, albeit darker (Frith 1982; Marchant & Higgins 1990). Both the Grey Teal and the Pacific Black Duck have a widespread distribution throughout Australia (Phillips 1923; Delacour 1956; Frith 1982; Marchant & Higgins 1990). In contrast, the range of the Chestnut Teal is more restricted to the southeast

and southwest of Australia (Phillips 1923; Delacour 1956; Frith 1982; Marchant & Higgins 1990).

The Pacific Black Duck and the Grey Teal undergo natural and captive hybridisation, as do the introduced Mallard and Chestnut Teal (e.g., Phillips 1923; Lavery 1966; Whatmough 1978; Marchant & Higgins 1990; Paton 1991; Guay et al. In press). Despite this, no cases of hybridisation between Pacific Black Duck and Chestnut Teal has been scientifically reported (see McCarthy 2006), however, anecdotal evidence suggests that it does occur (e.g., Smith 2011; Guay unpublished data). The aims of this study were therefore to (1) describe the phenotype of a putative Chestnut Teal - Pacific Black Duck hybrid, and (2) genetically confirm the hybrid status of the putative hybrid using mtDNA and microsatellite markers.

Methods

Putative Chestnut Teal – Pacific Black Duck hybrid specimen

The putative hybrid was shot by a hunter from Richmond Hill, Macquarie River, Tasmania, during May 2012 and the sample was provided by Queen Victoria Museum, Hobart, Launceston (Specimen number: QVM:2012:2:1). A photo of the specimen was supplied (Figure 1).

To describe the phenotype of the hybrid specimen, I adapted the identification keys of two similar duck identification keys; Rhymer et al. (1994) hybrid index for Pacific Black Duck – Mallard hybrids and the Urdiales and Pereira (1993) identification key of *Oxyura jamaicensis* and *Oxyura leucocephala* and their

hybrids. The identification keys provided a scoring technique that is based on the similarity of the specimen's phenotype to each of the parental species. The method used by Urdiales and Pereira (1993) was adapted for this study because it considered most features when distinguishing between parent species and hybrids. However, here the phenotype of the hybrid specimen using the photograph provided in figure 1 was assessed. The phenotype analysis was therefore more limited to the body parts visible in the photograph. Here the colouration of the face, neck, head, upper body and breast was considered.

Figure 1. Chestnut Teal – Pacific Black Duck hybrid specimen (bottom) and a putatively pure Pacific Black Duck (top).

Laboratory analyses

A total of 20 ducks (10 putatively pure Pacific Black Ducks and 10 putatively pure Chestnut Teals) collected from Tasmania, Australia, between 2006 and 2007 were used to establish the effectiveness of the genetic system established in Chapter 3 at distinguishing between Chestnut Teals, Pacific Black Ducks and their hybrids. Due to the low sample size used in this study, it is recommended that any future studies should include a larger sample size (25-30 samples per population) (see Hale et al. 2012).

The specimens were either caught by hand (see Chapter 4) and had 50μl of blood taken from the tarsal vein using a 26-gauge needle or tissue samples were provided by hunters. Samples were stored in 70% ethanol to prevent deterioration of the tissue or blood. DNA was extracted using a salting-out procedure (Bruford et al. 1992).

The nine microsatellite loci that were identified (see Chapter 3) to distinguish between domestic Mallards, Pacific Black Ducks and their hybrids were successfully amplified in the putatively pure Tasmanian Chestnut Teal, with the exception of Bcaμ4. Polymerase chain reactions (PCR) were performed and, and microsatellites were visualised and scored, as per Chapter 3.

To identify the sex of the hybrid sample the primers P8 and P2 were used according to the method described by Griffiths et al. (1998). mtDNA was used to identify the direction of hybridisation due to its maternal inheritance. Specifically, a 667bp fragment of the 5' end of the mitochondrial genome

control region was amplified by PCR from the putative hybrid sample using primers L78 (Sorenson & Fleischer 1996) and H774 (Sorenson et al. 1999). PCR were set up as 50μL reactions containing 2 μL of DNA, 300 pmoles of each primer (L78 and H774), dNTPs (200 nM), $MgCl_2$ (2.5 mM), Colorless GoTaq Flexi Buffer (1X) and 2.5 units of Taq DNA Polymerase (Promega). Betaine (1.0M) was added to the PCR reaction to increase yield (Johnson & Dunn 2006). PCR amplifications were performed at 1 cycle of 7 minutes at 94°C, 45 cycles of 20 seconds at 94°C, 20 seconds at 52°C, 60 seconds at 72°C and 1 cycle for 7 minutes at 72°C using a MyCycler thermal cycler (Biorad).

PCR products were separated by agarose gel electrophoresis. The bands were excised and gel purified using a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA), and sequenced commercially (Australian Genome Research Facility Pty Ltd, Melbourne, Australia). Fragments were sequenced in both directions using the primers L78 and H774. Complementary sequences of mtDNA were reconciled using SEQUENCHER 3.1 (Gene Codes, Ann Arbor, MI USA) and discrepancies were reconciled visually. The sequence was uploaded to Genbank (Accession number: KJ866053).

Phylogenetic analyses

Phylogenetic analyses were conducted with both maximum likelihood and Bayesian methods used to identify the maternal line of the hybrid specimen. A maximum-likelihood tree was constructed in Treefinder (Jobb et al. 2004) using the nucleotide substitution model suggested by the Bayesian

information criterion. Bootstrap support was estimated after 1000 replicates. A Bayesian phylogeny was then constructed using the same substitution model as previous in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The posterior probabilities were estimated after 2 million generations, sampling every 1000 generations. In our analysis the mtDNA sequence of the hybrid individual, several sequences of both parental species and the sequences from *Anas discors* and *Anas cyanoptera* to help resolve the phylogeny was included. Finally, the tree was rooted with *Aythya americana* and *Aythya nycora*. All additional sequences were obtained from the GenBank database by the National Center for Biotechnology Information.

Data analyses

GENEPOP 4.2 (Raymond & Rousset 1995; Rousset 2008) was implemented to test for linkage disequilibrium among polymorphic loci and MICROCHECKER (van Oosterhout et al. 2004) was used to detect null alleles. FSTAT 2.9.3 (Goudet 1995) to characterise loci, including the number of alleles, observed and expected heterozygosity and loci were tested for allele frequency differences at each locus to determine a genetic difference between the two species by calculating loci-specific pairwise F_{ST}.

To assess the assignment power of the loci, assignment tests for 20 ducks (10 putatively pure Pacific Black Ducks and 10 putatively pure Chestnut Teals) collected from Tasmania were conducted to determine the reliability of the microsatellites identified in Chapter 3 to distinguish between putatively pure Chestnut Teals and Pacific Black Ducks. Assignment tests, without prior

information, were performed with STRUCTURE 2.3.4 (Pritchard et al. 2000) with a burn-in period of 10,000 and 1,000,000 replicates, conditions used by Williams et al. (2005). Genetic cluster assignment was performed using the method described by Steeves et al. (2010), where 10 independent replicates, using the same burn-in period and number of replicates as above, with different random seeds were run for $K = 1$ and $K = 2$ (see Chapter 3).

The assignment power of our loci was assessed using four different categories; Pacific Black Duck, $q < 0.10$; backcross F_1 x Pacific Black Duck, $0.15 < q < 0.35$; F_1, $0.40 < q < 0.60$; F_1 x Chestnut Teal, $0.65 < q < 0.85$ Chestnut Teal, $q > 0.90$. Other hybrid combinations were not tested for as it was unlikely that they would be detected given the phenotype of the specimens and the number of microsatellite markers used (Vaha & Primmer 2006). Fifty hybrids for each of the two hybrid categories was simulated using HYBRIDLAB 1.0 (Nielsen et al. 2006) and following Vali et al. (2010) the hybrid detection power of the set of loci using detection efficiency, accuracy and overall performance was tested.

Efficiency is defined as the proportion of individuals in a group that were correctly identified (i.e. the number of individuals correctly identified within a group divided by the true number of individuals within that group). Accuracy is defined as the proportion of individuals assigned to a particular group that actually belong to that group (i.e. number of individuals assigned to a group divided by number of individuals that actually belong to that group). The overall performance is the mean of the efficiency multiplied by the mean of the

accuracy.

Results

Putative hybrid specimen

The putative hybrid specimen was genetically identified as male and had both features of a male Chestnut Teal and a Pacific Black Duck. For example, the specimen had facial markings similar to those of a pure Pacific Black Duck and a chestnut coloured chest of a male Chestnut Teal. The full phenotype description of the hybrid specimen is provided in Table 1.

Table 1. Phenotype description of the Chestnut Teal – Pacific Black Duck hybrid specimen compared to that of a putatively pure Chestnut Teal and Pacific Black Duck as described by Frith (1967) and Marchant and Higgins (1990).

Body part	Chestnut Teal	Pacific Black Duck	Chestnut Teal – Pacific Black Duck hybrid specimen
Face	**Adult male –** Glossy green. **Adult female –** Face fawn with blackish streaks. Throat is a pale brown.	Cream face and throat. Face has two dark stripes; one extending from the bill through the eye; and the other below the eye mottling at the cheek.	The face and throat of the hybrid specimen was much more orange in colour compared to that of a Pacific Black Duck. The lower facial stripe appears much more mottled than that of a putative Pacific Black Duck.
Head	**Adult male –** Glossy green. **Adult female** – Dark brown	Dark brown.	The upper head of the hybrid specimen appears to be dark brown – black in colour, typical in Pacific Black Duck.
Upper body	**Adult male –** Dark brown, each feather scalloped by chestnut colouration. **Adult female –** Dark brown.	Dark brown, scalloped by lighter brown.	The body of the hybrid specimen appears to be dark brown scalloped by chestnut colouration.
Breast	**Adult male –** Chestnut coloured. **Adult female –** Pale brown, each feather having a dark centre.	Brown.	The breast of the hybrid specimen is chestnut in colour, typical of a male Chestnut Teal.

Phylogeny

The mtDNA sequence of the hybrid specimen clustered with sequences derived from Chestnut Teals, indicating that the mtDNA of the hybrid specimen was that of a Chestnut Teal (Figure 2).

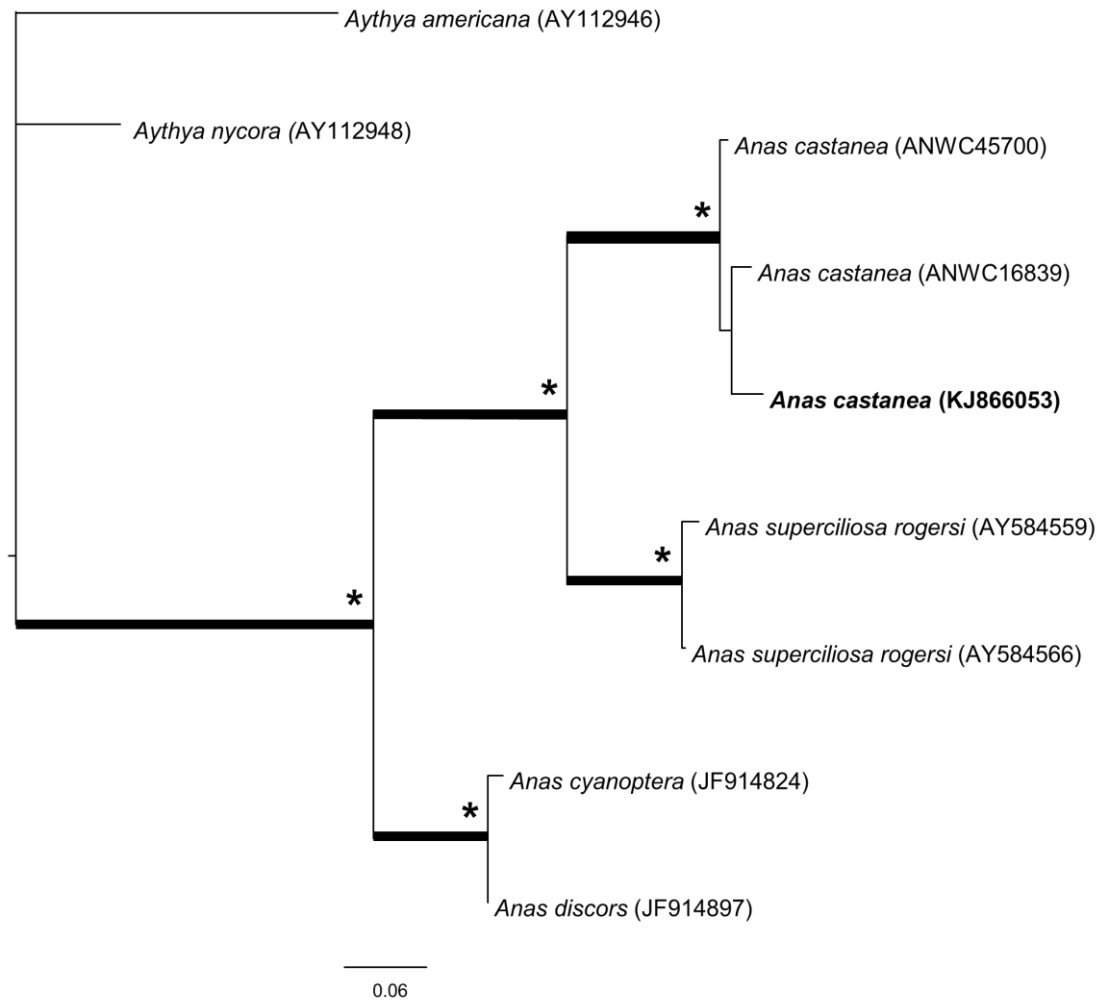

Figure 2. Phylogenetic tree outlining the relationship between the Chestnut Teal - Pacific Black Duck hybrid (accession number: KJ866053) and the parental species inferred using 284 bp of the mtDNA control region. Phylogenies were inferred using Maximum Likelihood and Bayesian methods. Thick-lined nodes represent those with high Maximum Likelihood bootstrap support (≥70%) and asterisks highlight nodes with Bayesian posterior probabilities ≥95%.

Microsatellite marker characteristics

Following Benjamini-Yekutieli correction for multiple tests (σ = 0.05; Narum 2006), no significant linkage disequilibrium and no significant deviation from Hardy-Weinberg equilibrium for either species was detected. Significant pairwise F_{ST} were detected between species for each locus except for Smo7 (Table 2). No null alleles were detected.

Table 2. Microsatellite loci with number of alleles, allele size, expected and observed heterozygosity and pairwise F_{ST} for putative Tasmanian Pacific Black Duck and Chestnut Teal.

Locus	**Pacific Black Duck**				**Chestnut Teal**				**F_{ST}**
	No. of alleles	**Allele size range (bp)**	**H_e**	**H_o**	**No. of alleles**	**Allele size range (bp)**	**H_e**	**H_o**	
Aph16	5	160-166	0.389	0.328	2	166-168	0.327	0.444	0.596
Aph25	3	182-187	0.010	0.010	3	183-189	0.612	0.625	0.962
Apl11	9	111-137	0.458	0.463	5	115-129	0.635	0.700	0.448
CM09	10	120-140	0.739	0.718	6	116-130	0.658	0.800	0.149
Cam3	5	167-177	0.326	0.279	4	167-177	0.420	0.400	0.639
Sfiμ2	6	383-403	0.448	0.266	2	379-383	0.164	0.000	0.602
Smo7	9	202-224	0.592	0.570	3	202-206	0.426	0.556	-0.020
JCC1	17	159-183	0.831	0.683	6	165-193	0.612	0.600	0.223

Hybrid detection performance

The detection of pure Chestnut Teals and Pacific Black Ducks was the most reliable and accurate at assigning the hybrid to its correct group (efficiency = 100%; accuracy = 100%), followed by F_1 hybrids (efficiency = 100%; accuracy = 92%), while Chestnut Teal backcrosses were the most difficult to accurately categorise (efficiency = 0%; accuracy = 0%; Table 3).

Table 3. Characteristics of the hybrid detection power of the eight microsatellite loci.

Class	ASS	EFF	ACC	PERF
Chestnut Teal	1.0	1.0	1.0	1.0
Pacific Black Duck	1.0	1.0	1.0	1.0
F_1	1.0	1.0	0.92	0.92
Chestnut Teal backcross	1.0	0	0	0
Pacific Black Duck backcross	1.0	0.98	0.24	0.24
Hybrid	1.0	0.99	0.58	0.57

Variables are: percentage of individuals assigned to a group regardless of whether this assignment was correct (ASS), detection efficiency (EFF), detection accuracy (ACC) and overall performance of the loci (PERF). Refer to main text for a detailed explanation of each variable. The 'hybrid' class represents all hybrid individuals grouped together (i.e. F1 plus backcrosses).

Assignment testing

Using STRUCTURE, the putative number of clusters was calculated to be two. Assignment tests confirmed that all putatively non-hybrid individuals were allotted to the expected population (Figure 3). The hybrid sample had an assignment probability of 0.31, which suggests that the specimen is a Pacific Black Duck backcross (Figure 3).

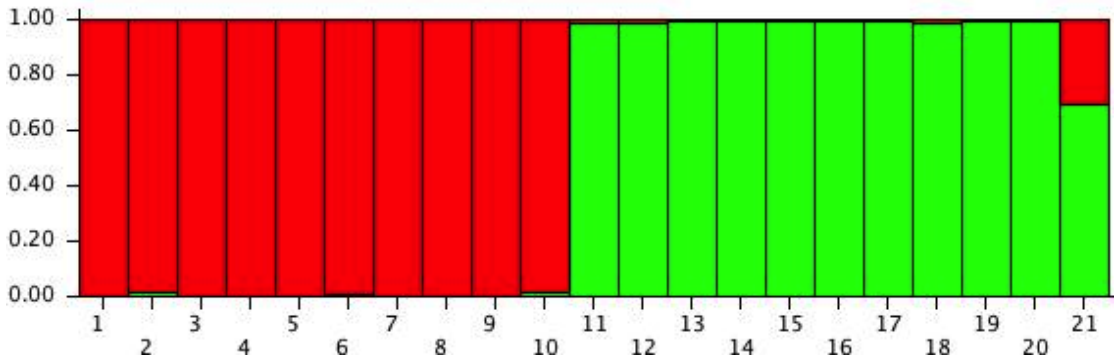

Figure 3. STRUCTURE 2.3.4. (Pritchard et al. 2000) output shows the proportion of membership based on genetic information for 2 groups, where individuals 1-10 are Chestnut Teals and 11-20 are Pacific Black Ducks. Specimen 21 is a putative hybrid, where the assignment probability was 0.31.

Discussion

This study has provided the first formal description and genetic verification of natural hybridisation between a Chestnut Teal and a Pacific Black Duck. The hybrid specimen described had intermediate plumage between that of a male Chestnut Teal and a Pacific Black Duck (Table 1). These genetic results together with the phenotype description support the case that a copulation occurred between a female Chestnut Teal and Pacific Black Duck drake.

The eight microsatellite markers used to detect hybridisation were most effective at distinguishing pure Chestnut Teals and Pacific Black Ducks. The efficiency of detecting a F_1 hybrid did not differ from that of pure Chestnut Teals and Pacific Black Ducks, but the accuracy was slightly reduced. The putative hybrid specimen had an assignment probability of 0.31, indicating that the specimen was a Pacific Black Duck backcross. Contrarily, the distinct chestnut phenotype indicates that the specimen is a F_1 hybrid, however, the reduced accuracy of the microsatellite markers used for detecting Pacific Black Duck backcrosses means that the genetic data is inconclusive. Additional genetic data is required to determine the category of the hybrid. In addition, the hybrid specimen was genetically confirmed to be male. The chestnut phenotype of a male Pacific Black Duck backcross may be less obvious.

Interspecific hybridisation is generally the result of mistaken identity, interspecific mate preferences or forced extra-pair copulations (FEPCs). While errors in species recognition can result in interspecific hybridisation,

hybridisation between the Grey Teal and the Pacific Black Duck is believed to occur through heterospecific mate choice that may result from brood parasitism or mixed flocking (Guay et al. In press). Brood parasitism is known to be common in many *Anas* species and individuals raised by heterospecifics are known to prefer the other species with which it was raised (Brodsky et al. 1989; Lyon & Eadie 1991). On the other hand, female Chestnut Teals, similar to other dabbling duck species, may have a preference for larger and potentially more aggressive Pacific Black Duck males compared to intraspecific pairing (Brodsky et al. 1988; Marchant & Higgins 1990). It may also be possible that the pairing was the result of FEPCs as in most duck species sex-ratios are male-biased, leaving an excess of drakes that may not be able to pair intraspecifically (Bowler 2005). Extra males may then try to pair with unpaired females from other species through forced extra-pair copulation (Brodsky & Weatherhead 1984). Furthermore, FEPCs are known to be common in most *Anas* species, including both Chestnut Teals and Pacific Black Ducks (McKinney et al. 1983; McKinney & Evarts 1997; Coker et al. 2002). To determine the mechanism behind Chestnut Teal – Pacific Black Duck hybridisation, if it persists, further genetic analyses of putative Chestnut Teal and Pacific Black Duck populations are required. Considering the poor detectablity of Chestnut Teal backcrosses using the current microsatellite loci, a greater number of loci need to be included in future studies in order to detect the presence of backcrosses in the wild.

The microsatellites used here are limited by their inability to distinguish between Chestnut and Grey Teals (Taysom et al. unpublished data). The

Chestnut Teal and the Grey Teal are two very closely related species which cannot be confidently distinguished from their mtDNA (Johnson & Sorenson 1999; Joseph et al. 2009; Dhami et al. 2013), suggesting that they have only recently diverged (Johnson & Sorenson 1999; Joseph et al. 2009; Dhami et al. 2013). Therefore, a greater number of microsatellite markers to distinguish between the three *Anas* species need to be incorporated in future studies.

While putative Chestnut Teal – Pacific Black Duck hybrids have been observed and shot in the wild (Smith 2011; Guay unpublished data) there are presently more records of Grey Teal - Pacific Black Duck hybridisation events than records of Chestnut Teal and Pacific Black Duck hybridisation (McCarthy 2006). Most of these reports of Grey Teal - Pacific Black Duck hybridisation are based on phenotype, however, more recently the direction of Grey Teal - Pacific Black Duck hybridisation has examined using the mitochondrial control region (e.g., Phillips 1923; Lavery 1966; Marchant & Higgins 1990; Guay et al. In press). The greater number of records of Grey Teal - Pacific Black Duck hybridisation may partly be due to the fact that sexually dichromatic male Chestnut Teal hybrids are more easily distinguished, while female Chestnut Teal and Grey Teal hybrids would be almost identical. It is therefore possible that female Chestnut Teal – Pacific Black Duck hybrids had been misidentified as Grey Teal – Pacific Black Duck hybrids. The further development of the genetic system to incorporate more microsatellite markers may lead to the genetic distinction between the two teal species.

Overall, the origin of a putative hybrid specimen was successfully identified

using genetic techniques supported by a relevant phenotype description. Additional sightings of birds with similar phenotypes (Guay unpublished data) suggests that this hybrid combination is not a one-off occurrence. The mechanisms behind Chestnut Teal – Pacific Black Duck hybridisation warrants further investigation.

Chapter 8

General Discussion

Hybridisation: a cause of extinction?

Overall, a low frequency of hybridisation was detected between introduced Mallards (*Anas platyrhynchos*) and the Australian Pacific Black Duck (*A. superciliosa rogersi*) (Chapters 4 & 5). A low level of hybridisation is known to be beneficial to some populations with little genetic diversity as it facilitates gene flow and thus increases genetic diversity (Pierotti & Annett 1993; Veen et al. 2001; Lengagne et al. 2006). For example, hybridisation between two species of frogs, *Rana lessonae* and *Rana esculenta*, was found to be facilitated by a higher mating success of *Rana lessonae* males, increasing genetic diversity (Lengagne et al. 2006). Furthermore, populations of Mottled Duck with low genetic variability in Lousiana and Texas, USA, are thought to benefit from genetic exchange with Mallards (Peters et al. 2014). However, the large haplotype diversity of the Pacific Black Duck compared to the Mallard (Chapter 6) suggests that Pacific Black Ducks across Australia are genetically diverse and are therefore not at risk of inbreeding or a genetic depression. Thus, hybridisation would largely be a negative outcome and, if left unchecked, could eventually threaten the species (as has occurred in New Zealand).

Hybridisation between introduced Mallards and Pacific Black Ducks is of greater conservation concern than hybridisation between the Pacific Black Duck and the Chestnut Teal, as the former was facilitated by humans. Furthermore, Mallards have been known to cause extinctions and endanger

other dabbling duck species worldwide (reviewed in Chapter 2). However, the first record of Chestnut Teal and Pacific Black Duck hybridisation is important as it demonstates genetic exchange between two species that co-evolved and have therefore had more time to evolve mechanisms to prevent interspecies matings. Conversley, larger sampling of putative Chestnut Teals and Pacific Black Ducks may be used to indicate a loss of biodiversity that could potentially be due to habitat modification and climate change (Chapin et al. 2000).

Hybridisation with Mallards appears to be widespread in areas where it cannot be controlled (e.g., large continents like North America) and where it involves wild Mallards that are able to disperse long distances (Chapter 2). For example, wild Mallards released in New Zealand have colonised the entire country and consequently hybridised extensively with the Pacific Black Ducks to the point where it is now considered Nationally critical and few pure Pacific Black Ducks are believed to remain (Rhymer et al. 2004; Muller 2009; Robertson et al. 2012). Furthermore, Mallards and their hybrids from New Zealand have also colonised Lord Howe Island and eventually outcompeted native Pacific Black Ducks (Chapter 6). In contrast, the frequency of hybridisation between domestic Mallard and the Australian Pacific Black Duck was found to be relatively low, with 1.5% of the sample genetically classified as hybrids compared to 90% in New Zealand (Chapter 4; Muller 2009). Therefore, it appears that in larger landscapes, domestic Mallards do not pose an overall threat to native ducks. Hybridisation was more common in urban areas (Chapter 5) and therefore hybridisation in Australia appears to be more

isolated, but is still an overall threat to the genetic integrity of the Pacific Black Duck.

Hybridisation in urban areas

Within Victoria there was an overall tendency for hybrids to be more common in urban areas compared to rural areas (Chapter 5). The observed dominance of Mallards over Pacific Black Ducks living in metropolitan Melbourne supports the genetic data of hybridisation between the two species because Mallard drakes may force copulation with Pacific Black Duck females (Chapter 5). Furthermore, Mallard drakes were observed in a much larger ratio compared to females, which could be a factor influencing the frequency of interspecific forced extra-pair copulations among Mallard drakes (e.g., Brodsky & Weatherhead 1984; Ankney et al. 1987). While it is suspected that these behaviours in Mallards may lead to interspecific hybridisation, it has not yet been confirmed and thus the mechanisms behind hybridisation between domestic Mallards and the Australian Pacific Black Duck warrants further research.

In addition, the overall incidence of hybridisation may be low or go undetected in areas where the number of resident Mallards are relatively low. For example, Mallards and their hybrids were visually detected in much greater numbers in Adelaide compared to Brisbane or Melbourne (Chapter 5; Paton et al. 1992; Sinden et al. 2003). Thus, it appears that where Mallards are more common, the detection of their hybrids is more likely.

What are the effects of artificial feeding on hybridisation?

The presence of Mallards in urban wetlands and the resulting hybridisation with the Pacific Black Duck could be exacerbated by human activities, such as feeding. Hybrids in this study were found to be more common in urban areas (Chapter 5), where Mallards are also known to be more common (Braithwaite & Norman 1974; Marchant & Higgins 1990; Guay & Tracey 2009). It has been suggested that artificial feeding may support domestic Mallard populations as well as influence the frequency of hybridisation by encouraging flocks to mix unnaturally (Brodsky & Weatherhead 1984; Chapman & Jones 2010). The impacts of artificial feeding in Australia are just starting to be explored (e.g., Chapman & Jones 2009; 2011; Jones 2011). To investigate impacts of artificial feeding in regards to hybridisation, I suggest observing the rates of artificial feeding and the activity budgets of birds both in parks where hybrids are common (e.g., Torrens River, Adelaide; Paton et al. 1992) and in areas where they are not (e.g., Albert Park Lake, Melbourne; Chapter 5).

Do hybrids disperse as far as pure Pacific Black Ducks?

This study found a tendency for hybrids to be more common in urban areas compared to rural regions throughout Victoria (Chapter 5). While no hybrids were detected in rural areas in Chapter 5, other studies have identified hybrids in rural areas throughout Australia (Chapter 4; Paton et al. 1992; Guay & Tracey 2009). The Pacific Black Duck is known to have a great capacity for flight, with records of Pacific Black Duck movements across the Tasman sea (Marchant & Higgins 1990). In contrast, domestic Mallards, typical in Australia, are sedentary as they were originally bred for meat and

eggs and therefore domestic birds are much heavier than their wild ancestors (Braithwaite & Miller 1975; Frith 1982; Marchant & Higgins 1990; ABBBS unpublished data). The flight capacity of domestic Mallard and Pacific Black Duck hybrids is currently unknown. It is thought that Pacific Black Duck backcross hybrids would be more capable of long distance flights because the majority of their genes would be from Pacific Black Ducks (Guay & Tracey 2009). Satellite trackers, or the cheaper alternative of tracking using PVC identification legbands, can be used to assess the flight capacity and the dispersal ability of genetically identified hybrids where they are common (e.g., Adelaide; Paton et al. 1992). This information can then be used to determine the risk of hybrids, rather than pure Mallards, spreading the Mallard genome throughout Australia.

Is Australia at risk of invasion from wild Mallards from Lord Howe Island?

Lord Howe Island is situated halfway between mainland Australia and New Zealand and was colonised by Mallards shortly after their release in New Zealand (Chapter 6). Wild Mallards in New Zealand have hybridised with a subspecies of the Pacific Black Duck, *A. s. superciliosa*, and wild Mallards on Lord Howe Island are thought to have outcompeted native Pacific Black Ducks (Chapter 6; Gillespie 1985; Rhymer et al. 1994; Muller 2009). It is believed that Mallards could colonise Australia from their stronghold on Lord Howe Island and their arrival on the Australian mainland is not unprecedented with records of New Zealand-banded immigrants already reported in Australia (Paton 1991; Anonymous 2013; ABBBS unpublished data). I suggest that Mallards on Lord Howe Island should be fitted with satellite tracking

technology, or PVC identification legbands, to assess their movements to determine whether resident Mallards move locally or whether they are likely to disperse over long distances. While the Australian Pacific Black Duck population is not under immediate threat of introgressive hybridisation with domestic Mallards, mainland Australia could be at risk of invasion by wild Mallards, which could increase the risk of hybridisation.

Management decisions

While the frequency of feral Mallard and Pacific Black Duck hybridisation appears low and not of concern in Australia, changes in duck behaviour, prevalence of Mallards or the environment may increase the rate of hybridisation. Therefore, I suggest a precautionary method of Mallard management by eradicating all feral Mallards from parks where they are known to exist to ensure the genetic integrity of the native Pacific Black Duck.

In other cases of hybridisation, involving species from different groups, the risk of hybridisation appears to generally be increased when the invading species have a great capacity for dispersal (see Chapter 2). Furthermore, certain behavioural traits, like dominance and aggression, may negatively impact the population size of a species by interspecific hybridisation and competition (Chapter 4 & 6). Overall, the characteristics of each invading species should be taken into consideration when considering the risk to the native species. Each potential case warrants further research to reduce the loss of biodiversity.

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Appendices

Appendix 1. Taysom, A. J., Johnson, J. & Guay, P.-J. (2014) Establishing a genetic system to distinguish between domestic Mallards, Pacific Black Ducks and their hybrids. *Conservation Genetics Resources*. **6**, 197-199.

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MICROSATELLITE LETTERS

Establishing a genetic system to distinguish between domestic Mallards, Pacific Black Ducks and their hybrids

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Abstract Dabbling ducks are subject to many threatening processes. Hybridisation with introduced Mallards (*Anas platyrhynchos*) is a threat that is commonly overlooked. Mallards, both domestic and wild, have been introduced in Australia and New Zealand. While hybridisation with Mallards caused significant decline in the New Zealand populations of Pacific Black (Grey) Ducks (*Anas superciliosa*), the degree of hybridisation between Mallard and the Pacific Black Duck in Australia is currently unknown, largely because hybrid backcrosses are difficult to visually identify. We screened 27 cross-amplifying waterfowl microsatellite markers and developed a set of 9 markers that can be used for genotyping and assignment tests to identify cryptic hybrids. Assignment tests, performed with the program Structure 2.3.4, had a 99 % likelihood that specimens of a known species (Mallard or Pacific Black Duck) were assigned to the correct group. The system was applied used to confirm the hybrid status of two putative hybrids identified phenotypically. The successful application of this system demonstrates its potential use in determining the rate of hybridisation between introduced Mallards and Pacific Black Ducks throughout Australia.

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Introduction

The Mallard, native to the northern hemisphere, is known to hybridise with various other dabbling duck species (reviewed in Guay et al. In Press). Mallards have been introduced to many foreign countries as game and ornamental birds where they encounter, hybridise and consequently threaten many native dabbling duck species (Guay et al. In Press). In New Zealand, introduced Mallards have hybridised with the native Pacific Black (Grey) Duck (*Anas superciliosa*) to the extent that the latter is now considered endangered. Unlike Mallards introduced to New Zealand that were of wild origin, introduced Mallards remaining in Australia today are typically of domestic origin and have not yet colonised the whole country (Guay et al. In Press). Due to the domestic ancestry of Mallards in Australia, hybridisation between Mallards and the Pacific Black Duck was originally believed to be restricted to urban areas, where domestic Mallards are prevalent (reviewed in Guay et al. In Press). Until now, Pacific Black Duck-Mallard hybrids have been identified phenotypically, however this method can underestimate the true frequency of hybrids as cryptic hybrids are generally overlooked Our aim was to establish a robust genetic system that could accurately discriminate between domestic Mallards, Pacific Black Ducks and their hybrids using DNA microsatellite markers.

Methods

Genetic samples (blood or tissue) from 10 putatively pure Pacific Black Ducks, 10 domestic Mallards and two

putative hybrids were collected throughout Australia from 2006 through 2012 (Online Resource 1). DNA was extracted using a salting-out procedure (Bruford et al. 1992).

We screened a total of 27 waterfowl microsatellites for cross-amplification in Pacific Black Ducks and Mallards (Online Resource 2). Primer pairs that consistently amplified a product were screened for polymorphism. The forward primer of each primer pair was synthesized with a 5′-M13 Tag (5′CACGACGTTGTAAAACGAC) to use in the universal dye labelling method (Boutin-Ganache et al. 2001). PCR reactions (10 μL) contained 0.25 units Taq polymerase (Promega), $MgCl_2$ (Table 1), 1× reaction buffer, 200 μM dNTPs, 300 nM of an M13 primer 5′-labelled with an ABI dye (NED, FAM, VIC and PET), the locus specific tailed (20 nM) and untailed (300 nM) primers and approximately 100 ng of genomic DNA. The reactions were heated to 95 °C for 60 s, followed by 40 cycles of 94 °C for 20 s, 55 °C for 30 s, 73 °C for 90 s, with a final extension step of 73 °C for 5 min on a Biorad MyCycler Personal Thermal Cycler. PCR product sizes were scored commercially (Australian Genomic Resource Facility, AGRF). Genotyping for all specimens was completed twice to ensure an accurate result. We tested polymorphic primer pairs for linkage disequilibrium using GENEPOP 4.2 and for deviation from the Hardy–Weinberg equilibrium using FSTAT 2.9.3. Finally, we tested each primer pair for allele frequency differences between the two species by calculating loci specific pairwise Fst using FSTAT 2.9.3.

Assignment tests were performed with Structure 2.3.4 (Prichard et al. 2000) using the conditions described by Williams et al. (2005). Any individual displaying a proportion of membership (q) greater than 0.9 for either species was considered to be of pure heritage and any individual with $q < 0.9$ for either species was considered a hybrid (see Williams et al. 2005).

Results

Of the 27 polymorphic loci screened for selection, a total of 9 yielded consistent results and significant allele frequency differences between the two species and were selected for further analyses. No significant linkage disequilibrium or allele frequency differences between the two species were

Table 1 Microsatellite loci used in this study with magnesium chloride concentrations, allele size, number of alleles, calculated expected and observed heterozygosity and F statistic

Locus	$MgCl_2$ (μM)	Pacific Black Duck				Mallard			
		No. of alleles	Allele size range (bp)	H_e	H_o	No. of alleles	Allele size range (bp)	H_e	H_o
Aph16	450	2	162–164	0.267	0.300	3	162–166	0.194	0.200
Aph25	225	1	185	0.000	0.000	2	185–187	0.478	0.500
Apl11	225	4	111–131	0.578	0.100	5	109–139	0.833	0.778
Bcaμ4	450	4	207–213	0.783	0.200	1	211	0.000	0.000
Cam3	225	4	167–177	0.561	0.500	3	168–175	0.361	0.200
CM09	450	5	120–140	0.761	0.600	5	120–128	0.811	0.500
Sfiμ2	450	2	391–393	0.500	0.100	1	383	0.100	0.000
Smo7	450	4	202–224	0.694	0.400	2	202–206	0.522	0.500
JCC1	450	7	167–181	0.833	0.800	6	165–180	0.772	0.600

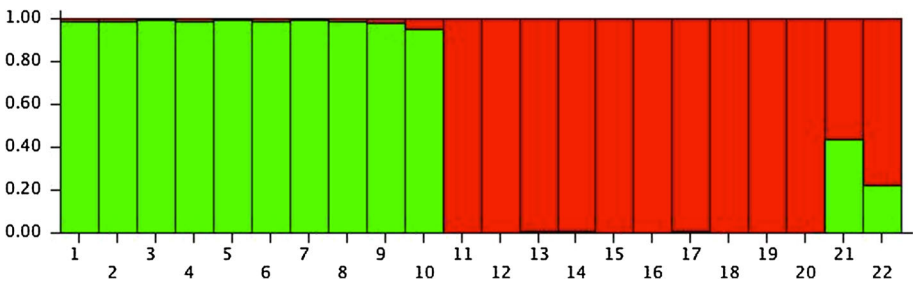

Fig. 1 Structure (Prichard et al. 2000) bar graph output shows the proportion of membership based on genetic information for 2 groups, where individuals 1–10 are domestic Mallards and 11–20 are putatively pure Pacific Black Ducks. Specimens 21 and 22 are putative hybrids, where the proportion of membership to the putatively pure Pacific black Duck group is 0.56 and 0.77 respectively

detected. No significant deviation from the Hardy–Weinberg equilibrium was detected. Significant differentiation in allele frequencies was also detected for each primer pair.

Assignment tests confirmed that all individuals were allotted to the expected population (Fig. 1). Pure Pacific Black Ducks and domestic Mallards were assigned to their species cluster at a probability of $q > 0.99$. The two hybrids had assignment probability to the Pacific Black Duck cluster of 0.56 and 0.77 respectively (Fig. 1).

Discussion

The microsatellite markers selected allowed successful assignments of ducks to either of the two species studied. Furthermore, using our set of markers, we were able to confirm the hybrid ancestry of ducks putatively identified as hybrids based on phenotype. Both putative hybrids had assignment probability lower than 0.90, which has been suggested to result from mixed ancestry (Williams et al. 2005). Our results demonstrate that the set of markers described here will allow the unambiguous identification of hybrids, including cryptic hybrids, thereby permitting the evaluation of true hybrid frequency in Australia. This set of markers will also have wide applicability as hybridisation between native dabbling ducks and domestic Mallards is a worldwide problem. Our genotyping system thus has the potential to be applied to a number of other hybrid crosses in various part of the world, including southern Africa where domestic Mallards have been introduced.

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Appendix 2. Locations within Melbourne where the surveys in Chapter 4 took place and the estimates of Pacific Black Duck and Mallard numbers.

Location	Bearing	Pacific Black Ducks	Pacific Black Duck ducklings	Mallards		
				Mallard males	Mallard females	Total Mallard count
Albert Park Lake	37°84'S 144°97'E	11		2		2
Alistair Knox Park	37°72'S 145°14'E	11				
All Nations Park	37°77'S 145°01'E			2		2
Arthur Streeton Drive	37°73'S 145°09'E	20		8		9
Binnak Park	37°70'S 145°08'E					
Botanic Boulevard	37°68'S 145°05'E	30		5		5
Bundoora Park	37°71'S 145°05'E	1				
Burndap Park	37°78'S 144°90'E	5	6	3	2	6
Cairnlea Lake	37°76'S 144°80'E	10		6	4	10
Carlton Gardens	37°81'S 144°97'E	13		3		3
Caulfield Park	37°87'S 145°03'E	5		6	2	8
Cherry Lake	37°86'S 144°84'E					
Coburg Lake	37°73'S 144°97'E	30		5		5
Darebin Parklands	37°77'S 145°03'E	4				
Edwardes Lake	37°71'S 144°99'E					
Elsternwick Park	37°88'S 144°99'E	7				

Location	Bearing	Pacific Black Ducks	Pacific Black Duck ducklings	Mallards		
				Mallard males	Mallard females	Total Mallard count
Fitzroy Gardens	37°81'S 144°98'E					
Glenbrook Waters, Cairnlea	37°77'S 144°79'E					
GL Basterfield Park	37°94'S 145°03'E	14			1	1
Hedgeley Dene Gardens	37°87'S 145°06'E	12	9			
Highbury Road Park	37°86'S 145°14'E					
Iramoo Lakes	37°75'S 144°79'E	1				
Jack Ropper Reserve	37°69'S 144°94'E	10		12	1	13
Jawbone Conservation Reserve	37°86'S 144°88'E					
Jones Reserve	37°76'S 144°98'E					
Kalparrin Gardens	37°70'S 145°10'E	3				
Karkarook Park	37°94'S 145°08'E	2				
Lakeside Drive, Burwood East	37°86'S 145°17'E	5		1		1
Landcox Park	37°91'S 145°01'E	12	10			
Latrobe University	37°72'S 145°05'E	1				
Main Drive, Bundoora	37°71'S 145°05'E	1				

Location	Bearing	Pacific Black Ducks	Pacific Black Duck ducklings	Mallards		
				Mallard males	Mallard females	Total Mallard count
Malvern Public Gardens	37°84'S 145°03'E	1				
Monash University	37°91'S 145°13'E	3				
Newells Paddock Reserve	37°80'S 144°91'E	2				
Newport Lakes	37°84'S 144°87'E	2		1	1	2
Northcorp Business Park	37°69'S 144°95'E					
Peter Hopper Lake	37°67'S 145°06'E	20		3	1	8
Princes Park	37°78'S 144°96'E					
Princeton Park Wetlands	37°68'S 145°08'E	5				
Queens Park	37°76'S 144°92'E	20		6	4	10
Royal Botanical Gardens	37°83'S 144°98'E	17	3			
Ruffey Park Lake	37°77'S 145°14'E	1			1	1
RMIT Bundoora East Campus	37°68'S 145°07'E					
Sir William Fry Reserve	37°95'S 145°05'E	8	18			
Surrey Park	37°83'S 145°11'E	8		3	1	4
Taylors Lakes	37°70'S 144°79'E	3				3

Location	Bearing	Pacific Black Ducks	Pacific Black Duck ducklings	Mallards		
				Mallard males	Mallard females	Total Mallard count
Tresury Gardens	37°81'S 144°98'E	1				
Treyvaud Memorial Park	37°88'S 145°08'E					
Trin-Warren Tam-Boore Wetlands, Parkville	37°78'S 144°94'E	1				
TB Drews Walk, Footscray Park	37°79'S 144°90'E	4		1		1
Shearwater Meadow	37°76'S 144°79'E	5	4			
Station Waters	37°76'S 144°78'E	20		8	2	11
Waterfield Park	37°76'S 144°78'E	2				
Westgate Park	37°83'S 144°91'E	2				
Whittlesea Public Gardens	37°67'S 144°98'E					
Woodlands Park	37°74'S 144°91'E	30		1		1
Valley Lake	37°74'S 144°87'E					
TOTAL		**275**	**44**	**50**	**14**	**72**

Appendix 3 Specimens used in Chapter 5 and their rural or urban specification.

Rural			
Specimen	**Status**	**Date**	**Locality**
AT001	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT002	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT003	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT004	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT005	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT006	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT007	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT009	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT010	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT011	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT012	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT014	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)

Rural			
Specimen	**Status**	**Date**	**Locality**
AT015	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT016	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT017	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT018	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT019	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT020	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT021	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT022	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT023	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT024	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT025	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT026	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)

Rural			
Specimen	**Status**	**Date**	**Locality**
AT027	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT028	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT029	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT030	Pacific Black Duck	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
AT032	Pacific Black Duck	23 March 2011	Victoria, Australia (37°24'S,144°82'E)
AT033	Pacific Black Duck	23 March 2011	Victoria, Australia (37°24'S,144°82'E)
AT034	Pacific Black Duck	23 March 2011	Victoria, Australia (37°24'S,144°82'E)
AT035	Pacific Black Duck	23 March 2011	Victoria, Australia (37°24'S,144°82'E)
AT036	Pacific Black Duck	22 March 2010	Victoria, Australia (37°24'S,144°82'E)
PJG1016	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1017	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1019	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1021	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1022	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)

Rural			
Specimen	**Status**	**Date**	**Locality**
PJG1023	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1024	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1025	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1026	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1027	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1028	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1035	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1036	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1037	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)
PJG1038	Pacific Black Duck	March 2010	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)

Urban			
Specimen	**Status**	**Date**	**Locality**
AT251	Pacific Black Duck	13 May 2013	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT253	Pacific Black Duck	13 May 2013	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT254	Pacific Black Duck	13 May 2013	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT255	Pacific Black Duck	19 February 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT256	Pacific Black Duck	19 February 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT257	Pacific Black Duck	23 February 2014	Lake Esmond, Victoria, Australia (37°57'S, 143°87'E)
AT258	Pacific Black Duck	23 February 2014	Lake Wendouree, Victoria, Australia (37°55'S, 143°83'E)
AT259	Pacific Black Duck	3 March 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT260	Pacific Black Duck	3 March 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT261	Pacific Black Duck	3 March 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT262	Pacific Black Duck	6 March 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT263	Pacific Black Duck	6 March 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)

Urban			
Specimen	**Status**	**Date**	**Locality**
AT265	Hybrid	6 March 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT266	Pacific Black Duck	12 March 2014	Queens Park, Victoria, Australia (37°76'S, 144°92'E)
AT267	Hybrid	12 March 2014	Queens Park, Victoria, Australia (37°76'S, 144°92'E)
AT268	Pacific Black Duck	12 March 2014	Queens Park, Victoria, Australia (37°76'S, 144°92'E)
AT269	Pacific Black Duck	12 March 2014	Queens Park, Victoria, Australia (37°76'S, 144°92'E)
AT270	Hybrid	12 March 2014	Queens Park, Victoria, Australia (37°76'S, 144°92'E)
AT273	Pacific Black Duck	14 March 2014	Queens Park, Victoria, Australia (37°76'S, 144°92'E)
AT274	Pacific Black Duck	14 March 2014	Queens Park, Victoria, Australia (37°76'S, 144°92'E)
AT275	Pacific Black Duck	16 March 2014	Burndap Park, Victoria, Australia (37°78'S, 144°90'E)
AT276	Pacific Black Duck	16 March 2014	Burndap Park, Victoria, Australia (37°78'S, 144°90'E)
AT277	Pacific Black Duck	16 March 2014	Burndap Park, Victoria, Australia (37°78'S, 144°90'E)
AT279	Pacific Black Duck	19 March 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)

Urban			
Specimen	**Status**	**Date**	**Locality**
AT280	Pacific Black Duck	19 March 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT289	Pacific Black Duck	24 March 2014	Queens Park, Victoria, Australia (37°76'S, 144°92'E)
AT293	Pacific Black Duck	30 March 2014	Albert Park Lake, Victoria, Australia (37°85'S, 144°97'E)
AT295	Pacific Black Duck	31 March 2014	Queens Park, Victoria, Australia (37°76'S, 144°92'E)
AT296	Pacific Black Duck	31 March 2014	Queens Park, Victoria, Australia (37°76'S, 144°92'E)
PJG017	Pacific Black Duck	Unknown	Healesville, Victoria, Australia (37°69'ES, 145°54'E)
PJG019	Pacific Black Duck	Unknown	Healesville, Victoria, Australia (37°69'ES, 145°54'E)
PJG380	Pacific Black Duck	Unknown	Lake Wendouree, Victoria, Australia (37°55'S, 143°83'E)
PJG398	Pacific Black Duck	Unknown	Lake Wendouree, Victoria, Australia (37°55'S, 143°83'E)
PJG399	Pacific Black Duck	Unknown	Lake Wendouree, Victoria, Australia (37°55'S, 143°83'E)
PJG475	Pacific Black Duck	Unknown	Lake Wendouree, Victoria, Australia (37°55'S, 143°83'E)
PJG1010	Pacific Black Duck	Unknown	Melbourne Zoo, Victoria, Australia (37°78'S, 144°95'E)

Urban			
Specimen	**Status**	**Date**	**Locality**
PJG1105	Pacific Black Duck	Unknown	Melbourne Zoo, Victoria, Australia (37°78'S, 144°95'E)

Appendix 4. Specimens used in Chapter 6 with their haplotype and Genebank accession numbers.

Specimen	Date collected	Locality	Haplotype	Genbank accession number	Reference
CG1	17 October 2007	Lord Howe Island (31°33′S 159°05′E)	G	KJ755786	This study
CG4	17 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CG9	17 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CG40	18 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CG41	18 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CG43	Unknown	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CG85	18 October 2007	Lord Howe Island (31°33′S 159°05′E)	Z	KJ755801	This study
CG86	18 October 2007	Lord Howe Island (31°33′S 159°05′E)	Z	KJ755801	This study
CG87	18 October 2007	Lord Howe Island (31°33′S 159°05′E)	R	KM099389	This study
CGX	Unknown	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CH85	12 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CH88	18 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CH90	12 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CH91	12 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CH93	19 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CH96	15 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CH97	15 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study

Specimen	Date collected	Locality	Haplotype	Genbank accession number	Reference
CI1	15 October 2007	Lord Howe Island (31°33′S 159°05′E)	Z	KJ755801	This study
CI2	15 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CI6	15 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CI28	15 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CI31	15 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CI39	15 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CI67	16 October 2007	Lord Howe Island (31°33′S 159°05′E)	Y	KM099391	This study
CI79	16 October 2007	Lord Howe Island (31°33′S 159°05′E)	Z	KJ755801	This study
CI83	16 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CI84	16 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
CI89	16 October 2007	Lord Howe Island (31°33′S 159°05′E)	W	KJ755825	This study
PJG01	May 1998	Waitaki Valley, New Zealand (44°79'S 170°79E)	W	KJ755825	Guay *et al.,* 2015
PJG02	May 1998	Kaikohe, New Zealand (35°42'S 173°80'E)	W	KJ755799	Guay *et al.,* 2015
PJG03	May 1998	Kaikohe, New Zealand (35°42'S 173°80'E)	Y	KJ755796	Guay *et al.,* 2015
PJG04	May 1998	Hokitika, New Zealand (42°72'S 170°97'E)	W	KJ755818	Guay *et al.,* 2015
PJG05	May 1998	Waitaki Valley, New Zealand	T	KJ755826	Guay *et al.,* 2015

Specimen	Date collected	Locality	Haplotype	Genbank accession number	Reference
		(44°79'S 170°79E)			
PJG06	May 1998	Wellington, New Zealand (41°28'S 174°76'E)	Z	KJ755801	Guay *et al.,* 2015
PJG07	May 1998	Hokitika, New Zealand (42°72'S 170°97'E)	W	KJ755819	Guay *et al.,* 2015
PJG08	May 1998	Kaikohe, New Zealand (35°42'S 173°80'E)	W	KJ755797	Guay *et al.,* 2015
PJG09	May 1998	Wellington, New Zealand (41°28'S 174°76'E)	W	KJ755800	Guay *et al.,* 2015
PJG10	May 1998	Wellington, New Zealand (41°28'S 174°76'E)	W	KJ755803	Guay *et al.,* 2015
PJG11	May 1998	Wellington, New Zealand (41°28'S 174°76'E)	W	KJ755804	Guay *et al.,* 2015
PJG13	May 1998	Wellington, New Zealand (41°28'S 174°76'E)	Y	KJ755805	Guay *et al.,* 2015
PJG14	May 1991	Taranaki, New Zealand (39°37'S 174°22'E)	Z	KJ755710	Guay *et al.,* 2015
PJG15	May 1998	Northland, New Zealand (35°57'S 174°01'E)	Z	KJ755793	Guay *et al.,* 2015
PJG16	May 1998	Hamilton, New Zealand (37°80'S 175°26'E)	W	KJ755817	Guay *et al.,* 2015
PJG17	May 1998	Te Awamutu, New Zealand (38°00'S 175°31'E)	W	KJ755815	Guay *et al.,* 2015
PJG18	May 1991	Manawatu, New Zealand (40°16'S 175°63'E)	Z	KJ755784	Guay *et al.,* 2015

Specimen	Date collected	Locality	Haplotype	Genbank accession number	Reference
PJG19	May 1998	Northland, New Zealand (35°57'S 174°01'E)	Z	KJ755792	Guay *et al.,* 2015
PJG20	May 1991	Manawatu, New Zealand (40°16'S 175°63'E)	Z	KJ755783	Guay *et al.,* 2015
PJG21	May 1998	Wellington, New Zealand (41°28'S 174°76'E)	U	KJ755807	Guay *et al.,* 2015
PJG22	May 1991	Taranaki, New Zealand (39°37'S 174°22'E)	G	KJ755786	Guay *et al.,* 2015
PJG23	May 1998	Auckland, New Zealand (36°86'S 174°76'E)	Z	KJ755812	Guay *et al.,* 2015
PJG24	May 1998	Northland, New Zealand (35°57'S 174°01'E)	X	KJ755795	Guay *et al.,* 2015
PJG25	May 1998	Morrinsville, New Zealand (37°68'S 175°54'E)	T	KJ755809	Guay *et al.,* 2015
PJG26	May 1991	Raetihi, New Zealand (39°44'S 175°21'E)	W	KJ755732	Guay *et al.,* 2015
PJG27	May 1998	Kaikohe, New Zealand (35°42'S 173°80'E)	W	KJ755798	Guay *et al.,* 2015
PJG28	May 1998	Wellington, New Zealand (41°28'S 174°76'E)	W	KJ755806	Guay *et al.,* 2015
PJG29	May 1998	Morrinsville, New Zealand (37°68'S 175°54'E)	Z	KJ755810	Guay *et al.,* 2015
PJG30	May 1998	Northland, New Zealand (35°57'S 174°01'E)	W	KJ755794	Guay *et al.,* 2015
PJG31	May 1998	Te Awamutu, New	Z	KJ755816	Guay *et al.,*

Specimen	Date collected	Locality	Haplotype	Genbank accession number	Reference
		Zealand (38°00'S 175°31'E)			2015
PJG32	May 1998	Auckland, New Zealand (36°86'S 174°76'E)	P	KJ755811	Guay *et al.,* 2015
PJG33	May 1998	Auckland, New Zealand (36°86'S 174°76'E)	W	KJ755814	Guay *et al.,* 2015
PJG34	May 1998	Auckland, New Zealand (36°86'S 174°76'E)	Z	KJ755813	Guay *et al.,* 2015
PJG35	May 1998	Northland, New Zealand (35°57'S 174°01'E)	W	KJ755790	Guay *et al.,* 2015
PJG36	May 1998	Wellington, New Zealand (41°28'S 174°76'E)	Y	KJ755802	Guay *et al.,* 2015
PJG37	May 1998	Northland, New Zealand (35°57'S 174°01'E)	V	KJ755791	Guay *et al.,* 2015
PJG38	May 1998	Horotiu, New Zealand (37°70'S 175°19'E)	W	KJ755808	Guay *et al.,* 2015
AT029	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)	E	KM099378	This study
AT030	17 March 2011	Reedy Lake, Victoria, Australia (38°19'S,144°42'E)	G	KJ755786	This study
AT035	23 March 2011	Victoria, Australia (37°24'S,144°82'E)	C	KM099376	This study
AT036	22 March 2010	Victoria, Australia (37°24'S,144°82'E)	D	KM099377	This study
AT037	5 April 2011	Western Treatment Plant, Victoria, Australia (37°89'S,144°64'E)	G	KJ755786	This study

Specimen	Date collected	Locality	Haplotype	Genbank accession number	Reference
AT099	19 October 2005	Narrandera, NSW, Australia (34°64'S,146°55'E)	A	KM099374	This study
AT100	19 October 2005	Narrandera, NSW, Australia (34°64'S,146°55'E)	C	KM099376	This study
AT109	2 November 2005	West Tocumwal, NSW, Australia (35°75'S,145°56'E)	C	KM099376	This study
AT110	2 November 2005	West Tocumwal, NSW, Australia (35°75'S,145°56'E)	O	KM099387	This study
AT111	2 November 2005	West Tocumwal, NSW, Australia (35°75'S,145°56'E)	G	KJ755786	This study
AT112	2 November 2005	West Tocumwal, NSW, Australia (35°75'S,145°56'E)	B	KM099375	This study
AT113	2 November 2005	West Tocumwal, NSW, Australia (35°75'S,145°56'E)	I	KM099381	This study
AT114	2 November 2005	West Tocumwal, NSW, Australia (35°75'S,145°56'E)	H	KM099380	This study
AT115	2 November 2005	West Tocumwal, NSW, Australia (35°75'S,145°56'E)	F	KM099379	This study
PJG511	10 June 2006	Victoria, Australia (37°24'S,144°82'E)	G	KJ755786	This study
PJG512	2006	Victoria, Australia (37°24'S,144°82'E)	G	KJ755786	This study
PJG513	2006	Victoria, Australia (37°24'S,144°82'E)	N	KM099386	This study
PJG514	2006	Victoria, Australia (37°24'S,144°82'E)	G	KJ755786	This study
PJG515	2006	Boolarra South, Victoria, Australia	O	KM099387	This study

Specimen	Date collected	Locality	Haplotype	Genbank accession number	Reference
		(38°44'S,146°27'E)			
T21	2006	Cressy, Tasmania, Australia (41°66'S,147°08'E)	G	KJ755786	This study
T22	2006	Cressy, Tasmania, Australia (41°66'S,147°08'E)	J	KM099382	This study
T23	2006	Moulting Lagoon, Tasmania, Australia (42°024S,148°19'E)	O	KM099387	This study
T24	2006	Lake Tiberius, Tasmania, Australia (42°43'S,147°36'E)	K	KM099383	This study
T25	2006	Tasmania, Australia (42°43'S,147°36'E)	M	KM099385	This study
T44	2006	Tasmania, Australia (42°43'S,147°36'E)	L	KM099384	This study
T47	2006	Tasmania, Australia (42°16'S,146°61'E)	S	KM099390	This study
T48	2006	Tasmania, Australia (42°16'S,146°61'E)	Q	KM099388	This study