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Hybridisation between related taxa has a range of possible biological consequences, ranging from the production of sterile offspring, through introgression of alleles into populations, to the formation of new species. Examples of plant and animal species hybridising with related taxa abound in the New Zealand region. We review New Zealand examples of hybridisation that have been veri î l Å e ķ þha ! © ain)nan)la ı e ꝏ in ¶ e ÿ vnom hybridisation has been demonstrated with cytogenetic and DNA sequence evidence. Thus the importance of hybridisation in the evolution of New Zealandôs þora and fauna is highlighted.

conservation; gene þow; hybrid; introgression; New Zealand; polyploidy; speciation

Hybridisation is the mating and production of offspring between individuals from genetically distinct populations (Harrison 1993). Hybridisation has been variously viewed as either an evolutionary dead-end, or an important evolutionary process, both in the formation of novel lineages and as a means of linking populations and species by gene þow (see Fig. 1). As an important evolutionary process hybridisation can create new species (Kraus & Miyamato 1990;

[1]Ecology Group/AWCMEE, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

[2]Landcare Research, PO Box 40, Lincoln 7640, New Zealand.

[3]Museum of New Zealand Te Papa Tongarewa, PO Box 467, Wellington 6140, New Zealand/AWCMEE, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

[4]Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand.

[5]Bioinformatics Institute, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand.

[6]Institute for Molecular Systematics, School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington 6140, New Zealand.

[7]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand.

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Arnold et al. 1991; DeMarais et al. 1992; Bullini 1994; Rieseberg et al. 1995; Coyne & Orr 2004; Schwarz et al. 2005), reinforce barriers between gene pools (Howard 1993; Coyne & Orr 1997, 2004; Servedio & Noor 2003), limit speciation and adaptation (Slatkin 1987), swamp endangered species (Rhymer & Simberloff 1996) or form a bridge for transfer of adaptations among lineages (Arnold 2004).

With the advent of new genetic tools, New Zealand biologists have taken the opportunity to investigate old hypotheses and erect new ones concerning hybridisation. Multilocus molecular markers permit detection of both ongoing and historical gene þow among lineages and detection of lineages that have arisen via hybridisation. New Zealand has a long history of hybridisation studies in plants especially but there are now many animal examples and even evidence of virus recombination on our shores. In addition, New Zealand has the advantage of good time keeping for constraining the age of ýrst contact for many hybridising taxa. The arrival of exotic species has been well documented and geological studies give us some ability to date the fragmentation, expansion and hybridisation of our native species. New Zealand mathematicians who are developing novel methods to study hybridisation will continue to give us impact in the international scientiýc community (for example Huson 2005; Winkworth et al. 2005; Baroni et al. 2006; McBreen & Lockhart 2006; Bordewich & Semple 2007; Joly et al. 2007, in press a; Holland et al. 2008).

Hybridisation has been at the centre of three debates in evolutionary biology: species concepts, species conservation, and origin of new þora and fauna. In each of these three areas, New Zealand studies offer new information or a different perspective.

The identiýcation and deýnition of species often refers to the ability of individuals from different populations to mate and produce at least some fertile offspring. The biological species concept (Mayr 1942) is based on the principle that different species do not exchange genes,

Hybridisation is a common and important evolutionary process worldwide. The long-term outcome of hybridisation is dependent on the relative ýtness of the hybrids and subsequent generations, compared to the parental taxa, as illustrated by the following New Zealand examples.
[1]*Hemideina ricta* and *H. femorata* (Morgan-Richards & Townsend 1995); [2]*Galaxias depressiceps* and *G. anomalus* (Allibone et al. 1996); [3]*Kunzea sinclairii* and *Leptospermum scoparium* (Harris et al. 1992); [4]*Asplenium ×lucrosum* (Perrie et al. 2005); [5]*Hemideina thoracica* (Morgan-Richards et al. 2000; Morgan-Richards & Wallis 2003); [6]*Hemideina maori* (King et al. 1996, 2003); [7]*Galaxias depressiceps* and *G.* sp D (Esa et al. 2000); [8]*Kikihia* species (Marshall et al. 2008); [9]*Pseudopanax lessonii* and *P. crassifolius* (Shepherd & Perrie unpubl. data); [10]*Phormium tenax* and *P. cookianum* (Smissen & Heenan 2007; Smissen et al. 2008); [11]*Carpophyllum angustifolium* and *C. maschalocarpum* (Zuccarello et al. unpubl.); [12]*Helichrysum lanceolatum* × *A. bellidioides* (Smissen et al. 2007); [13]*Nothofagus fusca*, *N. truncata*, *N. solandri* var. *cliffortioides* (Thomsen 2002; Knapp 2007); [14]*Himantopus novaezelandiae* and *H. leucocephalus* (Greene 1999; MacAvoy & Chambers 1999; Wallis 1999); [15]*Metrosideros* spp. (Gardner et al. 2004); [16]*Raoulia* spp. (Smissen et al. 2003; Ford unpubl. data); [17]*Hoheria glabrata* and *H. lyallii* (Heenan et al. 2005); [18]*Coprosma* spp. (Wichman et al. 2002); [19]*Grahamina capito* and *Forsterygion varium* (Hannan 2005); [20]*Brachaspis nivalis* and *B. collinus* (Trewick 2001); [21]*Anas chlorotis*, *A. superciliosa* and *A. platyrhynchos* (Kennedy & Spencer 2000; Barton 2003); [22]*Cyanoramphus forbesi* and *C. novaezelandiae chathamensis* (Chan et al. 2006); [23]*Acanthoxyla* (Morgan-Richards & Trewick 2005; Buckley et al. 2008). [24]*Pratia angulata* and *P. perpusilla* (Murray et al. 2004); [25]*Anaphalioides hookeri* (inferred parentage *A. bellidioides* and *A. trinervis*; P((Murray)-4C200On241j 05(Pratia)-4Six); ((eciT0 1; (of 1 Tf 11.61

so identiýcation of hybrids has been of fundamental importance in the resolution of speciesô boundaries. Sometimes distinct populations that are involved in hybridisation are regarded as members of different species and sometimes they are regarded as conspeciýcs. Those who investigate hybridisation do not usually hold to a strict version of the biological species concept, accepting that successful mating between members of different species is commonplace. Using the tools of molecular genetics, detecting gene þow has become straightforward, but delimiting species boundaries can become even more problematic as we strive to distinguish retained ancestral polymorphisms from those that have introgressed and understand the long-term consequences of limited gene þow. There is a perception that zoologists have accepted less gene þow than botanists when describing distinct species (but see Rieseberg et al. 2006). There are, however, many New Zealand examples of recognised animal species that have low levels of gene þow with parapatric relatives, including peripatus (Trewick 1998; Trewick 2000), tree weta (Morgan-Richards 1995; Trewick & Morgan-Richards 1995), brown teal (Barton 2003), parakeets (Kearvel et al. 2003) and ýshes (Esa et al. 2000) (Fig. 1). Although the New Zealand þora has been cited as having a high frequency of interspeciýc hybridisation

(III). Each section is further subdivided, but the physical location and consequences of hybridisation and backcrossing are too complex to be classiýed with ease (Fig. 1).

Human modiýcation of the environment began relatively recently in New Zealand (Anderson 1991), permitting inferences to be made on the subsequent responses of native þora and fauna. In particular, hybridisation of New Zealand species that in the recent (pre-human) past were geographically isolated has been well documented. Native species have come face to face with exotic species (Gillespie 1985; Gibbs 1987; Hitchmough et al. 1990) and range changes have brought together previously allopatric natives. For example, the cutting of water races by gold miners in Otago connected the galaxiid ýsh fauna of separate river systems (Esa et al. 2000). Exact dates of water race construction allow biologists to estimate gene þow on a background of a known number of generations since contact.

Plant interspeciýc hybrids have established in areas of signiýcant human-induced habitat disturbance. For example, the native ground covering plant *Pratia angulata* has hybridisedeesatia 7.91ata abundance (e.g., stilts (MacAvoy & Chambers 1999; Greene 1999) and parakeets (Chan et al. 2006)) and cross-fostering of black robins and tomtits (Ma & Lambert 1997). Humans are also implicated in the low numbers of weta on Banks Peninsula (Morgan-Richards & Townsend 1995) and fur seals on Macquarie Island (Lancaster et al. 2006) where hybrids have been detected using genetic tools. In both these examples it is thought that relative abundance of species affects the selection of mates, increasing hybridisation when one species is relatively rare (Hubbs 1955). Likewise, for albatross on Campbell Island interbreeding of two or three species is exacerbated by lack of conspeciýc mates for the rarer black-browed form (Moore et al. 2001).

Forest clearance on Mangere Island in the Chatham Islands group is thought to have promoted opportunities for hybridisation between Forbesô parakeets (*Cyanoramphus forbesi*) and Chatham Island red-crowned parakeets (*C. novaezelandiae chathamensis*). The former species generally prefers forest habitats to open vegetation, while the latter generally resides in open patches of grass, scrub and herbs (Taylor 1975). A survey of mitochondrial control region DNA sequence haplotypes detected gene þow between the two species of parakeets. Chatham Island red-crowned parakeet mtDNA haplotypes were identiýed in DNA samples obtained from Forbesô parakeet morphotypes (Boon et al. 2001; Ballantyne et al. 2004). Further examination with microsatellite markers has shown that the Mangere Island Forbesô parakeet population has hybridised extensively with Chatham Island red-crowned parakeets, to an extent that there may not even be a single true Forbesô parakeet without a history of hybridisation. The Mangere Island parakeet population is now composed predominantly of cryptic hybrids that reseex m ӟ M

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picornaviruses, coronaviruses, alphaviruses and retroviruses (Lai 1992). Retroviruses, in particular, are renowned for relatively rapid recombination rates, on the order of 2% per kilobase per replication cycle (Hu & Temin 1990). Retroviral recombination occurs in a host cell during reverse transcription when the infecting virion has a heterozygous genome (Hu & Temin 1990).

The retrovirus, FIV, a close relative of HIV, has been identiýed in domestic cats (*Felis catus*) in New Zealand (Swinney et al. 1989; Hayward et al. 2007). Phylogenetic tree construction of *envelope* (*env*) gene sequences has shown that two of the ýve possible FIV subtypes are found in New Zealand infected cats (Hayward et al. 2007). These two subtypes, A and C, co-occur in cat populations, leading to dual infection and consequently recombination/hybridisation. About 6.5% (n = 156) of New Zealand FIV-infected cats are infected with an A/C recombinant in the *env* gene (Hayward & Rodrigo 2008). These recombinant strains are circulating recombinant forms, that is, they are the viral progeny of the host cell where the recombination event occurred.

Viral recombination can repair substitution errors made by the enzyme reverse transcriptase, or can modify particular viral properties, such as virulence (Lai 1992). In this way, viruses are able to adapt to new environments, such as a new host species (Poss et al. 2007). Whatever the result of the crossover event, recombination is instrumental in the evolutionary history of viruses. In addition, viral recombination increases the genetic diversity of circulating viruses within a population, which has implications for vaccine use and development in New Zealand.

Although habitat modiýcation by humans often leads to, or exacerbates hybridisation, it is an important and common natural process too.

Genetically (and sometimes morphologically) distinct populations can meet and mate in spatio-temporally bounded regions called hybrid zones (Harrison 1993). The position and width of a zone is usually stable over many generations, due to equilibrium between the ability of organisms to disperse and the selective disadvantage suffered by the hybrid offspring (Barton & Hewitt 1985). Further stability is ensured when zones lie in density troughs (Barton 1979) or on ecotones (Moore 1977). Most hybrid zones involve secondary contact of populations that have diverged in isolation. For example, a species þock of galaxiid ýshes (*G. vulgaris sensu lato*) show some limited parapatric overlap in the South Island, as a result of natural secondary contact, and some of these contacts show occasional hybridisation (Allibone et al. 1996). Within the radiation of New Zealand cicadas many parapatric species form hybrid zones upon contact (Marshall et al. 2008). New Zealand tree weta hybrid zones have been described on mountain ranges (*Hemideina maori*, King et al. 1996, 2003) and in lowland forest (*H. thoracica*, Morgan-Richards et al. 2000; Morgan-Richards & Wallis 2003). The use of multiple hybrid zones within the same species has allowed inferences about relative disadvantage suffered by hybrid offspring within each zone. The dispersal ability of *H. thoracica* individuals from different chromosome races is assumed to be identical and thus the difference in zone width is

Because the majority of hybrid zones form following secondary contact and taxa are often inþuenced by the same vicariant events, it is common for multiple taxa to form hybrid zones at approximately the same location. Volcanic activity at the Lake Taupo caldera has repeatedly destroyed forest in the central North Island and a number of independent genetic studies have found that distinct populations meet near Lake Taupo (e.g., short-tailed bat Lloyd 2003; cabbage tree, Armstrong unpubl.; the parasitic plant *Dactylanthus taylorii* Holzapfel et al. 2002; fern *Asplenium hookerianum* Shepherd et al. 2007). In addition, two chromosome races of the Auckland tree weta (*Hemideina thoracica*) meet and interbreed on the shore of Lake Taupo (Morgan-Richards et al. 2000). Concordance of frequency clines for four other genetic loci (two allozyme, one microsatellite locus, mtDNA) conýrms that this is a secondary contact zone between two races of tree weta. The width and centres of the frequency clines of all ýve loci vary very little. The narrowest of the frequency clines is for the chromosome rearrangement. This rearrangement is either the direct cause of hybrid disadvantage, or is linked to loci that cause hybrid disadvantage. Chromosome heterozygotes often suffer reduced fertility compared to chromosome homozygotes due to mal-segregation of chromosomes during gamete production (meiosis). The narrowest of the other four frequency clines seen in the weta at Taupo is formed by the mtDNA; in contrast to the chromosomes, it is unlikely that the mitochondrial genome is linked to loci under selection. However, mtDNA is only maternally inherited and female tree weta may have lower dispersal rates compared to males, resulting in a narrow mtDNA cline relative to the clines in neutral nuclear loci seen at Taupo.

Historical gene flow (introgression)

Nothofagus is a major component of forests throughout the South Island of New Zealand, and several hypotheses have been proposed to explain its absence across the central portion of the South Island (reviewed by Wallis & Trewick 2001). A recent investigation using cpDNA to try to distinguish between hypotheses found that through hybridisation, red beech (*Nothofagus fusca*) and hard beech (*N. truncata*) have absorbed genetic material from mountain beech (*N. solandri* var. *cliffortioides*). A single insertion in the *trnL-trnF* intergenic spacer is found within introgression between *M. iolanthe* and members of the genus with similar songs, than lineage sorting (Buckley et al. 2006).

Contemporary gene f ow

Despite considerable differences in morphology, *Pseudopanax lessonii* (coastal ýve-ýnger, houpara) and *P. crassifolius* (lancewood, horoeka) form an array of morphological intermediates wherever they occur in close proximity. Preliminary genetic analyses using AFLPs and microsatellite DNA markers indicate that *P. lessonii* and *P. crassifolius* are genetically distinguishable as separate evolutionary lineages, and that the majority of their hybrids are later generation hybrids (Shepherd & Perrie unpubl.). F_1 (ýrst generation) hybrids appear to be uncommon, suggesting that *P. lessonii* and *P. crassifolius* only rarely cross directly and

When hybridisation results in a lineage reproductively isolated from its two parental taxa a new species is almost instantaneously produced. Due to the difýculty in reproducing without backcrossing to parental taxa, this form of speciation is rare compared to the rate of hybridisation without speciation. However, it is possible for hybridisation to be followed by chromosome doubling and the resulting individual to reproduce asexually or by selýng. Evidence for diploid hybrid species formation is weaker. Speciation via hybridisation is much less common in animals than in plants because isolating mechanisms (such as selýng) are less likely to evolve in concert with hybridisation. Chromosome doubling in animals can also have a dire effect on sex determination, and animals may be generally more susceptible to changes in gene dosage. *Pratia* discussed earlier is an example of new lineages arising from human induced range changes (Murray et al. 2004). In this case, hybrid lineages are recognised as distinct chromosome races (not new species), but are the result of interspeciýc crosses. Breitwieser et al. (1999) used evidence from additive ITS sequences to support the hypothesis that *Anaphalioides hookeri* is a hybrid species with parentage *A. bellidioides* × *A. trinervis.* Since it has a tetraploid chromosome count (2n=4x=56; Groves 1977), it is presumably an example of allopolyploidy (hybridisation followed by chromosome doubling to produce an independent hybrid lineage). In New Zealand buttercups, *Ranunculus nivicola* is an allopolyploid species with *R. verticillatus* and *R. insignis* parents. The degree of cpDNA sharing between *R. insignis* and *R. enysii* suggest that these two species have also been hybridising and *R. insignis* may even be of hybrid origin itself (Carter 2006).

Polyploidy is a common phenomenon amongst New Zealandôs ferns. All species of *Asplenium* native to New Zealand are at least tetraploid and, of the 17 species in the Austral group, nine are octoploid. cpDNA and nuDNA (*Leafy*) indicate that most of these octoploids are allopolyploids (Perrie & Brownsey 2005a; Shepherd et al. 2008a). cpDNA of the octoploids is very similar to their parental species, suggesting recent origins with little time to develop autapomorphies (Shepherd et al. 2008b). In some cases, octoploids share multiple chloroplast types with each other and their progenitors, indicating repeated polyploidisation events (*A. gracillimum*, *A. cimmeriorum* (Perrie & Brownsey 2005b)). Allopolyploidy in New Zealand ferns has also been documented using molecular approaches in *Polystichum*, where *P. neozelandicum* is an allo-octoploid of the tetraploids *P. wawranum* and *P. oculatum* (Perrie et al. 2003). Chloroplast sequences indicate that the tetraploid *Hypolepis ambigua* may be composed of independently derived allopolyploid lineages of unknown parentage (Perrie & Brownsey unpubl.), whereas morphological comparisons suggest that the tetraploid *Pteris macilenta* is almost certainly an allopolyploid derivative of *P. comans* and *P. saxatilis* (Braggins 1975).

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Alternatively, there may have been three (rather than two) sexual species involved in the multiple hybridisation and many origins creating the current diversity.

ýtness and adaptations, and provides raw data for analysis of genes involved in postzygotic isolation in hybrids, so-called ñspeciation genesò (Orr et al. 2004). For example, the new generation of sequencing technology will allow the biochemical pathway and alleles responsible for gamete (pollen, sperm) competition to be identiýed and thus their role in limiting hybridisation will be understood. Transcriptome analysis through isolation of mRNAs also allows estimation of gene expression, touted by many to be at least as important as structural changes to the genes in question (King & Wilson 1975). It could be, for example, that hybrid breakdown is attributable to changes in gene expression caused by novel interactions between two transcriptional networks (Landry et al. 2007).

In cases such as *Phormium* and *Pseudopanax* ecological selection may be critically important in maintaining species differences in the face of extensive hybridism and an apparent absence of robust intrinsic barriers to gene þow. In other groups, such as the *Raoulia* alliance, genetic divergence between hybridising species appears to be greater, and intrinsic barriers to gene þow are greater, but selection against recombinant genotypes is still likely to be important in limiting gene þow. In contrast, tree weta have relatively high levels of genetic diversity that date to geographic isolation during the Pliocene, yet populations with distinct karyotypes failed to speciate, possibly due to simple mate recognition systems in this genus.

The role of hybridisation in invasion, range expansion and adaptation to climate changes is another key area likely to provide stimulating research. The evolution of invasiveness is facilitated by hybridisation and the relationship between age of New Zealandôs biota and proportion of hybrid species could spark comparative studies of both island and continental ecosystems. One might view hybrid species as evidence of recent dispersal or invasion, but study of the genetics of weedy-ness and the hybrid genome will be more productive. From our understanding of the history and processes that have shaped the distribution and abundance of current taxa we can make predictions of how our þora and fauna will respond to the current

(3) The important role of hybridisation in the evolution of New Zealandôs endemic plants and animals has been highlighted by recent genetic studies. Recent hybrid origins of ferns, buttercups, everlasting daisies and stick insects indicate the ongoing generation of biodi-

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