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A review of genetic analyses of hybridisation in New Zealand

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Abstract 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 verified with chromosomal, protein or DNA data. Contemporary hybridisation has been studied at hybrid zones where distinct populations meet and mate in a defined and stable zone of contact. The role of human habitat modification is highlighted with examples of recent range changes that have led to hybridisation and subsequent conservation problems. Hybridisation can result in the swamping of endangered species, although it can also act as a bridge for the transfer of adaptations among lineages. Historical hybridisation in New Zealand has been examined with phylogenetics and there are many examples of organelle introgression or capture. The origin of new species of New Zealand stick insects, ferns and daisies via hybridisation has been demonstrated with cytogenetic and DNA sequence evidence. Thus the importance of hybridisation in the evolution of New Zealand's flora and fauna is highlighted.

Keywords conservation; gene flow; hybrid; introgression; New Zealand; polyploidy; speciation

INTRODUCTION

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 flow (see Fig. 1). As an important evolutionary process hybridisation can create new species (Kraus & Miyamato 1990;

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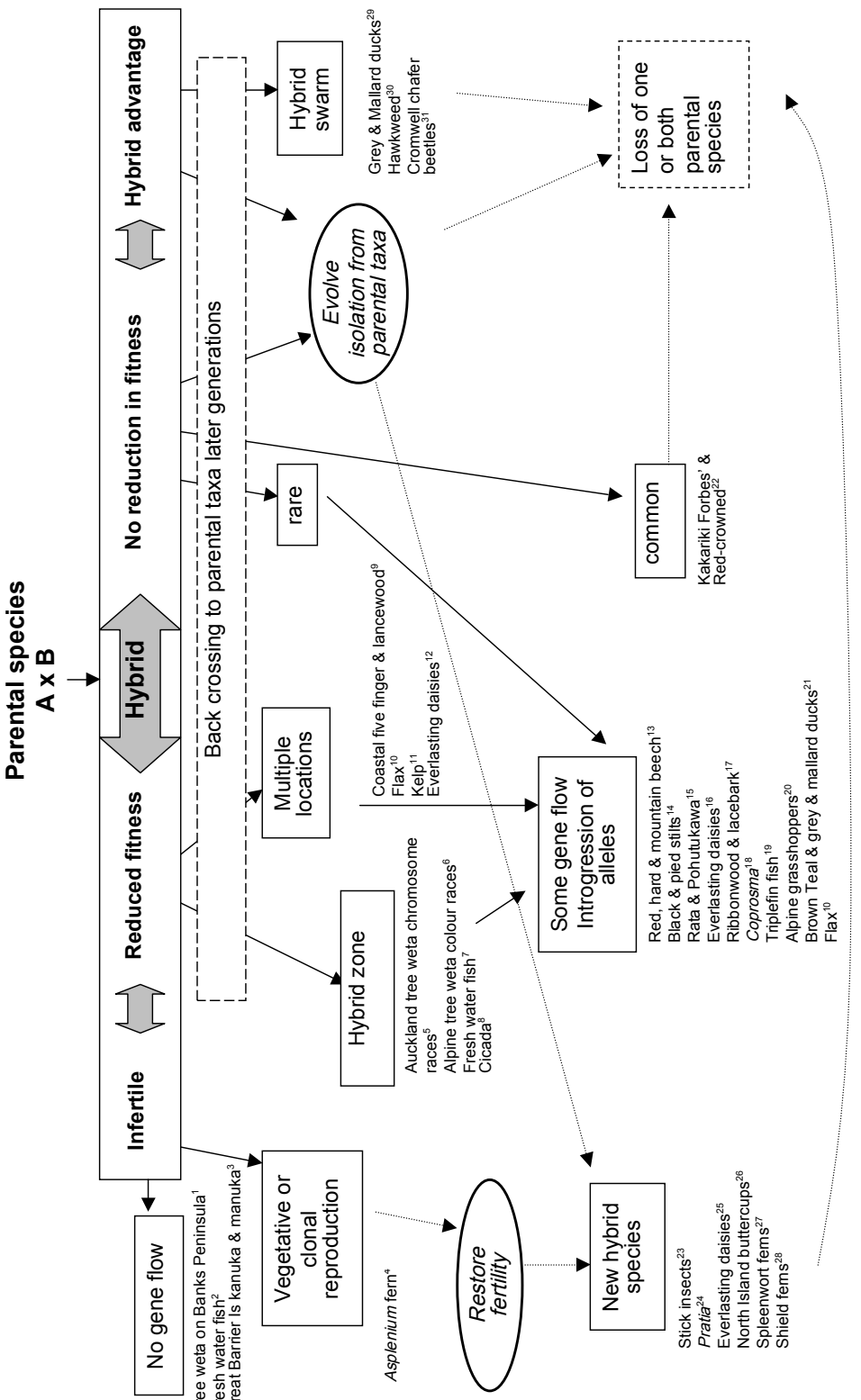
Parental species
A x B
Hybrid
Infertile
Reduced fitness
No reduction in fitness
Hybrid advantage
Back crossing to parental taxa later generations
No gene flow
Tree weta on Banks Peninsula[1]
Fresh water fish[2]
Great Barrier Is kanuka & manuka[3]
Vegetative or clonal reproduction
Asplenium fern[4]
Restore fertility
New hybrid species
Stick insects[23]
Prata[24]
Everlasting daisies[25]
North Island buttercups[26]
Spleenwort ferns[27]
Shield ferns[28]
Hybrid zone
Auckland tree weta chromosome races[5]
Alpine tree weta colour races[6]
Fresh water fish[7]
Cicada[8]
Multiple locations
Coastal five finger & lancewood[9]
Flax[10]
Kelp[11]
Everlasting daisies[12]
rare
Some gene flow
Introgression of alleles
Red, hard & mountain beech[13]
Black & pied stilts[14]
Rata & Pohutukawa[15]
Everlasting daisies[16]
Ribbonwood & lacebark[17]
Coprosma[18]
Triplefin fish[19]
Alpine grasshoppers[20]
Brown Teal & grey & mallard ducks[21]
Flax[10]
common
Kakariki Forbes' & Red-crowned[22]
Hybrid swarm
Grey & Mallard ducks[29]
Hawkweed[30]
Cromwell chafer beetles[31]
Evolve isolation from parental taxa
Loss of one or both parental species

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 flow 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 first 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 scientific 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 flora and fauna. In each of these three areas, New Zealand studies offer new information or a different perspective.

Species concepts

The identification and definition 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,

◀ **Fig. 1** Hybridisation is a common and important evolutionary process worldwide. The long-term outcome of hybridisation is dependent on the relative fitness 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 *Fosterygion 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*; Smissen et al. 2003; Smissen unpubl.; Breitwieser et al. 1999); [26]*Ranunculus nivicola* (Carter 2006); [27]Six species of *Asplenium* are of hybrid origin (e.g., *A. gracillimum* has inferred parents *A. bulbiferum* and *A. hookerianum*; Perrie & Brownsey 2005a; Shepherd et al. 2008a); [28]*Polystichum neozelandicum* (inferred parentage *P. wawranum* and *P. oculatum*; Perrie et al. 2003); [29]*Anas superciliosa* and *A. platyrhynchos* (Hitchmough et al. 1990); [30]*Hieracium pilosella* and *H. praealtum* (Morgan-Richards et al. 2004; Trewick et al. 2004); [31]*Prodontria modesta* and *P. bicolorata* (Emerson & Wallis 1994).

so identification of hybrids has been of fundamental importance in the resolution of species' boundaries. Sometimes distinct populations that are involved in hybridisation are regarded as members of different species and sometimes they are regarded as conspecifics. 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 flow 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 flow. There is a perception that zoologists have accepted less gene flow 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 flow 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 fishes (Esa et al. 2000) (Fig. 1). Although the New Zealand flora has been cited as having a high frequency of interspecific hybridisation in plants (Cockayne & Allan 1934; Anderson & Stebbins 1954; Rattenbury 1962; Dansereau 1964), no meaningful comparison with other floras or other analyses have been advanced to support the premise that hybridisation is of any more significance here than elsewhere in the world. Nonetheless, there are now many well documented natural interspecific and intergeneric plant hybrids occurring in New Zealand (e.g., Connor 1967; Drury 1973; Webb & Druce 1984; McKenzie et al. 2004; de Lange et al. 2005; Smissen et al. 2007). Phylogenetic analyses suggest that some intergeneric hybrids are part of poorly resolved and possibly recent species radiations (e.g., *Damnamenia* × *Pleurophyllum*; Wagstaff & Breitwieser 2004). In addition, there are a number of New Zealand animal examples of intergeneric hybridisation, such as stick insects and triplefin fish (Fig. 1).

Species conservation

The managers of endangered species often consider hybridisation to be a bad thing (Aviss 1995; Rhymer & Simberloff 1996; Wallis 1994, 1999) whereas evolutionary biologists might regard it as a positive event (Lewontin & Birch 1966; Fitzpatrick & Shaffer 2007). In New Zealand there are a number of detailed studies of endangered species that hybridise and the conservation and evolutionary consequences of gene flow are controversial (see below).

Origin of new flora and fauna

The importance of allopolyploidy (whole genome duplication accompanying hybridisation) in plant evolution is now well realised internationally with most or all angiosperm species probably ancient (paleo-) polyploids (Masterson 1994; Soltis & Soltis 1999). It has long been recognised that most New Zealand vascular plants are polyploids, and that among these are likely to be many relatively recently formed allopolyploids (neopolyploids) with affinities to extant diploid (or lesser order polyploid) species (Hair 1966). Hypotheses of allopolyploid species origin based on morphology together with cytology have been and continue to be made for a number of plants including ferns (Brownsey 1977), grasses (Connor 2004) and orchids (Dawson et al. 2007). Molecular genetic tools have proved very useful both in testing these hypotheses and revealing possible additional cases of hybrid species origin, including animal examples (see section III below). However, putative cases of diploid hybrid origins can sometimes be explained by incomplete lineage sorting, introgression of cpDNA and rapid species radiations (Lockhart et al. 2001; Gardner et al. 2004; Smissen et al. 2004).

Here we divide New Zealand studies of hybridisation into human-induced (I) and natural examples (II) plus the special cases where hybridisation leads to the formation of new species

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

I HUMAN-INDUCED HYBRIDISATION

Human modification of the environment began relatively recently in New Zealand (Anderson 1991), permitting inferences to be made on the subsequent responses of native flora 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 fish fauna of separate river systems (Esa et al. 2000). Exact dates of water race construction allow biologists to estimate gene flow on a background of a known number of generations since contact.

Plant interspecific hybrids have established in areas of significant human-induced habitat disturbance. For example, the native ground covering plant *Pratia angulata* has hybridised with another native *P. perpusilla* where they grow together at the Rotorua Golf Course forming plants with 91 chromosomes (13x) and 77 chromosomes (11x; Murray et al. 2004). The hybrid nature of these plants has been established using molecular cytogenetics. In this example, genomic *in situ* hybridisation (GISH) allowed the identification of parental genomes and sequencing of chloroplast (cpDNA) and nuclear (nuDNA) markers supported the hybrid origin hypothesis. The use of random amplified polymorphic DNA (RAPD) primers provided evidence that there have been multiple natural hybridisation events of these two parental species, followed by chromosome doubling.

The recently described fern *Asplenium* ×*lucrosum* has been widely confused with the indigenous Hen and Chickens fern *A. bulbiferum*. However, morphological and cpDNA analyses indicate that *A.* ×*lucrosum* is in fact a sterile hybrid between *A. bulbiferum* and a Norfolk Island fern, *A. dimorphum* (Perrie et al. 2005). The similarity in morphology between *A.* ×*lucrosum* and *A. bulbiferum* has led to the former being used mistakenly instead of the latter in restoration projects. Although sterile, *A.* ×*lucrosum* can spread vegetatively and has become a "casual" adventive in some areas (Perrie et al. 2005).

New Zealand's history of numerous plant introductions has produced a flora that is 50% alien species (Webb et al. 1988; Wilton & Breitwieser 2000). Such mixing has created unprecedented opportunities and pressure for hybridisation (Stace 1975; Arnold 1997). Even introduced species have hybridised with each other producing new genotypes in the novel environment. One of our most aggressive introduced weed species is the hawkweed *Hieracium pilosella*, which is vigorously invading New Zealand's high country. *Hieracium pilosella* mostly reproduces without sex, either by gametophytic apomixis or vegatatively by runners. However, there is a low percentage of sexual seed within populations (Chapman & Brown 2001). Genome size and cpDNA show that at least half of the plants in Canterbury that look like *H. pilosella* are in fact hybrids: pentaploid back-crosses with four sets of *H. pilosella* chromosomes and one set of *H. praealtum* chromosomes (Morgan-Richards et al. 2004; Trewick et al. 2004). Thus hybridisation between two introduced species has been implicated in the evolution of invasiveness in particular. This is a controversial topic internationally (Ellstrand & Schierenbeck 2000) but in New Zealand it appears that *Hieracium* has evolved invasiveness via hybridisation.

Ia Conservation

Habitat destruction in New Zealand has reduced the abundance of certain species, many of which are now threatened with extinction. Hybridisation between a number of pairs of bird species is thought to have resulted from human-induced changes of species' distributions and

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 conspecific 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 Forbes' 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 flow between the two species of parakeets. Chatham Island red-crowned parakeet mtDNA haplotypes were identified in DNA samples obtained from Forbes' parakeet morphotypes (Boon et al. 2001; Ballantyne et al. 2004). Further examination with microsatellite markers has shown that the Mangere Island Forbes' parakeet population has hybridised extensively with Chatham Island red-crowned parakeets, to an extent that there may not even be a single true Forbes' parakeet without a history of hybridisation. The Mangere Island parakeet population is now composed predominantly of cryptic hybrids that resemble Forbes' parakeets in appearance (Chan et al. 2006). This example clearly illustrates the negative impact that human-induced habitat modification can have on a distinctive member of the New Zealand fauna. On the other hand, immunological studies of these populations show that birds that look like hybrids have a stronger immune response than parental types (Tompkins et al. 2006). However, this work needs to be integrated with microsatellite identification of hybrids as plumage identification of hybrids might be biased by the health of the bird. Conservation managers face a dilemma: is reducing hybridisation the best long-term solution? It is possible that hybrid genomes improve the immunity of individuals which thus improves the long-term survival of the population, as has been found in North American salamanders (Fitzpatrick & Shaffer 2007). In these examples hybridisation could be viewed as a beneficial bridge for transfer of adaptations among lineages (Arnold 2004).

During the 19th century, black stilts (*Himantopus novaezelandiae*; kaki) were the dominant wader of the unstable braided rivers of the South Island. The introduction of willows and intensive lowland farming has favoured the spread of the self-introduced pied stilt (*H. leucocephalus*; poaka) from Australia to the extent that kaki are now restricted to the upper Waitaki where fewer than 20 pairs reside. Hybridisation between the species has compounded the problem, but is fortunately limited due to a difference in life history: poaka are migratory and kaki have usually formed pairs before their return. An active control programme against hybridisation has been in place for the last decade or more (Wallis 1999). Mitochondrial DNA studies showed that the two species are closely related but can be readily separated by PCR-RFLP (Restriction Fragment Length Polymorphism) assay, indicating that plumage of these stilts indirectly reflects their haplotypes (MacAvoy & Chambers 1999).

Ib Viral hybridisation

Hybridisation of viruses results in recombination between genetically-divergent virus strains, and has been detected in Feline Immunodeficiency Virus (FIV) in cats introduced into New Zealand. Viral recombination has been documented in certain RNA virus families, such as

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 identified 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 five 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.

II NATURAL HYBRIDISATION

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

IIa Hybrid zones (parapatry)

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 flock of galaxiid fishes (*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 inferred as the difference in hybrid fitness. For example, the chromosome hybrid weta produced in a narrow region at Waitangi River where the Northland tree weta (2n = 19XO, 20XX) meet and mate with the Whangarei tree weta (2n = 17XO, 18XX) suffer about 10 times the hybrid fitness disadvantage compared to that suffered by the hybrids produced where the Karikari Peninsula weta (2n = 23XO, 24XX) meet and mate with the Northland tree weta (2n = 19XO, 20XX; Morgan-Richards & Wallis 2003).

Because the majority of hybrid zones form following secondary contact and taxa are often influenced 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) confirms that this is a secondary contact zone between two races of tree weta. The width and centres of the frequency clines of all five 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.

IIb Sympatry

The backcrossing of hybrids to parental species can result in gene flow between species (introgression). Detecting introgression using only morphological characters can be difficult because backcrossed individuals can have similar morphologies to parent species. For example, some parakeet hybrids and backcrosses closely resemble parental species in plumage (Chan et al. 2006), but genetic markers can detect low levels of introgression resulting from rare hybridisation events among populations. In particular, organelle genomes (chloroplasts, mitochondria) are very useful since the non-recombining markers are not diluted out by backcrossing, but are inherited intact. They are thus a more reliable signal of introgression than rare introgressed alien alleles, which may be present at low frequency anyway. These genomes are also more likely than nuclear markers to move to fixation following introgression, owing to their smaller effective population size (Ferris et al. 1983).

A few New Zealand studies have inferred historical hybridisation using incongruence of phylogenies (for example, *Metrosideros* and *Nothofagus*) but in these cases lineage sorting of ancestral polymorphism is difficult to discount. More convincing cases include the black robin, whose mtDNA clusters closely with that of tomtits as opposed to mainland robins (Miller & Lambert 2006), and three species of alpine cockroach that share a similar group of mtDNAs (Chinn & Gemmell 2004). Most New Zealand hybridisation studies have employed a combination of organelle and nuclear markers such as AFLPs (Amplified Fragment Length Polymorphisms) and ITS (Internal Transcribed Spacer of the ribosomal cassettes). For example, hybridisation between two alpine grasshoppers at Mt Lyford (Kaikoura, *Brachaspis nivalis* × *B. collinus*; Trewick 2001) was inferred using both mitochondrial and ITS sequences, and frequent hybridisation and introgression between the two mountain ribbonwood species (*Hoheria glabrata* and *H. lyallii*; Heenan et al. 2005) was inferred from the combination of chloroplast and ITS sequences.

We can divide studies of gene flow into those that have detected historical hybridisation (introgression) and those that have detected ongoing gene flow—but sometimes both historical and contemporary gene flow have been revealed.

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 populations of all three species south of the beech gap, although north of the gap the species have their own haplotypes (Thomsen 2002; Knapp 2007). Shared chloroplast sequences have also been detected amongst five species of *Metrosideros* (Gardner et al. 2004). This chloroplast sharing, as well as the higher haplotype diversity detected in areas corresponding to putative Pleistocene glacial refugia, led the authors to suggest hybridisation and introgression during confinement to refugia. However, AFLP data did not reveal this geographic structure within pohutukawa (Broadhurst 2008). Environmental instability during the Pleistocene has also been suggested as a cause of introgression between diploid and polyploid *Coprosma* species, detected using ITS sequences (Wichman et al. 2002).

Interspecific and intergeneric hybrids among New Zealand everlasting daisies (Asteraceae tribe Gnaphalieae) in the *Raoulia* alliance of genera have long been recognised on the basis of morphology (Ward 1997). Some putative hybrids have been tested by rigorous morphological analysis (McKenzie 2001; McKenzie et al. 2003, 2004) and others are supported by the display of additive combinations of nrDNA ITS sequences (Smissen et al. 2003; Smissen unpubl.). The intergeneric status of many of the hybrids in the *Raoulia* alliance reflects marked morphological divergence between many of the species involved rather than phylogenetic relationships, as generic boundaries in the Gnaphalieae have not been aligned with robust phylogenetic analyses (the papers of Bayer et al. 2000, 2002; Breitwieser et al. 1999 not withstanding). Within the *Raoulia* alliance trans-specific plastid DNA polymorphism is prominent (Smissen et al. 2004) and may be the result of chloroplast introgression, but to date, no population level studies have detected contemporary gene flow. Available DNA sequence and AFLP fingerprint data suggest that at least some of the genomes of hybridising species in this group are moderately diverged, but there is little congruence between available plastid, nrDNA, and low copy nuclear gene trees (Smissen unpubl.). Combined with the reticulate pattern of morphological character state distribution in the group, this is suggestive of a role for introgression or homoploid hybrid speciation in the group, but evidence remains circumstantial.

Introgression between two species of New Zealand triplefin fish (family Tripterygiidae) has been detected in the southern parts of the South Island (Hannan 2005). The two species have different habitats, with *Grahamina capito* found typically in shallow harbours and inlets, while *Fosterygion varium* generally prefers subtidal clear water reefs. The hybrids are morphologically identical to *G. capito* but have mtDNA of *F. varium* (Hannan 2005). In addition, six nuDNA markers from hybrid individuals clustered with the *G. capito* lineage (Hannan 2005). These data suggest that *F. varium* females have mated with *G. capito* males, and then backcrossing has occurred with *G. capito* (Hannan 2005). Forty individuals from three localities show evidence of mtDNA introgression but the reciprocal situation, with *G. capito* females mating with *F. varium* males, has not been detected (Hannan 2005). In parakeets, evidence of historical mtDNA introgression has been detected (Boon et al. 2001) as well as ongoing hybridisation leading to gene flow (see above). *Pachycladon* shows both nuDNA sequence and cytogenetic evidence of historical hybridisation, revealing a hybridisation event about 1.6–0.8 million years ago at the origin of the radiation of nine allopolyploid species. (Heenan et al. 2002; Joly et al. in press b). In the case of the alpine cicadas (*Maoricicada*), incongruence among four gene trees is more likely the result of historical

introgression between *M. iolanthe* and members of the genus with similar songs, than lineage sorting (Buckley et al. 2006).

Contemporary gene flow

Despite considerable differences in morphology, *Pseudopanax lessonii* (coastal five-finger, 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 (first generation) hybrids appear to be uncommon, suggesting that *P. lessonii* and *P. crassifolius* only rarely cross directly and that the hybrids are primarily crossing with each other. Despite nuDNA differentiation, 3.3 kb of cpDNA sequence revealed no fixed differences between *P. lessonii* and *P. crassifolius*, but a number of substitutions distinguish these two species from *P. linearis* (Shepherd & Perrie unpubl.). This similarity in cpDNA could indicate cytoplasmic introgression, because the cpDNA phylogeny is in conflict with morphology and ITS relationships (Mitchell & Wagstaff 1997) where *P. linearis* and *P. crassifolius* are more closely related to each other than either is to *P. lessonii*.

The two widely recognised species of the endemic New Zealand genus *Phormium* (New Zealand flax) have been observed to hybridise in the wild when sharing the same habitat within the same geographical range (Cockayne & Allan 1934) and F_1 hybrids have been produced in controlled crosses (Allan & Zotov 1937; Houliston et al. unpubl.). Morphological evidence drawn from field observation has been advanced to suggest introgression between species in some parts of New Zealand (Wardle 1979). AFLP analysis of sympatric *Phormium tenax* and *P. cookianum* (Smissen & Heenan 2007) shows two genetic groups concordant with taxonomy, but also reveals a number of individual plants or populations combining genetic markers of both species.

Seven individual flax plants from a population at Okiwi Bay (Marlborough), referred to as *P. tenax* (Smissen & Heenan 2007), displayed AFLP markers more typical of *P. cookianum* suggesting introgression from the locally more common *P. cookianum*. A follow up study (Smissen et al. 2008) focusing on this site confirmed genetic admixture between the two species at Okiwi Bay. However, two populations can still be distinguished morphologically and genetically, indicating that either some level of reproductive isolation is present or that there has been insufficient time in the same habitat to allow homogenisation of the gene-pools. There is no evidence for any intrinsic barrier to gene flow between *P. cookianum* and *P. tenax* and hybridisation between them seems to be reasonably commonplace. However, across their overlapping geographic ranges, clear habitat preferences are evident, putting ecological factors firmly in the spotlight as the reproductive isolating mechanism.

The fertility and fecundity of wild F_1 hybrids between everlasting daisy species in the *Raoulia* alliance (Asteraceae tribe Gnaphalieae) has been examined in the cross *Anaphaloides bellidioides* × *Helichrysum lanceolatum* (Smissen et al. 2007) using multilocus DNA fingerprinting. This study presented some evidence of reduced seed set in hybrids but sampling was limited. Two wild back-crosses to *H. lanceolatum* were identified using AFLP profiles. Subsequent generations have been produced in the glasshouse and some come close to recovering the morphology of *H. lanceolatum* (Smissen unpubl.). However, F_1 hybrids were far more common than second and later generation hybrids in nature and introgression between the parental populations was not detected. Glasshouse grown backcrosses to *A. bellidioides* show marked morphological variation as would be expected in recombinant generations descended from a cross between two morphogically very different species.

III HYBRID SPECIES

When hybridisation results in a lineage reproductively isolated from its two parental taxa a new species is almost instantaneously produced. Due to the difficulty 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 selfing. 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 selfing) 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 interspecific 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 Zealand's 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).

Although hybrid speciation is less common in animals, stick insects provide one well documented New Zealand example. Their use of parthenogenetic reproduction makes stick insects one of the few animal groups with multiple examples of lineages of hybrid origin around the world (Bullini 1994). At least three New Zealand species are facultative parthenogens (*Clitarchus hookeri*, *Argarsarchus horridus* and *Tectarchus huttoni*) producing female offspring from unfertilised eggs. One endemic stick insect lineage within New Zealand, *Acanthoxyla*, has eight species but no males. Chromosome counts suggest they are diploid, but nuDNA suggests that some *Acanthoxyla* lineages might be triploid (Buckley et al. 2008). Sequence data indicate that all *Acanthoxyla* are of hybrid origin (Morgan-Richards & Trewick 2005; Buckley et al. 2008) but whether *C. hookeri* is the original paternal species, or involved in a more recent hybridisation with some lineages of *Acanthoxyla*, is in question. The ancestral sexual maternal species of *Acanthoxyla* is probably extinct (Trewick et al. 2008). In this stick insect it is possible that the whole genus arose via hybridisation and the morphological diversity currently present (and recognised as eight species) may be the result of rapid divergence.

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

FUTURE DIRECTIONS

Like most biological research, studies of hybridisation in New Zealand have led to more questions being asked than have been answered. For example, are the independently derived lineages of the allopolyploid *Asplenium gracillimum* reproductively isolated? Does *Acanthoxyla* have three parental species? Is the introgression that has been detected between numerous species of New Zealand's flora (e.g., within *Metrosideros*, *Coprosma* and *Pseudopanax*) typical of the flora as a whole and has hybridisation been more common here than overseas? Here we outline some areas of hybrid research that we expect to be both fruitful and exciting in the years ahead. We have focused on questions that are well suited to the New Zealand system and of general interest and most likely to push back the frontiers of biology.

Any history of hybridisation leads to conflict in gene trees (Ballard & Whitlock 2004). If hybridisation has generally been quantitatively underestimated, then molecular phylogenies based on single (or even a few) genes may not reflect species history. In these cases, multilocus phylogenies are required, as well as techniques to recover the reticulated species history (Holland et al. 2005; Baroni et al. 2006; Edwards et al. 2007). We expect to see progress in the near future as novel phylogenetic methods to detect hybridisation, both current and historical (Huson et al. 2005; Winkworth et al. 2005; Baroni et al. 2006; McBreen & Lockhart 2006; Holland et al. 2008; Joly et al. in press a), are applied to more species. Technical advances in multilocus genotyping methods and pyrosequencing make many more markers available, and expectations of molecular phylogenetic datasets will correspondingly increase dramatically. If disruptive selection promotes parental phenotypes at the expense of hybrid phenotypes we would expect to detect many examples of cryptic hybridisation as more multi-marker studies reveal competing gene trees for new taxa.

Pyrosequencing could prove to be the biggest innovation in genetics since DNA sequencing itself. The ability to sequence genomes of non-model organisms with little or no preliminary sequence information represents a major step in comparative genomics and evolutionary biology (Vera et al. 2008). Multiple markers not only allow distinction of more hybrid classes (e.g., F_1, F_2, backcrosses; Anderson & Thompson 2002; Shepherd & Perrie unpubl.), but permit demonstration of hybrid origin in cases where one parental species has made a relatively small contribution to the current nuclear genome. Differential rates of introgression may depend on size of linkage groups and a more detailed knowledge of mosaic genomes will allow description of co-adapted alleles, their regulation and their putative role in speciation. We will also be able to document differential introgression of neutral and adaptive markers at hybrid zones and determine how porous species boundaries are.

The immediate consequence of hybridisation (F_1) and long-term effects (reticulate evolution) need to be connected with an improved understanding of the selection pressure on F_2 and backcrossed individuals using fitness estimates based on lifelong reproductive output and compared to that of parental types. Ecological and experimental studies that make direct measures of relative fitness of hybrids and parentals in different habitats have the potential to contribute enormously to our understanding of hybridisation as a fundamental evolutionary process. In addition, comparisons of hybrid zones offer excellent biological systems to examine differential fitness, both multiple hybrid zone in the same location (e.g., Taupo, Northland Peninsula) and the same taxon involved in hybrid zones in different regions (e.g., cicadas, tree weta, fresh water fish, geckos). By keeping either age of zone constant (same location), or dispersal ability constant (same taxon) one can infer aspects of relative hybrid fitness. Deep sequencing of genomes will be a major step towards the detection of the molecular basis of

fitness and adaptations, and provides raw data for analysis of genes involved in postzygotic isolation in hybrids, so-called "speciation genes" (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 identified 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 flow. In other groups, such as the *Raoulia* alliance, genetic divergence between hybridising species appears to be greater, and intrinsic barriers to gene flow are greater, but selection against recombinant genotypes is still likely to be important in limiting gene flow. 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 Zealand's 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 flora and fauna will respond to the current rapid period of climate warming. The glacial cycles of the Pleiostocene must have seen major changes in ranges of many species, but not on the scale inferred in the Northern Hemisphere, as many New Zealand species have maintained diversity and (probably) their widespread distribution rather than skulking in refugia during cold periods. However, glacial refugia have been linked to times and places of high levels of hybridisation. We require better estimates of genetic potential within populations and measures of both intra- and interspecific gene flow in natural populations to allow us to predict the speed and potential of populations to adapt to environmental change. Hybridisation provides a means to transfer adaptive traits, but gene flow among locally adapted populations of different sizes can result in genetic swamping, slowing the process of evolutionary response to environmental change and adaptation to marginal habitats. Thus we need to measure density, diversity, dispersal and hybridisation over entire species ranges.

CONCLUSIONS

Hybridisation has been the focus of studies of species concepts, conservation and origins and will continue to be a key focus of evolutionary biology.

(1) We see in New Zealand that many distinct and well defined species can and do hybridise with related taxa, resulting in low levels of gene flow, and yet these species maintain themselves as discrete units. Hybridisation of at least 19 pairs of endemic New Zealand species, involving plants, insects, fish, and birds, has been confirmed with genetic markers (Fig. 1). Thus, recognition of species status does not rely on absence of gene flow.

(2) Hybridisation can be seen as a threat to biodiversity in some situations, for example stilts, ducks and parakeets, but it also has the potential to benefit populations by increasing genetic diversity as seen in cat viruses and many plant lineages.

(3) The important role of hybridisation in the evolution of New Zealand's 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 biodiversity via hybridisation. Evidence of hybridisation concurrent with climate cycles, such as seen in *Coprosma*, suggests natural range changes may increase levels of hybridisation. This finding is supported by examples of hybridisation where human habitat modification and disturbance has altered species distribution and abundance, for example fish, stilts, weta and parakeets. Hybridisation is a common and important evolutionary process here in New Zealand and elsewhere.

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