

Unravelling the fibres of harakeke evolution

Rob Smissen¹ and Peter Heenan²

Species and reproductive isolation

Charles Darwin and Alfred Wallace did taxonomists no favours. They supported the idea that evolution from a common ancestor, driven by natural selection, had brought about the diversity of species, overturning a view in which species were each 'specially created' with their unique characters.

This changed the job of taxonomy from the finite task of finding and cataloguing the forms of life, to the far more slippery task of fitting each organism into the complicated family history of living forms. Despite the great progress made in sketching these relationships over the centuries, in many groups of organisms deciding what is a distinct species and what is merely a variant of a wider species remains one of the most subjective questions in biology.

The view that species are 'groups of interbreeding natural populations that are reproductively isolated from other such groups' is a powerful guide, at least in sexually reproducing organisms. However, reproductive isolation is not always absolute and can be achieved in many ways, some of which are not obvious.

Often it is easier to show that groups of organisms are reproductively isolated than it is to find out how they are reproductively isolated (unless extensive breeding studies are conducted). A pragmatic approach is to study the variation within and across species, seek to understand its origins, and then impose a taxonomy that accommodates variability.

Hybridisation

Of course, some species are clearly defined by their reproductive potential, but this is not the case for many others. Some reproductively isolated species will hybridise, but their offspring may be sterile. In other species the hybrids have reduced fertility, but if they do manage to

breed, fertility can be restored in subsequent generations. In other pairs of species, first-generation hybrids are fertile and fit, but their offspring suffer poor fitness or fertility following the shuffling of the divergent sets of genes.

When interspecific hybrids have at least some level of fertility, then repeated backcrossing with one of the parent species can lead to the transfer of genes from one species to the other, a process called introgressive hybridisation or introgression.

Hybridisation can also lead to the formation of new species, and the conditions under which this can occur are the subject of much research internationally (Gross and Rieseberg, 2005).

Variability within species

Variability within species can be ascribed to at least four processes:

1. Organisms with the same or similar genes may look and behave differently due to the influence of their environment. This is often called phenotypic plasticity.
2. Mutations occur in individuals within species and, subject to natural selection and other population genetic processes, may be maintained in that species.
3. Most species inherit a level of genetic variation from their progenitors.
4. Species may hybridise with related species, and if the hybrids are fertile, may exchange genes through introgressive hybridisation.

Phormium – harakeke/New Zealand flax

New Zealand flax, comprising harakeke (*Phormium tenax*) and wharariki (*P. cookianum*), is one of the most distinctive and iconic New Zealand native plants. *Phormium* has had a chequered taxonomic history,

being placed in several plant families, but recent DNA sequencing studies have recognised it as a relative of the day lilies, *Hemerocallis*.

Phormium tenax and *P. cookianum* are readily distinguished by their leaves and fruit capsules, and these provide the most taxonomically informative and reliable characters to distinguish the two species (Table 1, Fig. 1 A, C).

Table 1 Morphological characters distinguishing *Phormium* taxa.

Character	<i>P. tenax</i>	<i>P. cookianum</i> subsp. <i>cookianum</i>	<i>P. cookianum</i> subsp. <i>hookeri</i>
Leaf	>1.5 m, erect	<1.5 m, drooping or upright	<1.5 m, drooping
Capsule orientation	upright	pendulous	pendulous
Capsule length	usually <10 cm	8–12 cm	10–22 cm
Capsule shape in cross section	triangular	round	round
Capsule twisting	no	strongly	strongly
Capsule tip	obtuse, abruptly tapered	acute, gradually tapered	acute, gradually tapered
Capsule fibrous with age	no	yes	yes
Flower colour	red or orange	red	yellow

The most recent taxonomic appraisal of *P. cookianum* recognised two subspecies, with subsp. *cookianum* from the South Island and southern North Island mountains and subsp. *hookeri* from coastal, lowland, and montane parts of the North Island (Wardle, 1979). More recent fieldwork has shown that subsp. *hookeri* also occurs in the upper part of the South Island. These two subspecies are distinguished by their capsules and flower colour.

Natural variation in *Phormium*

In the wild both *P. cookianum* and *P. tenax* are naturally variable. As discussed above, two subspecies of *P. cookianum* are recognized. Wardle (1979) also noted a coastal entity from the Cook Strait area but did not

¹ Landcare Research, PO Box 40, Lincoln 7640; smissenr@landcareresearch.co.nz;

² heenanp@landcareresearch.co.nz

give it any formal recognition, instead treating it within his concept of *P. cookianum*. We refer to these plants by the informal name *Phormium* “Coast”, and can distinguish them from *P. cookianum* and *P. tenax* by variation in the taxonomically important capsule characters.

The capsules of plants referable to *P.* “Coast” are notable in being intermediate between *P. cookianum* and *P. tenax*; capsules are horizontal, triangular-rounded, curved, gradually tapered toward the apex, moderately twisted, and with age they become paler and slightly fibrous (Fig. 1 A–C).



Fig. 1 A, fruit capsules of *P. cookianum* subsp. *hookeri*, Volcanic Plateau, central North Island; B, capsules of *P.* “Coast”, Ohau Point, Marlborough; C, capsules of *P. tenax*, Chatham Islands.

Phormium tenax has not been the subject of an intraspecific (within species) taxonomic study, but

some geographic variation is often recognised in the field by botanists. For example, plants from the Three Kings and Poor Knights Islands have orange or salmon-pink flowers, and leaves with a distinct golden-yellow marginal band. Some plants from the Chatham Islands are also different as they have flaccid and drooping leaves that are more like those of *P. cookianum*.

The habitats of the two species also differ, with *P. tenax* being characteristic of swamps, wetlands, damp seepages, and hillsides, whereas *P. cookianum* usually occurs on coastal and inland cliffs, and among montane scrub and grassland. Plants of *P.* “Coast” are mostly found on rock outcrops and hillsides in coastal parts of Wellington, Nelson, and Marlborough.

Cultural and economic uses

Phormium is important to Maori, who use leaf strips and fibre (muka) for weaving a range of products. More than fifty Maori weaving cultivars have been gathered together in the Rene Orchiston collection (Scheele, 2005).

Both *Phormium tenax* and *P. cookianum* are commonly planted in urban landscapes and subject to considerable horticultural selection with a wide range of cultivars being raised for variation of leaf colours and different growth habits (Heenan, 1991).

Recent studies have focused on the traditional weaving cultivars of *P. tenax*, and have looked at plant growth, fibre extraction, leaf exudates, and genetic interrelationships.

Breeding system

Phormium tenax can set seed by selfing but it has been shown to preferentially outcross. When different flowers on the same flower spike are either self-pollinated or cross-pollinated at the same time, the self-pollinated flowers abort whereas the cross-pollinated flowers continue to develop and form fruit and viable seed. However, if flowers are only self-pollinated, fruit and viable seed will often form. *P. tenax* is pollinated by New Zealand honeyeater birds (tui, bellbird). Honeybees are avid collectors of *Phormium* pollen, but it is not clear whether they are effective pollinators.

New research using DNA fingerprinting

Phylogenetic analyses of DNA sequences are very effective in fitting species into a broad ‘tree of life’, but to explore genetic relationships within species, or among closely related groups of species, a more discriminating approach is needed.

More often than not, when comparing closely related species, one finds that most genetic variation is attributable to *within* species, rather than *between* species diversity. In other words, for any given gene (including so-called ‘DNA barcode’ genes), two randomly chosen individuals of a species may resemble each other less than they resemble some members of another species. In such cases, it is important to study many genes (or many regions of non-coding DNA), rather than one or a few specific genes. The technique we are using, AFLP, can quickly produce hundreds of variable DNA markers. The resulting DNA fingerprints have the potential to discriminate the DNA of individuals and are similar to the forensic DNA fingerprints used to solve crimes.

P. tenax and *P. cookianum* can be distinguished genetically

We have been using AFLP DNA fingerprinting in *Phormium* with two main aims. Firstly, we wanted to evaluate how well the existing taxonomy matched broad patterns in genetic similarity between individuals and populations. Secondly, we wanted to assess how much of the morphological variation in *Phormium* species was produced by hybridisation as opposed to other sources of variation. In particular, we wanted to know if the plants of *P.* “Coast” had a hybrid origin (given their intermediate fruit characters), and whether they have a common genetic composition that might justify recognising them taxonomically.

Our DNA fingerprints were effective in distinguishing plants of *P. tenax* from *P. cookianum*, and reasonably good at distinguishing *P. cookianum* subsp. *hookeri* from subsp. *cookianum* (Fig. 2). However, several individual plants included in our study had the genetic characters of both species, consistent with a hybrid origin. These included plants we had called *P.* “Coast”. However, many *P.* “Coast” plants are more similar

in DNA fingerprint profiles to *P. cookianum* subsp. *cookianum* than to *P. tenax*, and on the whole, different populations of *P. "Coast"* have different *P. tenax* genetic markers. This suggests that, although at least a part of *P. "Coast"* has arisen from hybridisation between *P. tenax* and *P. cookianum*, this has occurred many times in different places and these hybrid populations are somewhat variable. It is still possible that part of *P. "Coast"* is a stable hybrid derivative that could be usefully recognised taxonomically, but more research is required to decide this. A number of other plants from around New Zealand that we considered to be *P. cookianum* also appeared in our DNA fingerprint study to be of hybrid origin, and it seems likely that the two species exchange genes from time to time when they grow together.

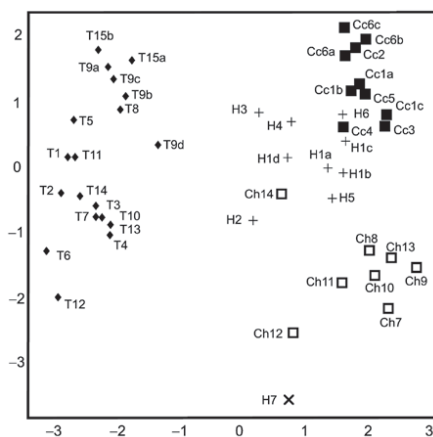


Fig. 2 Scatter plot from principal component analysis of *Phormium* AFLP profiles (from Smissen and Heenan, 2007). ■, *P. cookianum* subsp. *cookianum*; □, *P. cookianum* subsp. *hookeri*; ×, hybrid *P. cookianum* subsp. *hookeri* × *P. tenax*; +, *P. "Coast"*; ◆, *P. tenax*. We now believe the samples labelled Ch12 and Ch14 are hybrids.

Coastal Marlborough: a hotbed of genetic exchange?

We also found that one population of *P. tenax* sampled, from Okiwi Bay (on the Marlborough coast), included individuals with a number of *P. cookianum* genetic markers. We suggest that these markers were acquired through introgressive hybridisation with *P. cookianum*, which we knew to also grow at Okiwi Bay. To follow this up, we analysed further DNA fingerprint profiles from this population of *P. tenax* and compared them with other *P. tenax*, and with a few populations of *P. cookianum* from coastal Marlborough. We also compared the morphology of this

genetically unusual population to that of other *P. tenax* plants.

To minimise phenotypic plasticity we measured morphological characters from plants cultivated together at Lincoln, so that all the plants were growing under uniform conditions. The Okiwi Bay *P. tenax* proved to be small-statured by the standards of that species, but perhaps more significantly, individuals from this population displayed a number of *P. cookianum*-like characters, such as twisted capsules (Fig. 3) and capsules rounded in cross section.

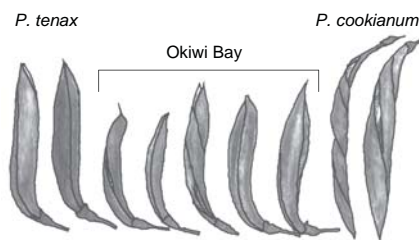


Fig. 3 Fruit capsules of 'pure' *P. tenax* (left) and *P. cookianum* (right), and representatives of the Okiwi Bay *P. tenax* (middle).

When we analysed DNA fingerprint profiles from *P. tenax*-like plants and *P. cookianum*-like plants at Okiwi Bay, we found that they could still be grouped according to the species they most resembled. However, they were much more genetically similar to each other than they were to typical *P. tenax* and *P. cookianum* populations growing in the absence of the other species. The profiles suggest that most, if not all, of the *P. tenax* plants at Okiwi Bay have some *P. cookianum* in their ancestry (Fig. 4). Many of the *P. cookianum* plants at Okiwi Bay also appear to be of mixed ancestry, but some are not significantly different from 'pure' *P. cookianum*. It appears that genes have been exchanged between the two species at Okiwi Bay. But for the moment at least, the two populations remain distinct from each other and genetic exchange may still be limited.

Future work

We believe we have shown that the two taxonomically recognised species of *Phormium* are genetically as well as morphologically distinct, but, at least sometimes,

can exchange genes locally. We now want to explore whether hybridised populations can persist and spread, are evolutionary 'dead ends', or become reintegrated into the parental species over time. Factors other than ecological specialisation may contribute to the reproductive isolation between the species, requiring investigation of rates of interbreeding in populations like that at Okiwi Bay and controlled breeding experiments to produce and examine second and later-generation hybrids.

We also plan to conduct DNA fingerprinting of the Rene Orchiston collection of *P. tenax* weaving cultivars in order to assess their relationships to each other and to wild collections.

We also hope to use DNA fingerprinting techniques on a very distinct form of *P. tenax* with drooping leaves that grows only on the Chatham Islands. These DNA fingerprints may shed light on whether it should be recognised as a distinct species.

References

Gross, B.L. and Rieseberg, L.H. (2005). The ecological genetics of homoploid hybrid speciation. *Journal of Heredity* 96: 241–252.

Heenan, P.B. (1991). Checklist of *Phormium* cultivars. RNZIH, Lincoln, New Zealand.

Scheele, S. (2005). Harakeke – the Rene Orchiston collection (3rd Edition). Lincoln, New Zealand: Manaaki Whenua Press. Available as a free download at http://www.mwpress.co.nz/store/downloads/Harakeke_3rdEd.pdf

Smissen, R.D. and Heenan, P.B. (2007). DNA fingerprinting supports hybridisation as a factor explaining complex natural variation in *Phormium* (Hemerocallidaceae). *New Zealand Journal of Botany* 45: 419–432.

Wardle, P. (1979). Variation in *Phormium cookianum* (Agavaceae). *New Zealand Journal of Botany* 17: 189–196.

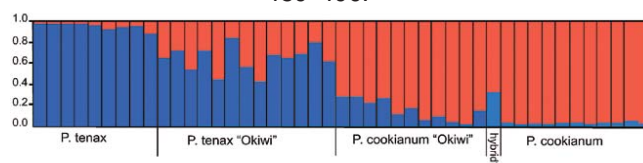


Fig. 4 Estimates of genetic admixture in Okiwi Bay *Phormium* populations. Each bar represents the estimated proportion of one plant's genome derived from *P. cookianum* (red) and *P. tenax* (blue). *P. tenax* (left) and *P. cookianum* (right) samples from other sites included in this analysis are not significantly different from 'pure'. The sample labelled 'hybrid?' was identified in the field as a putative first-generation hybrid between the Okiwi Bay *P. tenax* and *P. cookianum*, a hypothesis consistent with this analysis.