THE BIOGEOGRAPHY AND ORIGIN OF NEW ZEALAND SOPHORA (Leguminosae)

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Abstract

The application of DNA sequencing to studies of the biogeography and origin of New Zealand plant groups is illustrated by evolutionary relationships of *kowhai* (*Sophora* spp.; sect. Edwardsia; Sophoreae: Papilionoideae: Leguminosae). DNA sequences from an intergene region of the chloroplast *atp*B-*rbc*L were determined for 12 species by the use of the polymerase chain reaction. Signals in the molecular data were evaluated using phylogenetic algorithms to reconstruct the evolutionary history of the species.

The extremely high genetic similarity between Edwardsia *Sophora* resulted in an inability to fully resolve the phylogenetic tree. Three hypotheses are presented to account for the patterns of sequence differences between the New Zealand Edwardsia. One proposes a recent origin of *Sophora* section Edwardsia in New Zealand (4-10 million years ago), with subsequent dispersal of buoyant *Sophora microphylla* seeds to offshore and oceanic islands, where they might occasionally colonise. A second hypothesis suggests a recent radiation of *Sophora microphylla* and *Sophora prostrata* populations during the Pleistocene (0.1 - 1.6 million years ago), but is not well supported by the available sequence data. A third hypothesis proposes that the Lord Howe Island and New Zealand *Sophora* are derived from a Miocene (5-16 million years ago) oceanic migration of a Chilean ancestor of *Sophora* section Edwardsia. Predictions of the three hypotheses and strategies to test them are discussed.

Some of the conclusions derived from analyses of the chloroplast DNA sequences conflict with those obtained from morphological and chemotaxonomical studies. Analyses of all data sets indicates that the variations in morphology and secondary metabolitic constituents between *Sophora prostrata* and *Sophora microphylla* obscures a small amount of genetic diversity. The question of hybrid origins for *Sophora microphylla* is not supported by tree reconstructions from the molecular data set, and further genetic and ecological studies are required to investigate this.

Acknowledgments

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Much thanks to all my friends and family; Billy, Don, Conal, Marian & Lon, Lee-Ann, Brynn & Luke, Vivienne, Simon, Frances & Jason, Mum and Dad, who have managed to preserve my sense of humour.

But most of all I thank my parents, with much love.

Dedication

This thesis is dedicated in loving memory to my father, David James William Hurr, who passed away suddenly on September 16, 1995. He was a kind and intelligent man, who always took great interest in my university studies, and would have been very proud of my achievement. It is with much regret that he is unable to share this with me.

"The Gods of the earth and sea
Sought thro' nature to find the Tree;
But their search was all in vain:
There grows one in the Human Brain."
(Blake)

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Chapter One: **Introduction**

New Zealand's islands are particularly well suited to studies of dispersal and evolution. The New Zealand landmass has been isolated for 82 million years (Lawver *et al.* 1994; Storey 1995), and repeated episodes of migration, extinction and evolution have produced a unique variety of living and extinct organisms in New Zealand (Stevens 1980; Cooper and Millener 1993). The natural flora and fauna rank alongside life forms from the islands of Hawai'i, Madagascar, New Guinea and the Galapagos in providing examples of evolution in action (Hooker 1853; Hutton 1872; Wallace 1880; Fleming 1958; Gillet 1972; Carlquist 1974). This thesis illustrates the importance of the New Zealand legume flora for addressing biogeographical and evolutionary questions. It does this by examining chloroplast DNA sequences in the genus *Sophora* (Sophoreae: Papilionoideae: Leguminosae) and from this data inferring aspects of the evolutionary history and dispersal of the New Zealand and related species. The utility of this data for testing theories on the time of origin of plants is discussed. To provide a background to this study, the biogeographic, climatic, and geologic history of the New Zealand landmass is reviewed, along with a current understanding of systematics within the genus *Sophora*.

The major conclusions of this thesis are that firstly, phylogenetic trees are only hypotheses regarding the relationship between groups of taxa. DNA sequences must be scrutinized carefully in order to have confidence in the reliability of phylogenetic relationships. New algorithms are used which allow exploration of different types of information contained in sequences. Secondly, the use of molecular genetic techniques are valuable for investigations of evolutionary problems in New Zealand, particularly when applied in conjunction with the fossil record, population genetic and biochemical studies. Thirdly, the New Zealand *Sophora* have a relatively short evolutionary history in New Zealand, and have diversified in the southern hemisphere since the Miocene. Their disjunct distribution on islands of the Southern Oceans is explained by drift dispersal of buoyant seeds in the Antarctic-circumpolar current.

The Biogeography and Origins of the New Zealand Angiosperm Flora.

The study of the fossil record has provided insights into the timing of angiosperm origins, into the patterns and tempo of their early radiation and diversification, and into the geography of their early development (Doyle & Hickey 1976; Doyle 1977, Truswell 1987; Crane 1987). The principle radiation appears to have begun during the Early Cretaceous, coinciding with a decline in gymnosperm species numbers (Niklas *et al.*, 1980, 1983; Tiffney 1981; Crane 1987). Major evolutionary groups established by the end of the Cretaceous (Figure 1.1.) diversified further, and many modern angiosperm families and genera originate from the Early Tertiary (Muller 1970, 1981; Stebbins 1981; Krassilov 1991).

With the gradual fragmentation of Gondwanaland, once readily feasible overland or short-distance dispersal of structured communities gradually became more difficult and eventually impossible. Mass migration ceased, individual, long-distance migration was substituted (Truswell 1987; Schuster 1979; Crane 1987; Sanderson & Donoghue 1994). One of the major problems facing evolutionary studies in New Zealand is establishing from where, and when, many of our angiosperms came (Fleming 1962). New Zealand began its rapid drift away from the Australian-Antarctic margin of Gondwanaland with the formation of oceanic crust in the Tasman Sea and South Pacific Ocean in the Late Cretaceous period, 82 million years ago (Simpson et al 1979; Crook 1981; Cooper & Millener 1993; Lawver *et al* 1992; Storey 1995) - (Figure 1.2.). Biogeographic studies in New Zealand (see Fleming 1980; McGlone 1985; Craw 1989) have presented two different views of the origins of the biota. "Traditional" biogeographers have assumed much of the New Zealand flora and fauna arrived here after the separation of the land mass from Gondwana, and various means and routes of dispersal have been proposed to account for their arrival (Kuschel 1975; Fleming 1980; McGlone 1985). Alternatively, panbiogeographers emphasize a series of land bridges which occurred while continental areas and fragments were still drifting apart, now broken with greater isolation of the New Zealand landmass (see Craw 1989).

Fig. 1.1. A Partial Time-Scale (Cretaceous to present). From Herendeen & Dilcher 1992.

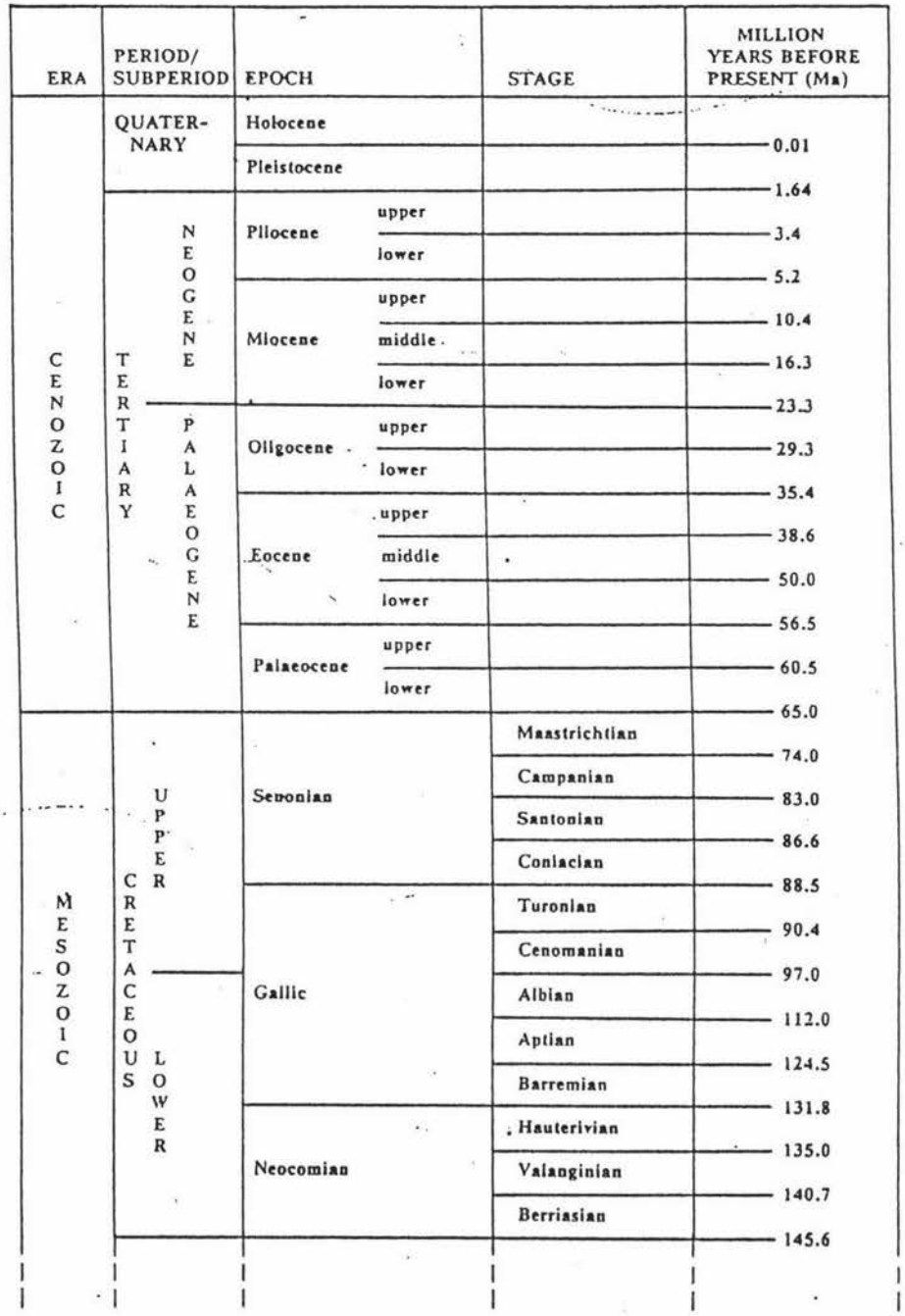
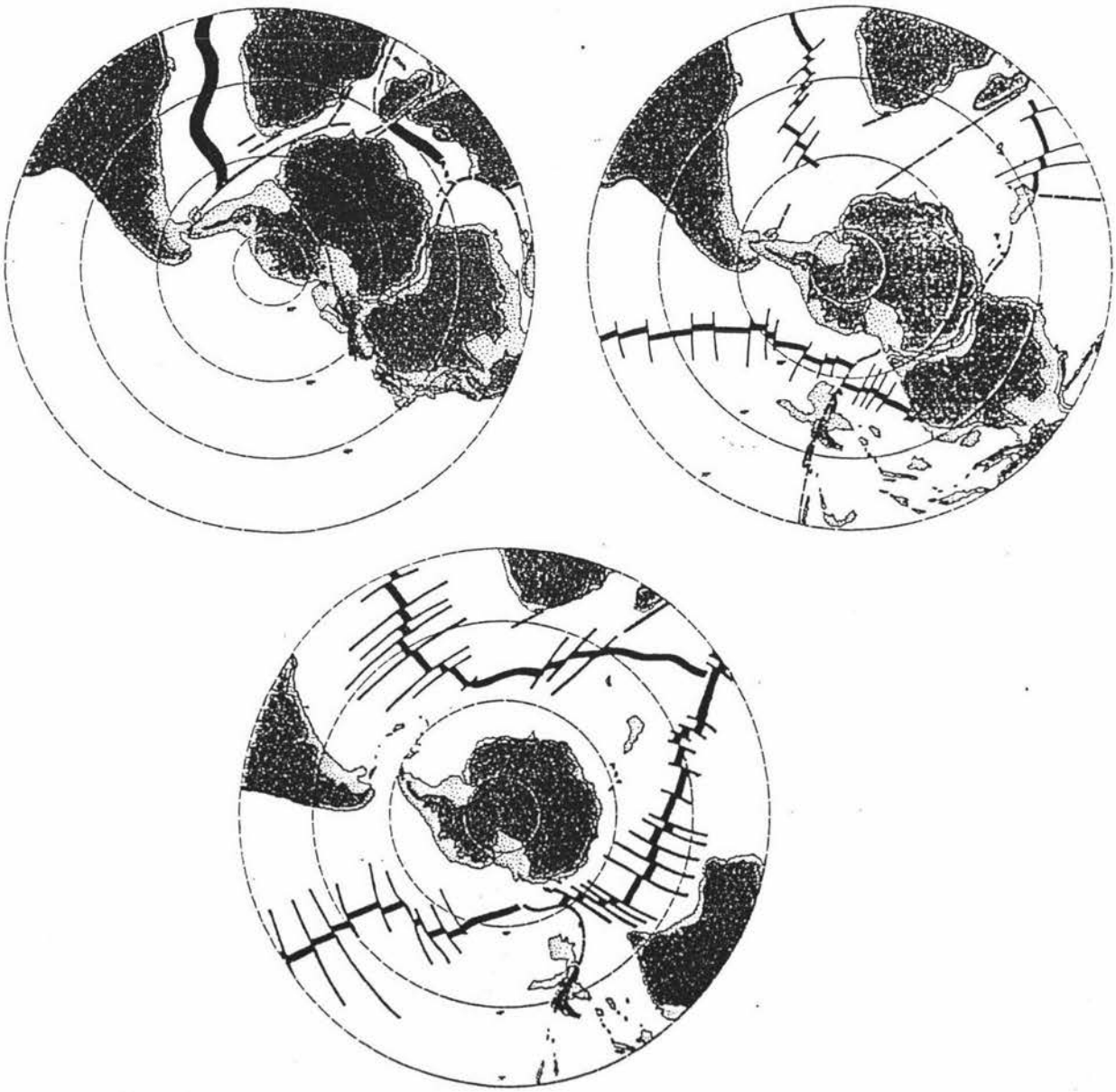


Fig. 1.2. Summarizing stages in the development of the Southern Ocean and the break-up of Gondwanaland during the Mesozoic and Cenozoic. The reconstructions are based on the theory of an expanding earth, and all landmasses have been drawn in their modern form to aid identification.
A.) The Southern Hemisphere landmasses 90 million years ago (late Cretaceous). Diameter of the earth is 90 percent of the modern globe. Continued rifting has separated South Africa, Malagasy, India and Australia. Sea-floor spreading has commenced in the Indian Ocean, between India and Antarctica. B.) The Southern Hemisphere landmasses 60 million years ago (Paleocene). Diameter of the earth is 93 percent of the modern globe. Sea-floor spreading is actively underway in all the major oceans, except the segment of the Southern Ocean between Antarctica and Australia. C.) The Southern Hemisphere landmasses today, with active spreading ridges in the Pacific, Indian and South Atlantic Oceans. (From Stevens 1980).



Previous Land Connections.

During the early evolution of angiosperms Australia, Antarctica and the New Zealand-New Caledonia crustal complex were still united as "Tasmantis" (Figure 1.3). These linked lands shared a type of forest dominated by gymnosperms and ferns with an increasing angiosperm component (Schuster 1979; Stevens 1980; Dawson 1988). The earliest angiosperms are known mostly from pollen that cannot be matched with present day types. The *brassi* group of *Nothofagus* (southern beeches) appears in the pollen record at about the same time in Australia, New Zealand and Antarctica (Holloway 1954; Wace 1965), and somewhat later in South America where it is associated with the *fusca* and *menziesii* groups (McGraff *et al.* 1995). Pollen assignable to the Proteaceae family is also found in Australia, New Zealand, Antarctica and South America (Schuster 1979). Other families from this age include the Winteraceae, Epacridaceae, Chloranthaceae (*Ascarina*), Loranthaceae (mistletoes), Lauraceae, Ranunculaceae, Haloragaceae (*Gunnera*), Cruciferae and Caryophyllaceae (Mildenhall, 1980). New Zealand and Australia share a strong floristic relationship (80% of vascular genera in common) that may have been much greater at times in the Tertiary when characteristically Australian genera such as *Eucalyptus*, *Casuarina*, and *Acaciapollenites* were also present in New Zealand (Pole 1993).

In the Late Cretaceous, the New Zealand region became separated from Australia-Antarctica by water of oceanic depth. A land connection between New Caledonia and New Zealand may have existed along the Norfolk Ridge during this period (Raven 1972). Many of the genera showing Australian-Malayan affinities may have reached New Zealand during Tertiary times via this warm northern route (Lord Howe Rise and Norfolk Ridge). Plant genera showing these affinities include *Agathis*, *Cordyline*, *Macropiper*, *Pittosporum*, *Corynocarpus*, *Elaeocarpus*, *Metrosideros*, *Meryta*, *Avicennia*, *Myoporum* and *Solanum* (Kuschel 1975; Fleming 1980). In the late Cretaceous and early Tertiary period, New Zealand was probably still in close contact with Antarctica, perhaps only by a chain of islands, and this permitted South American forms (such as *Fuchsia*, *Griselinia*, *Laurelia*, and *Jovellana*) to reach New Zealand (Fleming 1980). By 60-70 million years ago, westward drift of South America accelerated, leaving behind the chain of mountains extending southward from the tip of South America to west Antarctica, so that the widely spaced islands of the Scotia Arc began to form (Dalziel & Elliot 1971), and former land connections between Antarctica and South America were severed.

Fig. 1.3. Diagrammatic reconstruction of the southeastern margin of Gondwana in the early Cretaceous, ca. 120-110 Ma. The main uplift phases of the Rangitata Orogeny were continuing during this time. In the Aptian-Albian a rifting phase (indicated by thick lines) commenced along the west coast of New Zealand, along the western side of the Lord Howe Rise, and in the Bounty Trough. Major rifting also commenced between the African and South American plates (indicated by the toothed pattern). Ancestral angiosperms appeared at about this time and radiated through the Gondwana margins. Because of the disruption of land routes by the onset of rifting along the western edge of "Greater New Zealand" (indicated by the speckled pattern), heralding formation of the Tasman Sea, the ancestral angiosperms may have used a land route into New Zealand via Antarctica (as indicated by the solid arrow). Ancestral ratite birds, including moa, may have also used this route if they were flightless at this time. (From Stevens 1991).

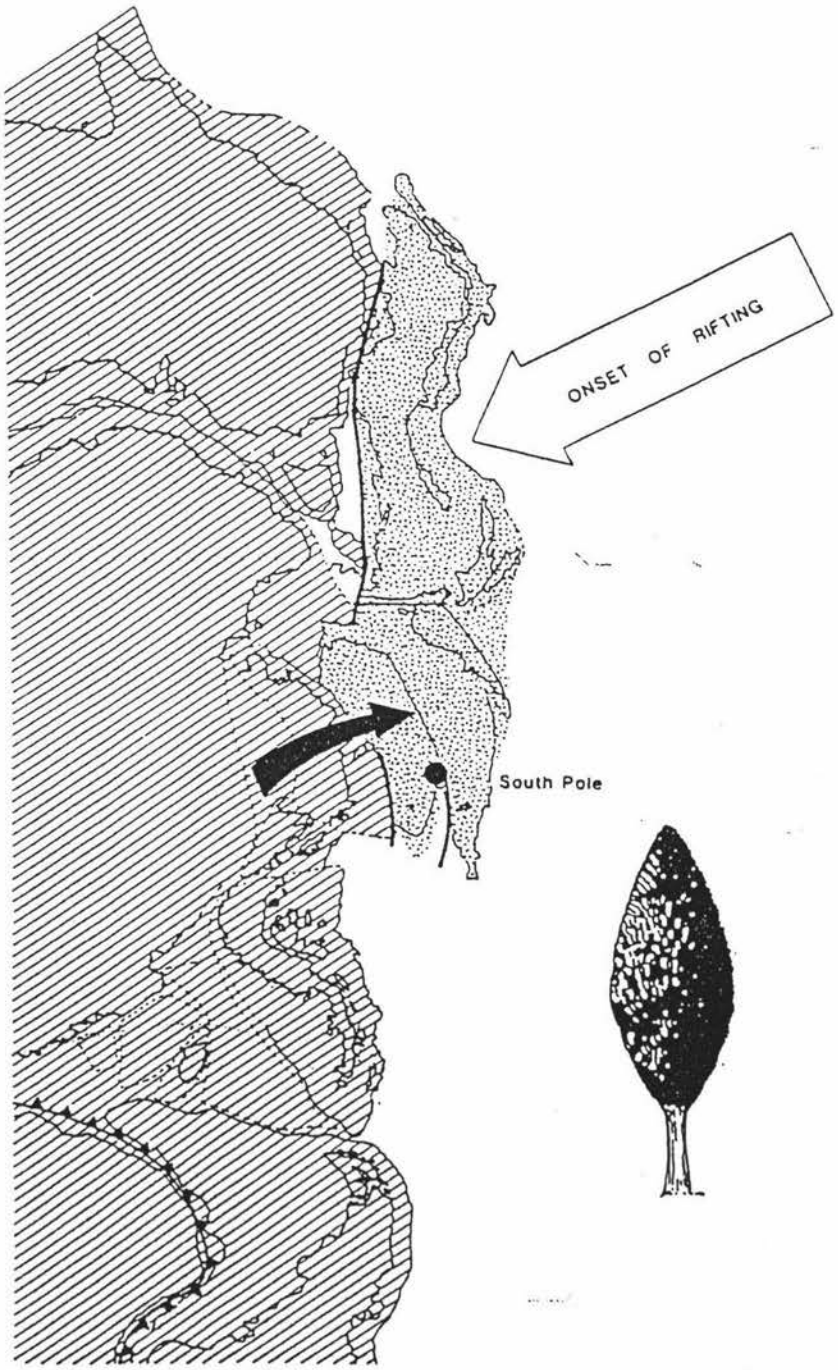


Table 1.1. General vegetation patterns and the times of appearance of taxa in the fossil record.

Period	Common Elements	First Appearance of Taxa	Reference
Oligocene 37 -25 MYA	*Nothofagus brassi and fusca* group, *Casuarina* Palmae, Myrtaceae and Podocarpaceae	*Weinmannia*, *Laurelia*, *Myrsine Elaeocarpus*, *Coprosma*, *Astelia*, *Dacrydium cupressinum*, *Nothopanax*, *Epilobium*, *Carpodetus*, *Pomaderris*, Compositae	Couper 1953, 1960 Mildenhall 1980 Dawson 1988 Pocknall 1989
Miocene 15-25 MYA	*Nothofagus brassi* groups, Araucariaceae Podocarpaceae, Palmae, Myrtaceae, *Eucalyptus, Casuarina* and tree ferns.	*Muehlenbeckia*, *Cordyline*, *Ripogonum*, *Dysoxylum*, *Alectryon*, *Macropiper, Cocos zeylanica*, *Acaciapollenites*, *Pittosporum, Fuchsia* and *Melicytus*	Mildenhall 1972 Mildenhall 1980 Mildenhall & Pocknall 1984 Pocknall & Mildenhall 1984
Pliocene 2-15 MYA	Podocarpaceae, *Dysoxylum*, *Knightia*, *Agathis*,	*Quintinia, Cyathea medullaris, Sarcocornia, Carmichaelia, Sophora*?	Raven 1973 Wardle 1968, 1978 Fleming 1963
Pleistocene 2 MYA	*Dacrydium*, *Astelia*, *Phyllocladus alpinus*,	*Libertia*, *Toronia*, *Dodonaea*, *Acacia*,	Fleming 1980
Holocene 10 000 yrs bp to present	Present-day flora	About 1700 adventives have been recorded in the wild.	McGlone 1985

Elements and Origins.

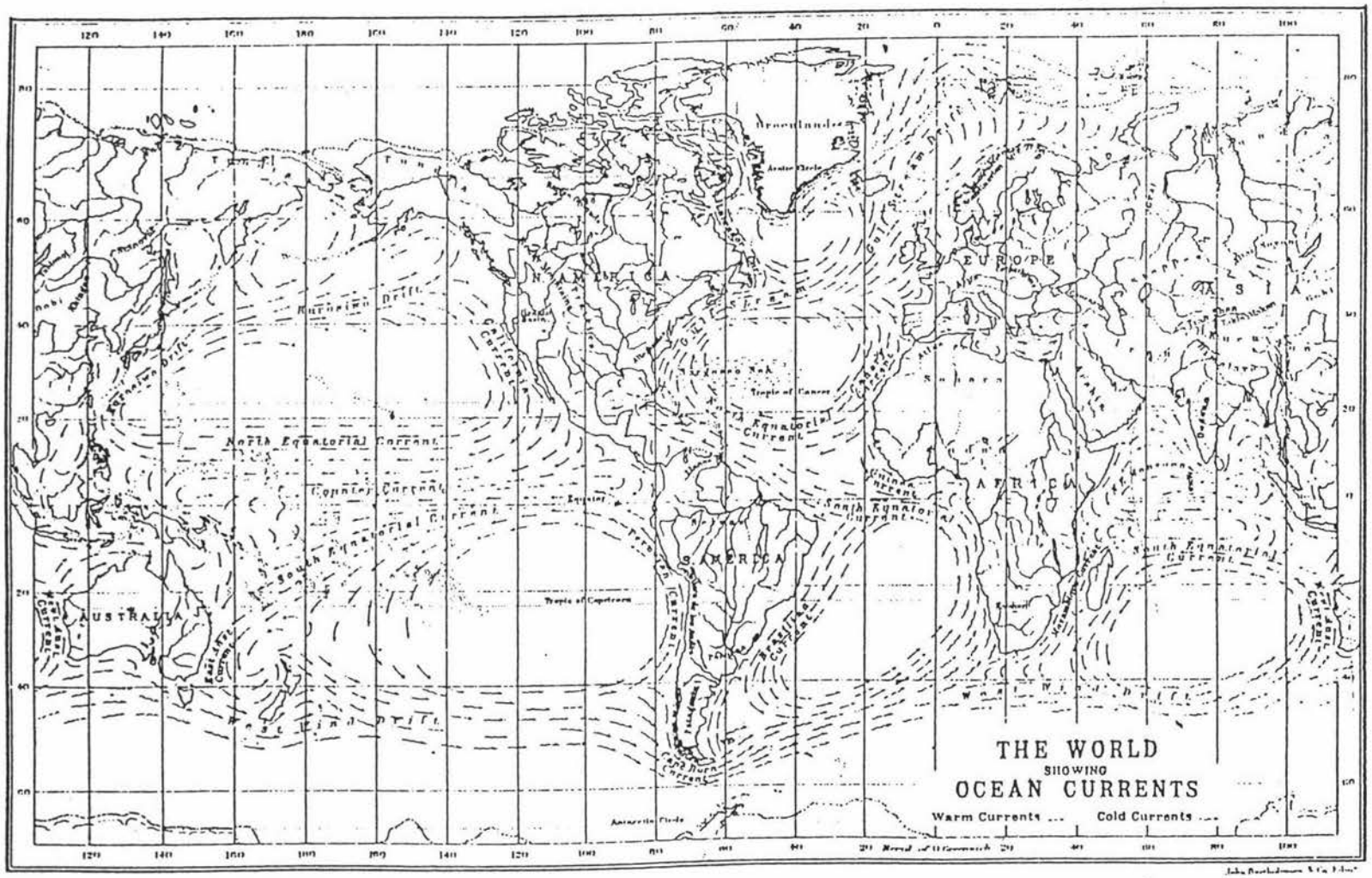
There are some 400 angiosperm genera in New Zealand, of which about 10% are endemic to New Zealand, about 80% are also found in Australasia (of this, only 12% are confined to Australia and New Zealand), and 25% are Malayan in their affinities (Laing and Blackwell 1964). The New Zealand flora has been placed into various groups (elements) according to geographic relationships and affinities and probable origins. These include: (1.) Palaeozelandic element; (2.) Palaeotropic element (Indo-Malayan or Indo-Pacific); (3.) Endemic element; (4.) Australian element; (5.) Cosmopolitan element; (6.) Holarctic element; and (7.) an Austral element, divided into i.) a Palaeoaustral group and ii.) a Neoaustral group (see Fleming 1962, 1963).

Trans-oceanic dispersal of plants to New Zealand.

There is abundant evidence for trans-oceanic dispersal in the New Zealand flora (Guppy 1906, 1917; Ridley 1930; Murray 1986; Sykes & Godley 1968; Carlquist 1981). In general insular areas are converging points for many surface ocean currents, some air currents, and migratory routes of birds (Schuster 1979). Among plants, those whose seeds had special modifications for dispersal (for example: flotation devices for sea transport; plumes of hairs for wind dispersal; hooks for attachment to bird's feathers; or hard seeds in berry fruits eaten by birds and eventually excreted intact) were able to utilize these transports, and are strongly represented in the modern flora (Lloyd 1985; Dawson 1988).

Many of the flowering plants to appear in the Early Tertiary fossil record were probably carried by birds such as parrots and pigeons; especially those with orange, red and black fruits (Clout & Hay 1989). The plants *Rhopalostylis*, *Astelia*, *Hedycarya*, *Coprosma* and *Podocarpus* are still dispersed by birds in New Zealand (Preest 1963). The migrations of pelagic birds between sub-Antarctic islands may also have been an important source of propagules during various times in New Zealand's history. The chief New Zealand sub-Antarctic groups, Auckland, Campbell, and Macquarie Island as well as Patagonia, southern Chile, Tierra del Fuego, the Falkland Islands, Tristan de Cunha, the Crozets, Kerguelen Land, and the mountains of Victoria and Tasmania, have been important sources for the sub-Antarctic and alpine plants in New Zealand (Wace 1960; Wardle 1978). The buttercups *Ranunculus*, *Acaena*, *Callitriche*, *Cotula* and *Hebe* are all

Fig. 1.4. Map of the World showing the major ocean currents. In the Southern Oceans, the dominating current is the West Wind Drift, which is able to transport buoyant objects around the Antarctic Circle. (From Knox 1960).



suggested to have reached New Zealand by wind dispersal and mountain-hopping (Davis 1950; Raven 1973; Wardle 1978).

The opening of the southern ocean between Australia and Antarctica in the late Eocene, and of the Drake Passage south of South America at the end of the Oligocene, established the antarctic circumpolar current. This current, together with the westerly wind that drives it, has been paramount in the west-to-east dispersal of many southern plant species to New Zealand since late Oligocene times (Fleming 1962). The New Zealand coconut, *Cocos zeylandica* was undoubtedly transported by ocean currents during the Tertiary (Dawson 1988). The dominating feature of the current system is the West Wind Drift (Figure 1.4). Off the coasts of the major land masses it branches into currents flowing in a northerly direction (Knox 1960). The general eastward drift in the southern Pacific is interrupted by the southern extension of South America and is deflected southwards through the Drake Passage, as the Cape Horn Current.

Evolutionary History of the New Zealand Vegetation.

An understanding of past environments and geography is fundamental to the study of New Zealand plant biogeography, and there has been much interest in trying to relate certain disjunct distributions to prehistorical events (e.g. Rattenbury 1962; Wardle 1963; Burrows, 1965; Raven 1972; McGlone & Moar 1977; Fleming 1980; McGlone & Webb, 1981; Mildenhall & Pocknall, 1984; Lloyd 1985; McGlone, 1985; Wardle, 1988; Ogden, 1989; Pocknall, 1989; Flenley, 1992). The geological and palaeological records indicate that extensive geological, climatic and ecological disturbances have been a feature of the New Zealand landmass for at least the last 130 million years (Gage 1961, Suggate et al, 1978, Fleming, 1980, Burrows and Greenland, 1979, Mildenhall 1980, Stevens 1980, Pocknall 1989, 1992). In addition to the origin and separation from Gondwanaland, four periods are thought to have had important influences on the evolution and distribution of plant groups in New Zealand since the Oligocene (Cooper and Cooper 1995?):

The Miocene period (15-25 million years ago).

Increasing topographic relief, local volcanic outbursts, and the consequent increase in sedimentation during the Miocene raised most of the Northland, parts of the King Country, and parts of Southland above sea level (Fleming, 1962). Apart from volcanoes, the land was not yet

mountainous. In the marine animal fossil record of New Zealand, successive occurrences of cool water forms during the period from Mid to Late Miocene suggest that marked fluctuations in temperature took place (Fleming 1975). This is reflected in the vegetational changes during the same period, from a predominantly warm temperate to subtropical vegetation in Mid Miocene to cool temperate vegetation on uplands in the Late Miocene. Most of the fossil species cannot be identified with modern plants, and much of the extant angiosperm taxa are not found in the Miocene fossil record, and Pole (1989) concludes that a rapid turnover of species and genera has occurred since the early Miocene.

The Kaikoura Orogeny and it's implications for the evolution and distribution of plants.

The change to cooling palaeoclimates continued into the Pliocene period, and may have been largely responsible for the extinction of a number of forest genera and species, including most of the *Nothofagus brassi* group and many palms. A geographical differentiation of vegetation is indicated by the pollen record (Couper & McQueen 1954) together with the occurrence of mixed cool and warm floras indicating some altitudinal zonation (McQueen *et al.* 1968). This is understandable, for the earth movements through Kaikoura Orogeny to which New Zealand owes its present geography became most intense during the Pliocene, 15 - 5 million years ago (Suggate *et al.* 1978; Fleming 1979; Cooper & Millener 1993). Extensive development of alpine vegetation would have commenced from this time. Raven (1973) suggested that the alpine flora came from Australia, but other authors (Fisher 1965; Wardle 1978; Fleming 1979) argue that adaptation of indigenous plants is more likely.

During the Pliocene the north of the North Island was a series of archipelagos, and sea level fluctuations changed their size and number. The diversity of beetles and land snails found in northern areas may be due to isolation on such scattered islands (Climo 1978, 1989, Solem *et al.* 1981, Powell 1949), which favours race and species formation (Climo 1989).

Fig.1.5. Changes in land area and relief of the New Zealand region through geologic time. (Adapted from Cooper *et al.* 1992).

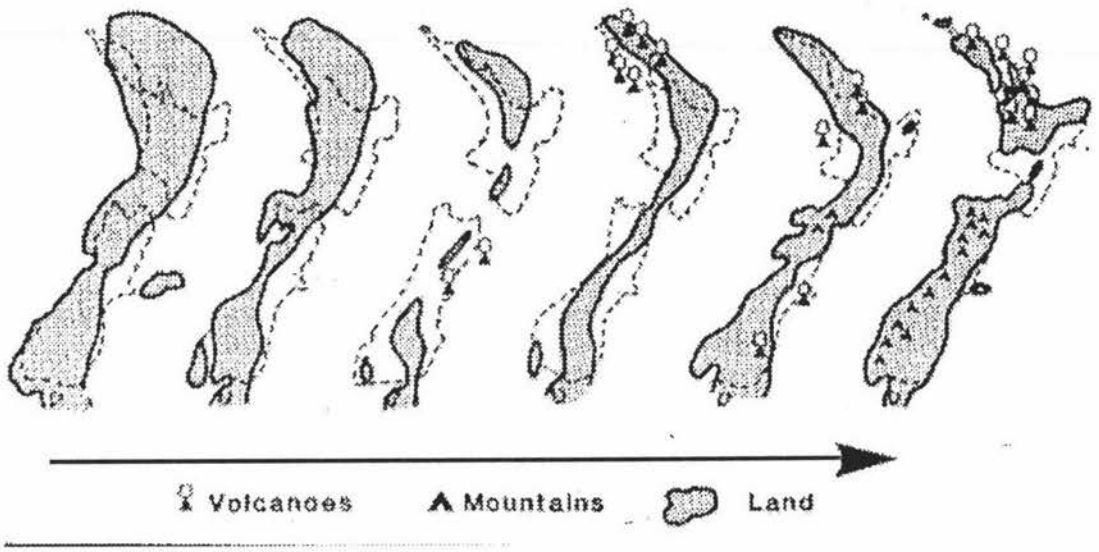
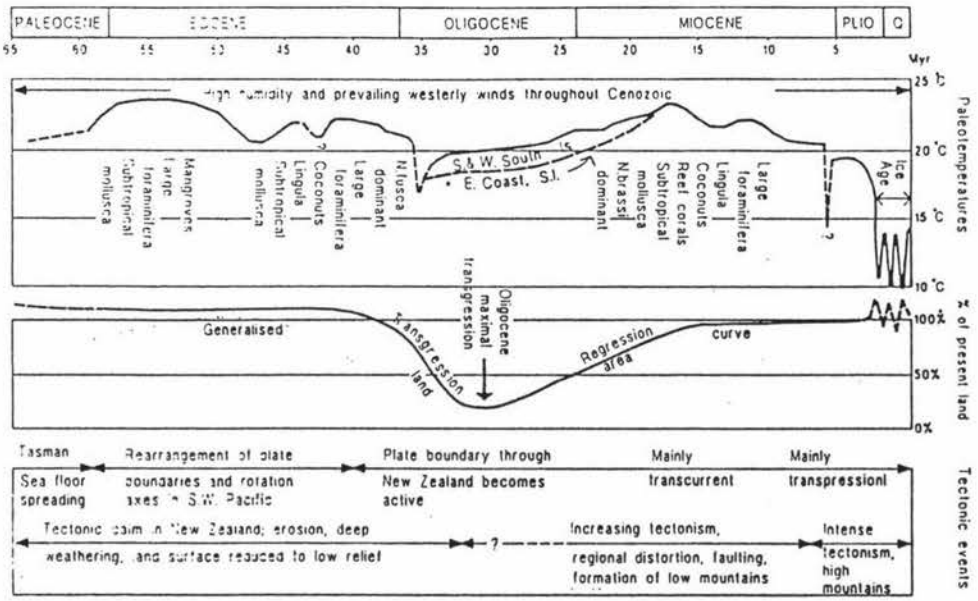


Fig. 1.6. Climatic and Tectonic History of New Zealand: main events. Sea surface paleotemperature curve (normalized to latitude of Wellington) for New Zealand Cenozoic; judging from the fossil pollen record the terrestrial air temperature curve generally accords with the trends shown, but differs in detail. Note that there are at least nine glacial cycles (only four shown). (From Cooper & Millener 1993).



Evolution and distribution of the flora as affected by Quarternary Climates.

Over the last 700 000 years, since the last major palaeomagnetic reversal, nine regular and well-marked glacial/interglacial intervals, comparable in duration and severity to the Last Otiran glacial/interglacial cycle, have been documented in the marine isotopic record (Shackleton & Opdyke 1973; Leg 90 1983; Nelson *et al.* 1985; Markgraf *et al.* 1995). New Zealand partly to largely escaped glaciation (Schuster 1979; Markgraf *et al.* 1995). However, the Pleistocene is considered to have had an important effect on the distribution and speciation of many groups both here (Bull & Whitaker 1975; Burrows 1965; Fleming 1979; Petersen 1968; Wardle 1963b, Wardle *et al.*, 1988; Willet 1950), and elsewhere (for example, Haffer 1969; Mayr & O'Hara 1986). During cooler cycles there was a general absence of tall forest taxa, and a subalpine grassland/shrubland was present at sites from present sea level to 800m in the central and southern parts of both North and South Islands. Factors such as exposure to wind, frost, and oscillating droughts, were important in controlling vegetation patterns during the glacial periods (Diels 1897; Cockayne 1928; McGlone & Webb 1981; Pillans *et al.* 1993). With each glacial the alpine vegetation expanded and diversified; with each interglacial the forests expanded from refugia (reduced in diversity) to recover the landscape (Flenley 1992).

The Arrival of the Bipeds.

Human settlement of New Zealand over the last millennium has also had a major influence on the biota (Molloy *et al.* 1963; Anderson 1983; McGlone 1983b; Holdaway 1990). When the first outrigger canoes arrived, approximately 1000AD (Suggs 1960; Sutton 1994), New Zealand was largely covered in evergreen forests. Much of the dryland forest in the eastern parts of both main islands was probably destroyed by the fires of the early Polynesians, and short tussock grassland developed in its place (Cameron 1964; McGlone 1978, 1983b). The destruction of mainland habitats contributed greatly to the extinction of many native species, including about 25 species of birds (McGlone *et al.*, 1994). After 1400 AD, with the extinction of many of the avian herbivores, the predominant browsing and grazing systems ceased to exist, and the unbrowsed vegetation had to adjust to the new regime (Caughley 1990). By the time Europeans reached New Zealand, only about 50% of the land was still in forest, and this has been reduced systematically over the past 150 years of settlement (McGlone 1983a). Many herbivorous and predatory animals have been introduced, each with feeding niches completely novel to the New Zealand biota, and many are now abundantly naturalised (King 1984).

Evolutionary Studies in New Zealand Plant Groups.

How and why species change have been illustrated by studies in the Galapagos and Hawaiian islands (*e.*g. Berry 1984; Baldwin & Robichaux 1995; Grant & Grant 1989; Lowrey 1995; Givnish *et al.*, 1995). Despite recognition that New Zealand is an important place to investigate evolutionary patterns and processes (Hooker 1853; Hutton 1872; Wallace 1889; Cockayne 1911; Godley 1949; Hair 1966; Fleming 1958), the biota has not yet been examined in sufficient detail to complement Hawaiian and Galapagan studies. New Zealand's antiquity, continental origins, and the large size of some of its islands make it different from other oceanic islands. Therefore, studies of the origins of New Zealand's biota and their evolution may offer different perspectives on patterns and processes of evolution.

Evolutionary studies have been conducted here, but few have addressed directly the evolutionary concepts established by Lyell (1830), Darwin (1959), and Wallace (1889), further elaborated on by Dobzhansky (1941), Stebbins (1950) Mayr (1942, 1963), Lewontin (1974), Kimura (1983) and Nei (1987). Exceptions are studies of the origin and evolution of alpine plants in New Zealand (Fisher 1965; Raven & Raven 1976; Ornduff 1964), and floral biology (Lloyd & Yates 1982; Lloyd & Webb 1986; Webb & Kelly 1993). Recent advances in molecular genetic techniques are leading to a renaissance in phylogenetic and evolutionary investigations, although little work has yet been carried out on specific New Zealand groups.

New Zealand's Offshore and Outlying Islands.

The New Zealand region was formerly a "continental" region which fissured to create a series of present-day isolated islands of small size (New Caledonia, Norfolk Island, Lord Howe Island, Campbell Island, Macquarie Island, Auckland Islands, New Zealand (North, South, and Stewart Islands), the Antipodes, and Chatham Islands, *etc.*). Some of these small, isolated islands, even though seemingly relatively recent, must have had a long complex history. Present day Campbell, Macquarie, and Auckland Islands and all or most of present-day New Zealand seem to represent the remnants of a large land mass, the Campbell Plateau (Griffiths 1971), now largely foundered.

The New Zealand botanical region includes over 660 islands ranging from the subtropical Kermadec Islands (960 km NE of Auckland) to the sub-Antarctic islands (six widely separated groups between 48°S - 55°S, 159°E - 179°E) and the Chatham Islands 770 km east of Christchurch (Figure 1.7.). About 630 of the islands lie within 50 km of the mainland, and some of the closer ones would have been more or less connected to New Zealand during the Last Glaciation (*e.g.* Hay *et al.* 1970). The separation and reunion of small peripheral populations during certain times is often considered to have had important influences on race and species formation in the flora and fauna (*e.g.* Finlay 1928; Lloyd 1982; Nixon 1982; Brownsey 1985; Wardle 1987; Craw 1988). The remaining islands - "outlying islands"- have been isolated for longer and tend to show a higher degree of endemism (Cheeseman 1909; Wace 1960; Lloyd 1982).

Hybridization in the New Zealand flora.

A notable feature of many New Zealand plant groups is their readiness to hybridize (see Allan 1961; Fisher 1965; Carlquist 1974). Hybridization may play a major role in the evolution of some plant groups, both here and elsewhere (Gillet 1972; Rattenbury 1962; Raven & Raven 1976; Ogden 1989; Arnold, 1992, 1994), and several authors suggests that the high frequency of hybridization in the New Zealand flora points to a recent and rapid evolution. However, Hair (1966) and Connor (1985) note that the failure to recognize that many of the hybrids occur in disturbed habitats may have over-emphasized the role of hybridization in New Zealand

Heteroblastic Development in New Zealand Plants.

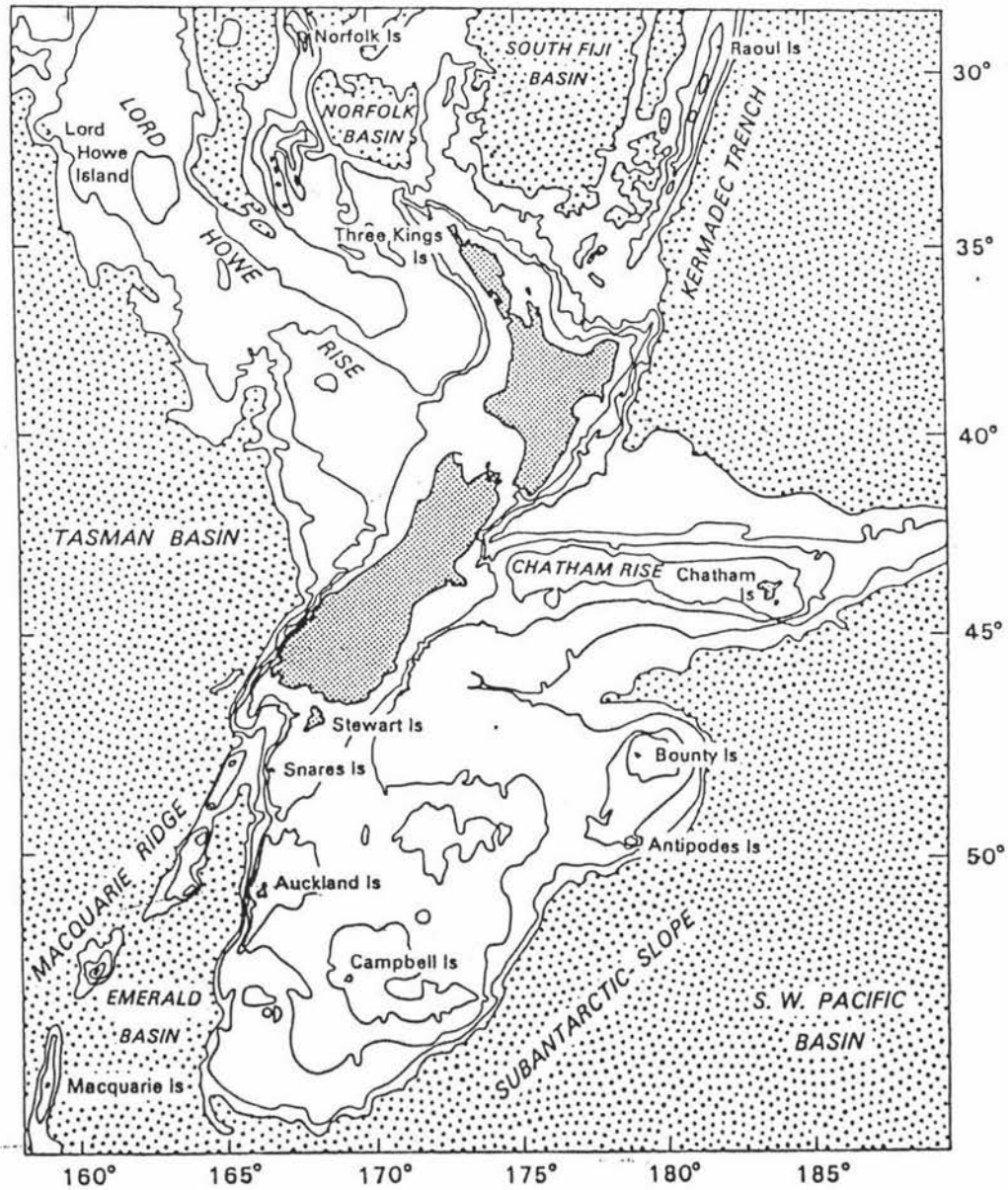
A high frequency of New Zealand forest species, including vines, trees and shrubs, have differing juvenile and adult stages. Cockayne (1928) estimates about 200 seed plants have heteroblastic development, of which 165 remain juvenile for a long period. The differences between juvenile and adult stages involve changes in leaf morphology (leaf-heteroblastism) and branching form (habit-heteroblastism), often in combination (Phillipson 1964). A few studies have tried to attribute heteroblastic changes in development with changing height in the canopy (much like the submerged/surface leaf form differentiation in aquatic plants), succession (Hollows 1978),

Fig. 1.7. Map of the New Zealand region, including Offshore and Outlying Islands. Isobaths (submarine contours) are drawn at 500 m, 1000 m, 2000 m and 3000 m. Oceanic areas deeper than 3000 m are stippled.

The Campbell Plateau includes the region south and south-east of New Zealand on which stand Auckland, Campbell, Antipodes, Bounty and the Snares Islands. The Bounty Trough lies between Bounty Islands and the Chatham Rise. The Solander Trough, a northern continuation of the Emerald Basin, lies to the west of Stewart Island.

The Norfolk Ridge swings in an arc between New Zealand's North Cape and Norfolk Island and continues onto New Caledonia. The Norfolk Ridge is flanked by the New Caledonia Basin to the west and the Norfolk Basin to the east. The Three Kings Rise extends northwards from the Three Kings Islands to lie between the Norfolk Basin and South Fiji Basin.

The Kermadec Trench continues northwards into the Tonga Trench, and fades southwards towards East Cape. A trough-like feature, the Hikurangi Trench, continues down the East Coast of the North Island, to end off the Marlborough coast, against the northern flank of the Chatham Rise. (From Stevens 1980).



and escape from moa-browsing (Greenwood & Atkinson 1977; Atkinson & Greenwood 1989). Preliminary studies have shown that the external environment is capable of influencing plant form and the degree of juvenile expression (e.g. Cockayne 1912; Philipson 1963; Denny 1964; Laing & Blackwell 1964; Keen 1970).

Divaricating Plants in New Zealand.

Approximately 10% of the woody flora of New Zealand consists of divaricating shrubs and tree juveniles belonging to many different genera and families. Divarication is a loosely applied term to describe a syndrome of characters such as intricate branching (with branches emerging at a more or less 90° angle), less than normal apical dominance, and microphylly (Figure 1.7.). Despite this general grouping there is considerable diversity in form (Tomlinson 1978). Comparable plant forms exist in south-west Madagascar (Koechlin *et al.* 1974), and various semi-arid regions of Australia and eastern Patagonia (Bartlett and Bartlett, 1976), although it is uncertain that these thorny-shrubs are exactly equivalent.

There has been much speculation about the evolutionary causes of the high frequency of heteroblastic development and the divaricating growth form in the New Zealand flora. The convergence among unrelated genera must reflect New Zealand's long isolation, and unique factors of the prevailing environment (Went 1971). Much debate has centered on the relative influences of physical versus biological factors in shaping the evolution of divaricating plants: The most commonly cited hypotheses are (i) that the juvenile form evolved in response to browsing by moa (Atkinson and Greenwood 1989); (ii) that the most xerophytic form (adult or juvenile) evolved in response to water stress imposed during glaciation in the Pleistocene (Cockayne 1911; McGlone & Webb 1981); (iii) that some heteroblastic species, possessing the distinct juvenile stage, are the result of interspecific hybridization between ancestral homoblastic trees (Godley 1985); or (iv) that viruses or bacteria acted as vectors for the lateral transfer of the genes for certain types of juvenile forms (Went 1971).

Owing to the extinction of moa some 500 years ago, the moa-browsing hypothesis is very difficult to test. Some authors suggest that the hypothesis is to some extent reviewable by examining the actual evidence of feeding abilities of moa and other ratites (*e.g.* Batchelar 1989; Cooper 1994). Wellman (1994) raises the possibility that the moa, like other birds, had dust baths on the river flats, and could be potential dispersers of the seeds caught in their feathers. Diet

sample information has previously been analyzed to infer the habitats persisted by moa, but may also indicate the potential for the moa as a dispersal agent of seeds for these species.

A few studies have been conducted to test aspects of the climate hypothesis (*e.*g. Kelly & Ogle 1990), but have so far contributed little to increasing scientific knowledge in this area. Cooper and Millener (1993) have suggested that accurate assessment of taxonomical relationships and the divergence times of a divaricating plant and a non-divaricating relative can provide a good test between the moa-browsing hypothesis and the climate hypothesis for the origin of divarication. The climate hypothesis predicts a divergence between species of about 1 to 2 million years ago, while the moa-browsing hypothesis predicts divergence times in the region of 20-30 million years ago, coincident with radiation of moa in New Zealand.

Assessing Taxonomical Relationships

Traditionally, angiosperm phylogenies and concepts of "ancestral" and "derived" characters, have been constructed entirely on the basis of comparative morphological studies (see Wallace 1889; Darwin 1859, 1875; Mayr 1942, 1963; Stebbins 1950). The presence of flowers and fruit defines the angiospermous condition, and floral structure provides the majority of characters used in angiosperm systematics (Kanis 1981). Taxonomical investigations in New Zealand have not always adequately resolved taxonomic relationships. Patterns of variation can change significantly between seemingly older and younger parts of various plant groups, and this poses a problem for formal systematics (*e.*g. Polhill 1981). Biochemical, genetical, and cytological investigations of taxonomy are essential complements to morphological studies (Godley 1949; Hillis 1987; Patterson 1987; Hillis *et al.*, 1996). These techniques are described below, and examples of their application in New Zealand are given.

Secondary metabolites

Analysis of plant secondary metabolites have been used for taxonomic studies (Cronquist 1980), and have been applied to several New Zealand plant groups (Taylor 1964, Briggs & Ricketts 1937; Markham & Godley 1972, Wilson 1984, Connor 1985). Chemotoxonomy has also been applied at deeper levels of evolution, such as between groups and their alliances, and between different families (*eg.* Ohyama *et al.*, 1995; Mears & Mabry 1971; Turner 1971). However, genetic methods are required for the identification of the basal (pleisomorphic) species of lineages, and for determining both the rate and direction of evolution within plant groups.

Cytological Studies.

Examination of chromosomal organisation can be useful both for systematics and for inferences about processes (Dobzhansky 1941, White 1978, Grant 1981, Carson 1983, Moritz 1986, Systma 1990), but it is difficult to establish whether such changes are causally related to the formation of new species (Bush *et al.* 1977, White 1978) rather than being subsequent effects of isolation (see Endler 1986). Chromosomal atlases for many New Zealand plant species have been compiled (see Frankel and Hair 1937, Hair 1966, 1977, Rendle & Murray 1989), but the range and patterns of chromosomal changes have not yet been extensively examined. Hair (1966) noted that chromosomal instability was associated with advancing fronts of podocarp dispersal but this has not been investigated thoroughly.

Macromolecular Sequences.

Application of molecular techniques to evolutionary questions has contributed much to an understanding of phylogeny (Wilson *et al.*, 1985; Hillis 1987; Patterson 1987; Wilson *et al.*, 1989; Moritz & Hillis 1990; Systma & Gottlieb 1986; Clegg & Durbin 1990; Hey 1992; Nee & Harvey 1994). They have been helpful in resolving relationships which have been difficult to address by other means, and can also provide a more reliable indicator of the degree of phylogenetic divergence (for example, King and Wilson 1975; Roberts & Maxson 1985; Wayne *et al.*, 1989; Meyer *et al.*, 1990; Martin *et al.*, 1993). DNA sequence data has the advantage of being independent of other biological characters, and patterns of character evolution can be examined within the independent context of molecular trees (Clegg & Zurawski 1992).

The invention of rapid DNA sequencing methods has seen an explosion in the use of molecular data for the study of plant evolutionary problems (Saiki *et al.* 1988, Gyllensten & Erlich 1988, Kocher & White 1989, White *et al.* 1989, Arnheim *et al.* 1990; Arnheim & Erlich 1992). The procedure (see Chapter Two) is, in theory, elegantly simple, allowing specific regions of DNA to be isolated and sequenced within hours, and from organisms for which no prior genetic information is available. Consequently, it has led to somewhat of a renaissance in systematics (Patterson 1987, 1990). In the last six years, genetic data have been used to test taxonomies of selected New Zealand animal groups (*e.*g. tuatara - Daugherty 1990a, 1990b; moa and kiwi - Cooper *et al.* 1992; and fur seals - Lento *et al.* 1993). Little work has yet been carried out on New Zealand plants.

Chloroplast DNA and Evolution

Chloroplast genes evolve slowly in comparison to nuclear genes and are not subject to the complex processes of conversion and intragenic recombination that often affect nuclear sequence evolution (Clegg *et al.*, 1987). This coupled with the fact that leaf cells typically contain several thousand of these plastids, has meant that chloroplast DNA has become the molecule of first choice for the plant systematist. It has been shown to be useful at a range of taxonomic levels within plants, and is now fairly well characterized among major plant group lineages (*e.g.* Palmer 1986; Palmer *et al.* 1988; Duvall *et al.* 1993a; Zurawski and Clegg 1987; Ritland and Clegg 1987; Golenberg *et al.* 1990; Doebley *et al.* 1990; Soltis *et al.* 1990; Olmstead *et al.* 1992; Bousquet *et al.* 1992a; Kim *et al.* 1992; Gaut *et al.* 1992; Savolainen *et al.* 1994; Plunkett *et al.*, 1996). Complete sequences for a few taxa are obtainable from genetic libraries, such as Genbank and Embo.

Chloroplast variation has also been used to study evolutionary interactions such as species introgression and hybridization, inversions and restriction site variation, and the origins of polyploidy (Clegg *et al.* 1987; Soltis *et al.* 1989b; Wagner *et al.* 1987; Johnson and Palmer, 1989; Doyle *et al.* 1992b; Delgado-Salinas *et al.* 1993).

Rates of Sequence Evolution

According to the molecular clock hypothesis (Zuckerkandl and Pauling 1965), genetic divergence is a linear function of evolutionary time. The more mutations that have accumulated in a specific protein sequence, the older its lineage. Average rates of synonymous substitution in cpDNA have been estimated to be about $1x10^{-9}$ per synonymous site per year (Zurawski and Clegg 1987; Wolfe *et al.* 1987). It is at least twofold, and probably fourfold, slower than estimated rates of synonymous substitution for plant nuclear genes (Wolfe *et al.* 1987, 1989a; Meagher *et al.* 1989). Conserved elements are usually thought to be of biological importance and under functional constraint; either metabolic (related for instance to the regulation of gene expression), or structural (related for instance to the packaging of DNA) (Manen *et al.* 1994). Noncoding regions of cpDNA tend to accumulate additions and deletions that eventually obliterate sequence similarity (Zurawski and Clegg 1987).

Using the principle of the molecular clock, there have been a number of attempts to estimate divergence times of major plant taxa from chloroplast DNA sequences, for example, Clegg *et al.*

(1987) and Wolfe *et al.* (1989). An implicit assumption of the molecular clock is an approximate constancy of evolutionary rate over time. However, several studies have reported heterogeneity of substitution rates among plants in chloroplast DNA, as well as among cpDNA genes (e.g. Wolfe *et al.*, 1987, 1989b; Doebley *et al.* 1990). Ritland and Clegg (1987) found that the rate of evolution of a chloroplast intron and the third position rate for the *atpB* gene were accelerated in the lineage leading to pea. Rate heterogeneity clearly has important implications for the use of cpDNA sequences as evolutionary clocks in phylogenetic analyses (Bousquet *et al.* 1992b).

Limitations of Sequence Data

Analysis of cpDNA sequences is a useful tool for the investigation of relationships among plants, but confidence in a phylogeny requires congruence from other data sets, such as from different genes, genomes, morphology and chemotaxonomy (Patterson 1987, Hillis *et al.*, 1996). Reliance on single genes can be misleading (Penny *et al.*, 1982; Wilson *et al.*, 1987). Examination of short regions of DNA also reflects the evolutionary history of that gene rather than the organism (Nei 1987, Pamilo & Nei 1988, Martin *et al.* 1990). Generally, sequence information from several thousand base pairs is preferred (Nei 1987; Martin *et al.* 1990), but sequencing studies employing PCR usually use sequences under one kilobase in length (Kocher *et al.* 1989).

Do molecules and morphology give the same picture of the history of life, or are the approaches sides of the same coin, with the same problems and limitations? (Systma 1990). Molecular data can only (realistically) be collected from living representatives, and thus molecular phylogenetic studies cannot provide direct information on the evolutionary position of extinct species or "intermediate types". Missing taxa, either undiscovered or extinct, may obscure patterns of origin and colonization in phylogenetic hypotheses (ref). Morphological convergence or parallelism is another problem that can result in misleading phylogenetic relationships (Systma 1990). Fortunately, molecular and morphological methods are more often in agreement than conflict (Hillis 1987; Moritz & Hillis 1990; Bousquet *et al.*, 1992a; Williams *et al.*, 1994).

Generating sequence data is now starting to become routine. The primary difficulties lie in data analysis. Most phylogenetic trees derived from sequence data are probably incorrect (Penny *et al.* 1990; Rohlf *et al.* 1990), since no current algorithm meets all the necessary criteria of being fast, efficient, consistent, robust, and falsifiable (Henderson *et al.* 1989, Penny *et al.* 1990). Reviews

of phylogenetic reconstruction generally recognize the limitations, though users of the programs may not (Swofford *et al.*, 1996, Cracraft & Helm-Bychowski 1991, Penny *et al.* 1992). Phylogenetic trees should be regarded as hypotheses and subject to error. Statistical analyses have been developed which help in the assessment of the reliability of phylogenies (Li & Gouy 1991; Penny *et al.*, 1992, 1993).

The Legume Flora of New Zealand

The study of the fossil legume record of New Zealand provides several evolutionary and biogeographical problems. All three legume subfamilies have been recorded by pollen, however only the Papilionoid legumes survive in the native extant flora. Pocknall (1989) records a brief appearance of Caesalpiniodeae (*Caesalpinia* group) during the middle to late Oligocene (23-29 MYA), and the subfamily Mimosoideae is represented by *Acaciapollenites* (early Miocene to early Pleistocene - 26-2 MYA - Mildenhall 1982, 1989), *Acacia* (early Pliocene to Last Glaciation - Mildenhall 1970), and other genera (*e.*g. Pole 1992). The Papilioinoideae do not appear to be common until the Pleistocene (2 MYA), with the appearance of fossil pollen of *Sophora* and *Carmichaelia* (Raine, pers. com. 1996; see also Dodson 1976; Pole *et al.* 1989; Pole 1992).

The modern New Zealand legumes consist of about 51 species belonging to seven genera of the subfamily Papilionoideae. Nearly all of the species are the endemic tribe Carmichaelieae, of which the oldest known genus is the extinct monotypic *Streblorrhiza* from Norfolk Island. Aside from one endemic species of *Carmichaelia* on Lord Howe Island, the remainder of the group, including 40 species of *Carmichaelia*, one of *Corallospartium*, three of *Notospartium*, and one of *Chordospartium*, is entirely confined to New Zealand, and the group is suggested to have radiated from the Late Pliocene onward in the newly available alpine and subalpine habitats of New Zealand (Raven, 1973). The genera *Clianthus* and *Swainsona* are each represented in New Zealand by one species, and are also present in Australia. The genus *Sophora* is represented by three species in New Zealand, all belonging to the section Edwardsia, which is also present on Lord Howe Island, islands of the circum-antarctic oceans, Hawai'i, Chile and Reunion. *Sophora* is also found in Australia (*Sophora fraseri*), but belongs to the section Disemaea of *Sophora*.

Origin of the Genus *Sophora*.

Relative timing of the three stages involved in fragmentation of Gondwanaland (Lawver *et al.* 1994), as well as the early fossil history of legumes (Herendeen *et al.* 1992) provide powerful tools for modern biogeographical and evolutionary studies. Legumes are generally regarded to have originated during the Upper Cretaceous in west Gondwanaland, diversifying into three subfamilies by middle Eocene (Crepet and Taylor 1985, 1986; Polhill 1981). Fossils representing all three subfamilies are present in Eocene deposits (56.5-38.5 Ma.) of Africa, North and South America, and by the Miocene (5-23 MYA) fossils resembling modern taxa are present. Herendeen *et al.* (1992) provides a summary of the systematic and biogeographic aspects of the fossil record in Leguminosae, and addresses the implications of the fossil record with respect to i) structural evolution within the family, ii) pattern and timing of diversification in Leguminosae, iii) biogeographic history, and iv) current hypotheses of phylogenetic relationships within the family.

The Sophoreae are described by Polhill (1981b) as "a tribe of convenience between the Caesalpinioideae and the bulk of the Papilionoideae, sharply defined from neither," and probably represents a grade rather than a clade (Doyle *et al.* 1996). Many of its genera are thought to form a paraphyletic assemblage near the base of the Papilionoideae, as suggested by phylogenies of the chloroplast gene *rbcL* (Doyle 1995), and by a largely morphologically based preliminary cladistic analysis of the Leguminosae (Chappill '1995). The continents of Africa and Central America appear to be the two main centers of radiation of the tribe, evidenced by the modern diversity of extant legumes on these two Gondwanan continents (Table 1.2). By the middle Eocene (50 Ma.), fossil fruits and leaflets assignable to *Sophora* are found in western Tennessee, Mexico, and Africa (*e*.g. Crepet and Herendeen 1992; Wolfe 1966; Wolfe & Tanai 1980; Graham 1992a; Herendeen 1992; Sousa & Delgado 1993; Magallon-Puebla & Cevallos-Ferriz 1994). The genus *Sophora* is distributed today in tropical and temperate regions of both hemispheres (Palomino *et al* 1993).

Views on the Origin of New Zealand *Sophora*

The New Zealand species belong to the section Edwardsia of *Sophora*, which yields one of the finest examples of an Antarctic-circumpolar distribution (Figure 1.8.). Seventeen closely related species (or subspecies) occur in Lord Howe Island, New Zealand, the Chatham Islands, Raivavae, Rapa, Marquesas, Masafuera, Masatierra, Chile, Easter Island, Gough Island, and Reunion, all once considered as a single species, *S. tetraptera* J.Mull., with further species in New Zealand, Hawai'i, and Chile (Guppy 1906, 1917; Skottsberg, 1953: Table 1.3). Most of these are now classified as *Sophora microphylla*, which produces buoyant seed (controlled by the density of the kernel) that can retain viability in sea water for at least three years (Sykes & Godley, 1968). Many legumes are well known for their ability to disperse across oceanic barriers and to tolerate saline conditions (Gunn & Dennis 1976; Raven & Polhill 1981a). And the wide geographical distribution of Edwardsia suggests that many of the island taxa were transported in this way (Sykes & Godley 1968; Raven 1973).

It is not known when the genus *Sophora* arrived in New Zealand. The limited fossil evidence (all of pollen) suggests that they were not common until the Pleistocene (Table 1.3), when several of the other papilionoid genera make their first appearance in New Zealand (Raven 1972; Fleming 1962, Rainer, pers. com.). *Sophora* pollen is a reticulate tricolporate type common among the dicotyledons, and might easily be misidentified or overlooked (Raine, 1996. pers. com.), and while *Sophora* tends to be locally abundant, it is not an overly dominant part of the vegetation. Thus, it is possible that the fossil data underestimates the time of origin of the genus in New Zealand.

Chemotaxonomic studies show that *Sophora microphylla* and *Sophora prostrata* are readily separated by the phenolic constituents of their seed coats, and both are readily distinguishable from *Sophora tetraptera* by the phenolic constituents of their leaves (Briggs & Ricketts 1937; Briggs & Taylor 1938; Briggs & Russell 1942; Briggs & Mangan 1948; Markham & Godley 1972). It was largely on the basis of these studies that the Chile and Gough Island populations were confirmed as *Sophora microphylla*; which is believed to have originated and subsequently dispersed from New Zealand (Markham & Godley 1972).

Table 1.2. Geographic centers of diversity for several legume tribes measured by the percent of the total number of genera in each tribe which occur only in a given geographic area. From Herendeen *et al.* 1992 (Data are from the individual tribal treatments in Polhill and Raven 1981).

Tribe	Africa-Madagascar	Tropical America	Asia-India	Australia	More than 1 Area	Number of Genera
Caesalpinieae	24	50	6	0	20	46
Cassieae	25	20	20	10	25	20
Cercideae	60	0	0	0	40	5
Detarieae	46	26	17	0	11	54
Amherstieae	84	8	8	0	0	25
Mimoseae	30	39	3	0	28	36
Ingeae	0	55	15	5	25	20
Swartzieae	27	64	0	0	9	11
Sophoreae	33	44	8	0	15	48
Dalbergieae	0	74	0	0	26	19
Millettieae	32	27	16	2	23	44

Fig. 1.8 Fragmentation of the tribe Sophoreae, as proposed by Yakovlev (1972) and others.

1. STYPHNOLOBIUM
2. CALIA
3. SOPHORA
    - Sect. 1. CEPHALOSTIGMATON
    - Sect. 2. WIGHTIA
    - Sect. 3. DISEMAEA
    - Sect. 4. PSEUDOSOPHORA
    - Sect. 5. HAMMERMANIA
    - Sect. 6. AMMOTHAMNUS
    - Sect. 7. SOPHORA
    - Sect. 8. EDWARDSIA
    - Sect. 9. KEYSERLINGIA

Fig. 1.9. Tribal Relationships in the Papilionoideae (From Polhill 1981).

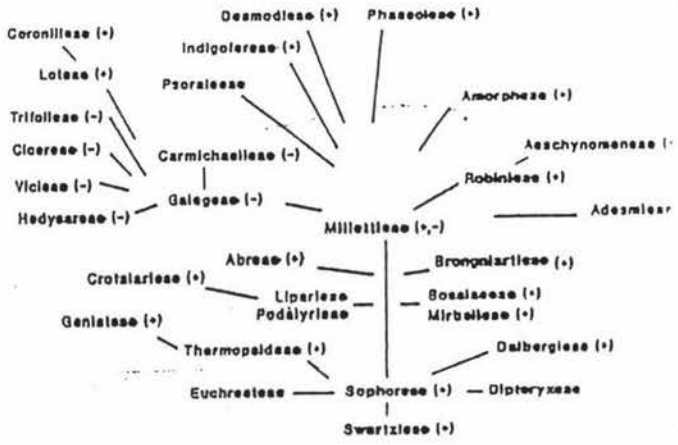


Fig. 1.10. Southern Hemisphere distribution of *Sophora* section Edwardsia. One Edwardsia species is also found in Hawai'i, located at 115°longitude: 20°N latitude.

1.) Lord Howe Island; 2.) New Zealand; 3.) Chatham Islands; 4.) Raivavae; 5.) Rapa; 6.) Marquesas; 7.) Easter Island; 8.) Masafuera; 9.) Masatierra, 10.) Chile; 11.) Gough Island; 12.) Reunion Island.

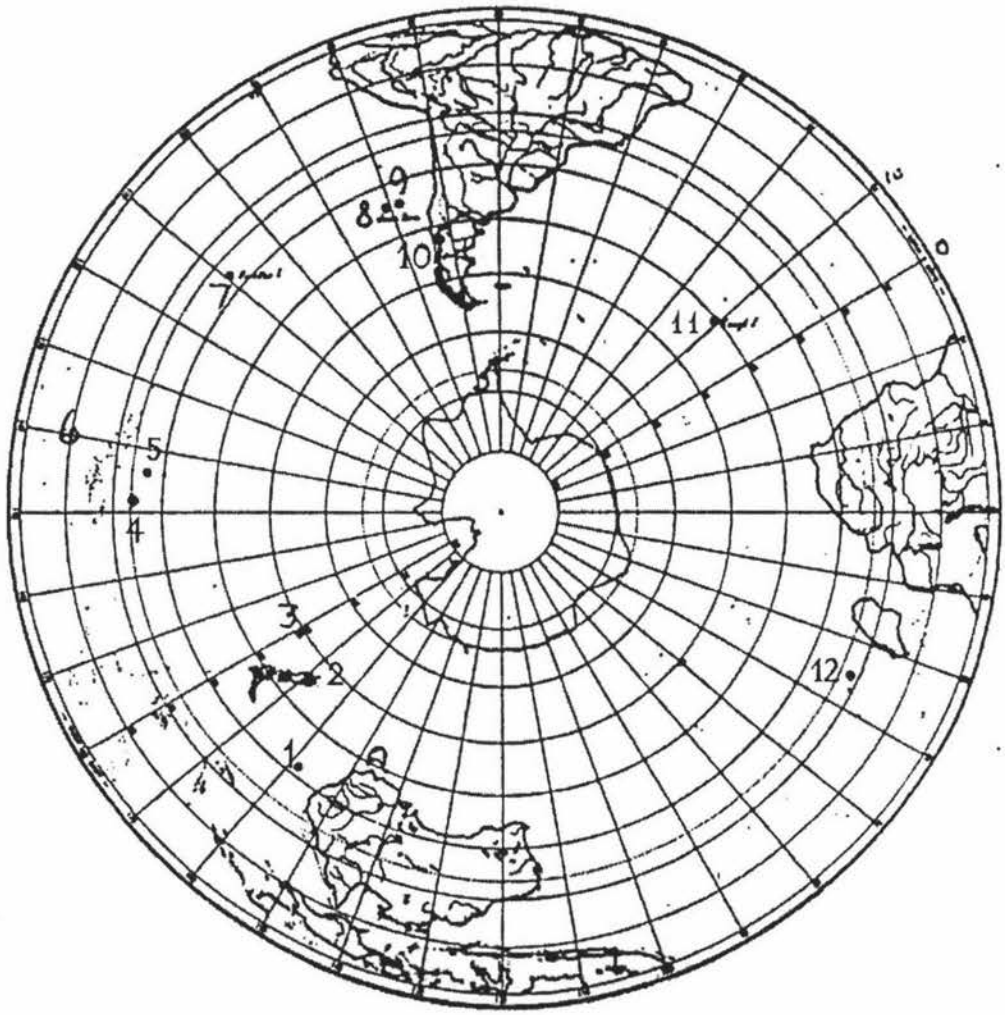


Table 1.3. Members of *Sophora* section Edwardsia, and their distribution. The symbol '≡' denotes where alternative nomenclature has been described.

Distribution	Edwardsia species	Reference.
Reunion & Mauritius	*Sophora denudata* a) *S. denudata* subsp. *denudata* b) *S. denudata* var. *sericea*	Bory, 1804 Yakovlev, 1972
Rapa	*Sophora rapaensis*	St. John, 1985.
Raivavae	*Sophora raivavaeensis*	St. John, 1985.
Mangareva Island	*Sophora mangarevaensis*	St. John, 1985.
Juan Fernandez	*Sophora fernandeziana*	Skottsberg, 1822
Juan Fernandez	*Sophora masafuerana*	Skottsberg, 1822
Easter Island	*Sophora toromiro*	Skottsberg, 1822
Hawai'i	*Sophora chrysophylla*	Chock, 1956
Lord Howe Island	*Sophora howinsula* ≡ *S. tetraptera* subsp. *howinsula*	Oliver, 1917
New Zealand	*Sophora microphylla* a) *S. microphylla* var. *fulvida* b) *S. microphylla* var. *longicarinata* c) *S. microphylla* var. *chathamica* d) *S. microphylla* var. *prostrata*	Ait, 1789 Allan, 1962 (Simpson) Allan, 1961 (Cockayne) Yakovelev, 1972 (Buchan.) Yakovlev, 1972
	Sophora prostrata ≡ *S. microphylla* var. *prostrata*	Godley, 1975
	Sophora tetraptera ≡ *S. tetraptera* subsp. *tetraptera*	Mill., 1780 Yakovlev, 1972
Gough Island	*Sophora microphylla*	Godley, 1975
Chile	*Sophora microphylla* ≡ *S. microphylla* var. *macnabiana*	(Grah.) Yakovlev, 1972
	Sophora macrocarpa	Rees, 1819
India?	*S. reediana*	.(Phil.) Yakovlev, 1967

Both *Sophora tetraptera* and *Sophora prostrata* readily hybridise with *Sophora microphylla*, and this may provide evidence for a relatively recent evolution in New Zealand taxa (Godley 1985). Cockayne (1912) noted the apparent similarity between the divaricating *Sophora prostrata* and the juvenile stage of *Sophora microphylla*, and suggested that it was a permanently neotonous form of the latter. Alternatively, Godley (1979, 1985) proposed that it is a distinct species which perhaps hybridized with *Sophora tetraptera* during the past to produce the intermediate-type swarm of *Sophora microphylla*. To test his argument, Godley has performed hybridization trials in the experimental gardens at Manaaki Whenua Landcare Research Inc., Christchurch (formerly Botany Division of DSIR.), and the F1 hybrids are still growing in the collection there.

New Zealand distribution of *Sophora*.

Both the distribution of genotypes and phenotypes of *Sophora* in New Zealand have attracted attention in previous evolutionary studies (*e.g.* Cockayne 1912; Godley 1985). The three New Zealand species occupy different geographical ranges, and are also differentiated morphologically and ecologically.

The North Island species, *Sophora tetraptera*, is found from East Cape to the Ruahine Mountains, and is regarded to have populations in Hawaii (Wagner *et al.* 1990), and in southern Chile, although Sykes and Godley (1968) consider this to be an incorrect identification. The tree species grows to about 14m, and is found in lowland and montane forest. Leaves are 7-15 cm long and pinnate, with 6-40 pairs of leaflets, and the yellow flowers are 4-5 cm long, produced during spring when the trees are often bare of leaves.

The endemic *Sophora prostrata* is occurs on the eastern side of the Main Divide, particularly around Banks Peninsula and the Canterbury foothills of the South Island. The seeds do not float in either fresh or sea water (Sykes & Godley 1968). It is an erect or prostrate, divaricating shrub, growing to two metres in height with densely twiggy and zigzag branchlets. These bear miniature leaves (8-12mm long) with 2-8 pairs of tiny leaflets. The yellow flowers are smaller than *S. tetraptera* (1-2 cm long).

Table 1.4. Fossil Pollen Records for *Sophora* in New Zealand. **Aranuian** = Postglacial, **Otiran** = Last Glacial, **Oturian** = Last Interglacial, **Hautawan to Castlecliffian** = Early Pleistocene, **Duntroonian** = Late Oligocene, **Kapitean to Opoitian** = Late Miocene to Early Pliocene. (Summary compiled by Dr J. Ian Raine, Paleontology Section, Institute of Geological & Nuclear Sciences Ltd).

FOSSIL LOCATION	NEW ZEALAND STAGES & RECORD FILE NUMBERS	POLLEN TYPE	IDENTIFIED BY
CHATHAM ISLANDS	Aranuian CH/f129 core 0.15-0.17 m	*Sophora tetraptera*	D.C. Mildenhall 1972
NORTHLAND	R12/f69; Hautawan to Castlecliffian	*Sophora*	D.C. Mildenhall 1990
	Otiran to Aranuian T11/f4 core;13.0-14.0 m	*Sophora*	D.C. Mildenhall 1976
WANGANUI	Aranuian R22/f85 core cuttings 14.0 m	?*Sophora*	D.C. Mildenhall 1983
WELLINGTON	Aranuian R26/f64 core 3.15 m	*Sophora*	D.C. Mildenhall 1976
	Aranuian R26/f65 core 2.85m	*Sophora*	D.C. Mildenhall 1976
	Aranuian R26/f67 core 2.25 m	*Sophora*	D.C. Mildenhall 1976
	Aranuian R26/f73 core 0.47 m	*Sophora*	D.C. Mildenhall 1976
	Aranuian R26/f75 core 0.25 m	*Sophora*	D.C. Mildenhall 1976
	Oturian R26/f7524	?*Sophora*	2 samples D.J. McIntyre & W.F. Harris 1961
CROMWELL GORGE	Aranuian G41/f28 exposure 0.0-0.10 m	*Sophora*	D.C. Mildenhall 1989
	Aranuian G41/f29 exposure 0.0-0.04 m	*Sophora*	D.C. Mildenhall 1989
	Aranuian G41/f29 exposure 0.18-0.24 m	*Sophora*	D.C. Mildenhall 1989
SOUTHLAND	Duntroonian F45/f8883	cf. *Sophora*	D.J. McIntyre 1961
	Kapitean to Opoitian F46/f8531	cf. *Sophora*	D.J. McIntyre 1960

Sophora microphylla overlaps the ranges of these other two species, and is found throughout the North Island with populations in the South Island and some offshore islands, as well as Pacific island, Chile, and Gough Island populations (Figure 1.9.). It is a heteroblastic tree species, possessing two distinct developmental stages. First, a flexous shrub with wiry, yellowish interlacing stems and a few small leaves, which resembles the divaricating shrub *Sophora prostrata*. From 2 to 3.5 metres in height, this is gradually replaced by the mature tree form, up to 10 m in height (Figure 1.10.). The yellow flowers are also smaller than *S. tetraptera* (2-3 cm long).

In New Zealand, *Sophora microphylla* demonstrates considerable phenotypic plasticity, and several well marked varieties of limited geographical distribution have been described, *eg*. var *fulvida* Allan, from the west coast near Auckland, and var. *longicarinata* (Simpson) Allan. from the Takaka area. Denny (1964) investigated populations of *Sophora microphylla* and found that the juveniles of the Canterbury plants were phenotypically more divaricating than the juveniles from the Auckland population. Her studies also found that the external environment is capable of altering the degree of divarication in juvenile plants of *Sophora microphylla*, with shaded conditions giving the least divarication while dryness and high light intensity both increased divarication. The distinctive juvenile stage is not present in offshore populations. Godley (1975) suggested that the variable degree of habit-heteroblasty, shown between different New Zealand and island populations in this species, is the result of hybrid interactions between the tree species, *Sophora tetraptera*, and the divaricating shrub, *Sophora prostrata*. He further suggests that if this idea is valid, it should apply to the origin of divaricating juvenile forms in general (Godley, 1979).

The hypotheses concerning the origin of divarication have also been discussed with respect to *Sophora prostrata* and *Sophora microphylla*. The climate hypothesis (Rattenbury 1979; McGlone & Webb 1981) predicts that the divaricating shrub and divaricating juvenile form, respectively, arose during the Pleistocene in response to inhospitable climatic conditions. Smaller leaves and reduced stature are considered to better adapt these plants to oscillating droughts and glacial conditions. The moa-browsing hypothesis (Greenwood & Atkinson 1977; Atkinson & Greenwood 1989) alternatively suggests that the divaricating form evolved in response to browsing pressure caused by moa, and point to the convergence among unrelated genera and absence of these forms on offshore islands as support for the hypothesis.

Fig. 1.11. Heteroblastic developmental change in *Sophora microphylla*, showing the remains of divaricate branching at the base, an intermediate pendulous stage, and adult fastigiate branching. (From Godley 1979).

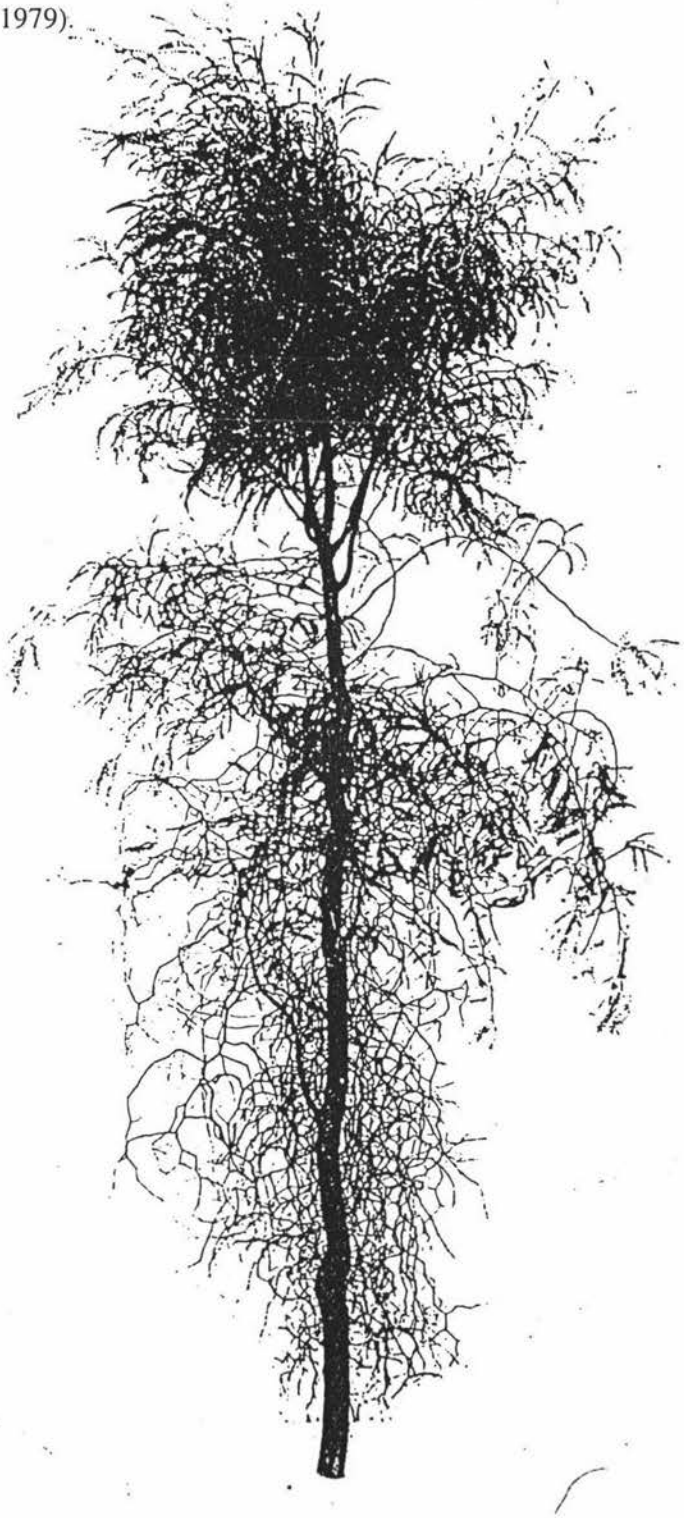


Fig. 1.12. Six-month old plants of *Sophora microphylla* showing presence or absence of divaricating juvenile form. Top (left to right): Cape Reinga; Mahurangi; north of Tongariro. Bottom (left to right): Chatham Island; Takaka (var. *longicarinata*); Herbert, North Otago. (From Godley 1979).

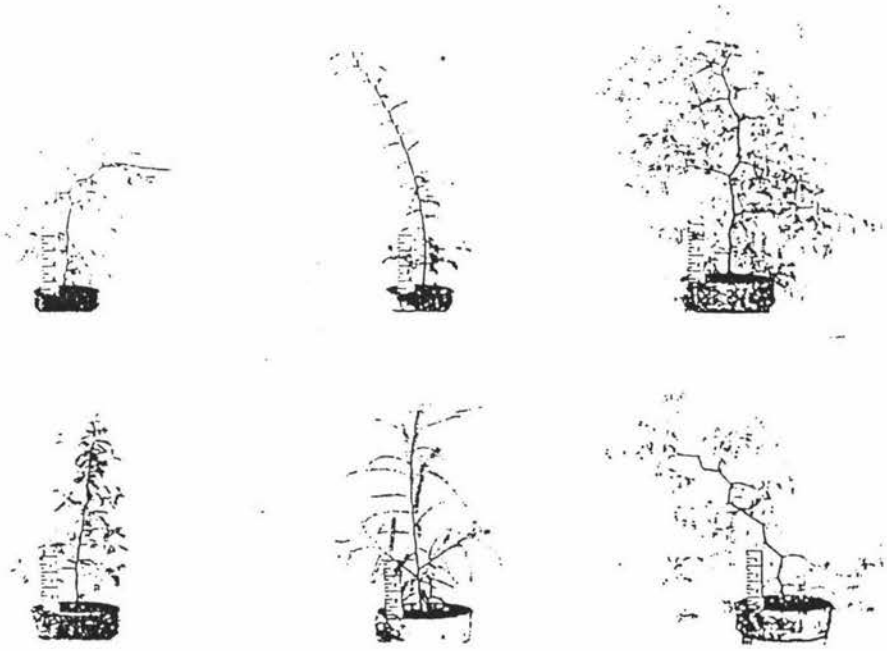
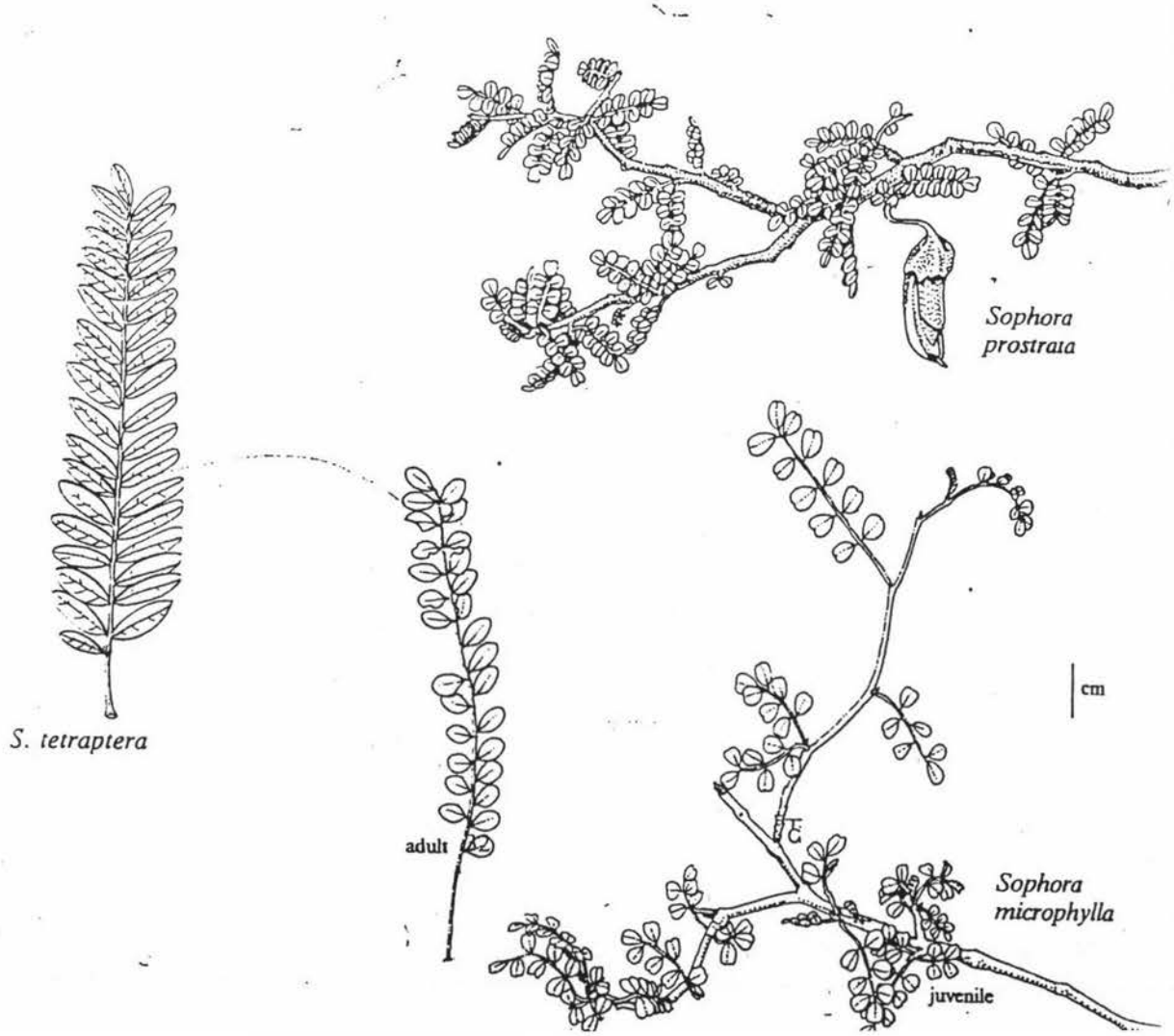


Fig. 1.13. The three New Zealand species of *Sophora*. (From Poole & Adams 1990).



Hypotheses for Testing the Origin and Evolution of New Zealand *Sophora*.

A phylogenetic tree is a hypothesis, and thus permits specific questions to be asked. These questions can now be rephrased around a molecular investigations framework. This thesis uses molecular data from New Zealand *Sophora* to reconstruct the branching order and edge lengths of the evolutionary tree.

Hypothesis One: Transoceanic Dispersal of Sophora to New Zealand.

The early fossil record and geographic distribution of *Sophora* suggests that they were not common before the middle Eocene, 50 million years ago. As the New Zealand landmass separated from Gondwana in the Late Cretaceous (80 Ma.), this evidence can be used as support for a long-distance migration of the genus to New Zealand. Seed buoyancy has been observed to be effective in the dispersal of *Sophora microphylla* to islands in the Pacific and Chile (Sykes & Godley 1968), and is hypothesised to be the main agent of dispersal in the Edwardsia.

Hypothesis Two: Miocene Dispersal of Sophora tetraptera to New Zealand.

The Antarctic circum-polar current, and the Westerly Winds that drive it, were formed by the end of the Oligocene, and have assisted in the east to west dispersal of many plant groups in the southern hemisphere (Fleming 1962). It is probable that the fossil record underestimates the time of origin of *Sophora* in New Zealand, and an origin as early as the Miocene is proposed. *Sophora tetraptera* has seeds that float in both fresh and salt water, and is considered to be the most ancestral taxon in New Zealand.

Hypothesis Three: New Zealand Origin and Diversification of Sophora microphylla.

The species *Sophora microphylla* is considered to have evolved in New Zealand, and subsequently dispersed to other islands in the Pacific and Atlantic oceans, as well as Chile. If this hypothesis is correct, phylogenetic trees would place the New Zealand populations basal to the offshore populations. Further, if *S. microphylla* arose from a hybridization involving *S. tetraptera* and *S. prostrata*, then we might expect the latter two species to have evolved earlier on the phylogenetic tree.

Hypothesis Four: Pliocene Origin of Sophora prostrata.

Sophora prostrata is endemic to New Zealand, and has seeds that do not float in either fresh or sea water. In this respect, it agrees with the records for *Sophora chrysophylla*, an upland Hawaiian species (1965, 1970; Godley 1985). Clearly, the divergence time of *Sophora prostrata* from other New Zealand species is important in determining its nearest relative, as well as for investigating the possible evolutionary causes of divarication. If moa were the dominant selective force, then the divergence times of divaricating plants might be expected to reflect the times of radiation of the moa (*ie.* from about 20 million years bp. Cooper *et al.* 1992). Alternatively, if climatic factors were significant, more recent divergence times associated with the beginning of the Quarternary, about 1 million years ago, might be expected.

This thesis uses the polymerase chain reaction (PCR) to amplify a specific fragment of chloroplast DNA from *Sophora* leaf material. The sequences of the DNA fragments are then determined and analyzed to make inferences about the evolutionary relationships between New Zealand and offshore populations of *Sophora*. While the principle of PCR is elegantly simple (Saiki *et al.* 1988, Arnheim *et al.* 1990), there can be several difficulties in its implementation. The sensitivity of PCR and therefore the possibility of amplifying the wrong DNA make it necessary to check the accuracy and identity of the DNA sequences. Advances in automated PCR technology are being made, but it may still be several years before reliable sequence data can be obtained. The procedures used to extract, amplify and sequence chloroplast *atpB-B*er sequences are described in this chapter, along with steps taken to ensure the correct identity and accuracy of the sequences.

Total cellular DNAs were isolated from 12 *Sophora* taxa and two legume outgroups, *Clianthus* and *Carmichaelia* by various modifications of a simple CTAB method (Doyle & Doyle 1987). Sequenced taxa, and their collection and taxonomic authorities for samples (if present) are given in Table 2.1. Three different sections of *Sophora* were surveyed, but sampling emphasized Edwardsia. Of the 17 currently recognised species and subspecies of that section, 11 were sampled, and included six different populations of the widely dispersed *S. microphylla*. Within Edwardsia, sampling was most extensive within New Zealand and allied species (Figs. 2.1 & 2.2).

The Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a simple and rapid means of amplifying specific regions of DNA from tissues, cells or other sources. Its versatility and relatively low cost make it suitable for the investigation of a variety of problems in biogeography and evolutionary biology (Arnheim *et al.*, 1990). The basic procedure starts with two short pieces (about 15 to 30 nucleotides long) of single-stranded DNA - the primers. These need sufficient sequence specificity to bind to the DNA tagging the region of interest; one primer for each of the complementary DNA strands. The first step in the polymerase chain reaction is a high temperature (usually between 90 and 94°C) which denatures the DNA molecule and separates the strands. This allows the primers access to

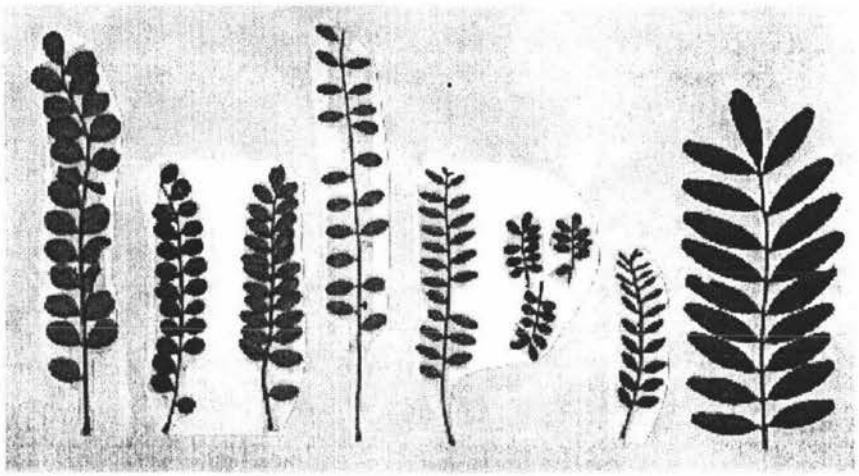
Table 2.1. Legume taxa from which chloroplast DNA sequences were obtained. Manaaki Whenua Landcare Research collection (Christchurch, NZ) and taxonomic authority is given for taxa A-I.

Taxon	Locality	Specimen obtained from:
Sophora microphylla	Gough Island, Sth. Atlantic	* A
S. microphylla	Chile	* B
S. microphylla	Chathams Island	* C
S. microphylla	Waiau, Canterbury	* D
S. microphylla	Stevens Island	* E
S. microphylla	Hen Island, Nth. Island	* H
S. howinsula	Lord Howe Island	* G
S. prostrata	Waiau, Canterbury	* I
S. tetraptera	Hawkes Bay, Auckland	* F
S. raivavaeensis	Raivavae, Austral Ridge	Peter Heenan, Landcare CHCH.
S. japonica	Japan	Massey University.
S. tomentosa	Port Vila, Vanuatu	Curry & Love, Vanuatu.
Carmichaelia arborea	NW Nelson to Stewart Island.	Massey University.
Clianthus puniceus	East Cape-Lk. Waikaremoana	Garden cultivar.

Overleaf: **Fig. 2.1** Map of New Zealand showing geographical location of *Sophora* samples.

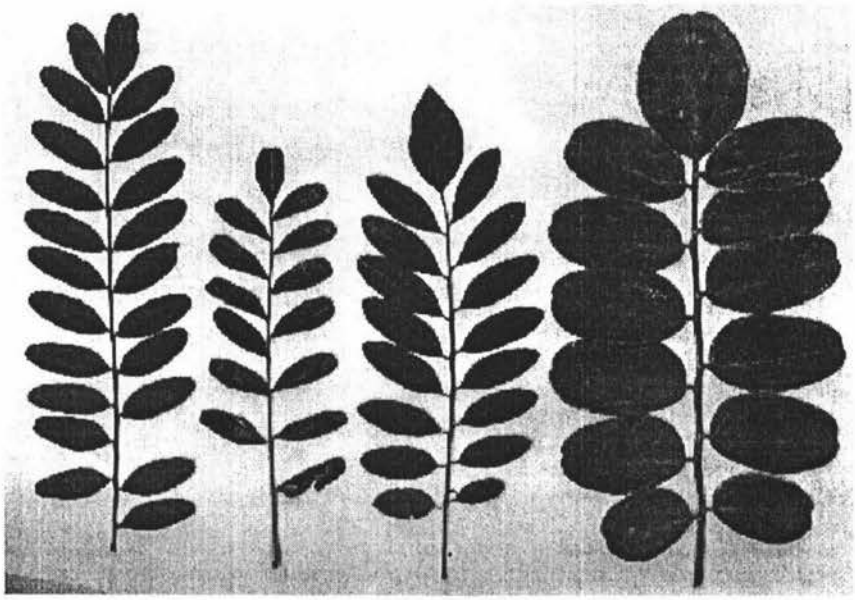

N
Hen & Chicken Island, Northland
Hawkes Bay
Stevens Island
Waiau, Canterbury
0
100 miles

Fig. 2.2 Typical leaves of *Sophora* species and populations sequenced in this study.



A B C D E I H G

A) *Sophora microphylla* (Gough Island); B) *S. microphylla* (Chile); C) *S. microphylla* (Chatham Islands); D) *S. microphylla* (Waiau, Canterbury); E) *S. microphylla* (Stevens Island); I) *Sophora prostrata* (Waiau, Canterbury); H) *S. microphylla* (Hen & Chickens Island, Northland); G) *Sophora howinsula* (Lord Howe Island).



F J K L

F) *Sophora tetraptera* (Hawkes Bay); J) *Sophora raivavaeensis* (Raivavae); K) *Sophora japonica* (Japan); L) *Sophora tomentosa* (Port Vila, Vanuatu).

their complementary binding sites. Rapid lowering of the temperature (to between 37 and 60°C - depending on the specificity of the primers) in the second stage permits the primers to bind with the complementary sequence on the template DNA. In the presence of the four nucleotide triphosphates the region of DNA between the pair of primers can be copied using a heat stable DNA polymerase, such as *Taq* polymerase (Saiki *et al.* 1988). After one round of amplification, the reaction mix is denatured through heating and allowed to reanneal with excess primer, and the polymerase reaction is carried out a second time. Repeated cycling of these series of reactions results in a geometric increase of the sequence bounded by the initial primers.

Having appropriate DNA primers is critical to the success of PCR (Kitchin *et al.* 1990). This information is obtained either by conventional cloning and sequencing of the region of interest, or by using information held in DNA sequence databases such as Genbank and EMBL. Highly conserved regions of DNA - such as the large subunit of Rubisco (*rbcL*) of the chloroplast genome - can be isolated from a wide range of plants using "universal primers" (*eg.* Zurawski & Clegg 1987), but faster evolving regions of DNA require more taxon-specific sequence data. This can pose difficulties for groups that have been less well characterized at the molecular level; for instance many plant groups.

Two difficulties of PCR is the risk of contamination by other tissue or DNA, and the requirement of DNA free from contaminating molecules, such as proteins and other secondary compounds. PCR's extreme sensitivity means that even slight contamination from another DNA source can lead to the amplification of the wrong sample, and extreme care must therefore be taken to ensure that the working area is "hygienic" in this respect. Some plant species may incorporate secondary metabolites in their tissues as they mature, and this can have a negative effect on the amplification process. In these circumstances, a clean-up step may be required to reduce the concentration of contaminants. If this fails to yield clean DNA, then young or seedling tissues can be tried, as these may not yet have incorporated the same levels of contaminants as more mature tissues.

Amplification of DNA by the Polymerase Chain Reaction.

The target chloroplast fragments were isolated during the PCR cycle using the specifically designed amplification primers;

BO1 5′ CACTCATAgCTACAgCTCTAATTC 3′

BO2 5′ TCTTTAACACCAgCTTTgAACCCAA 3′

which isolated an approximately 1000 nucleotide long region between the *atpB* and *rbcL* genes (Figure 2.3).

DNA amplifications were performed in 0.5 ml reaction tubes using a DNA thermal cycler (Corbett Research Ltd.). Reactions contained 2µl of pure DNA, 2µl of reaction buffer (Promega Corporation, Madison, USA; 50mM Kcl, 10 mM Tris-HCl, pH 8.8, 0.1% Triton X-100), 1-6 µl $MgCl_2$ (depending on the concentration of DNA), 2µl of each deoxynucleotide (dNTPS - 1mM), 1µl of each primer (1mM), and 0.5µl Taq polymerase. The reaction was made to a total volume of 20µl with sterile milliQ water (dH_2O).

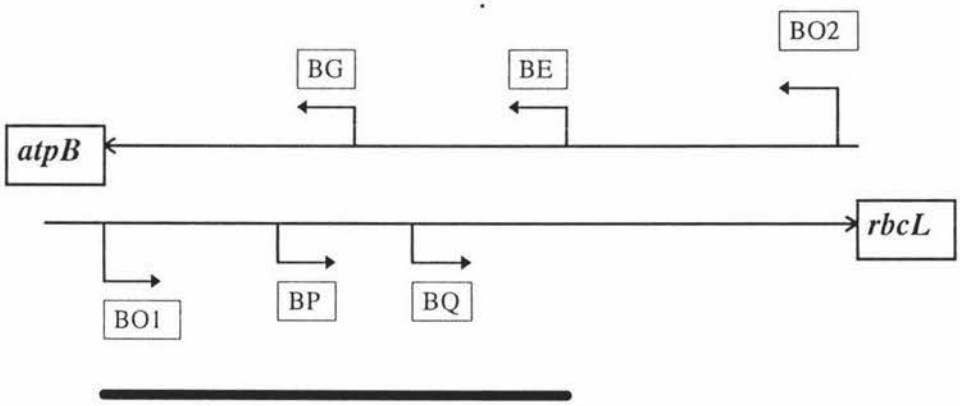
Several factors influenced the success of the PCR (including the quality and quantity of the template DNA, primer specificity and concentration, magnesium concentration, and the type of thermal cycler used). A series of reactions was often required to fine-tune the amplification mixtures for each sample, particularly to adjust the volume of $MgCl_2$ depending on the final quantity of pure DNA. Various temperature and time settings need to be trialed to obtain the optimal specificity and amplification of the product (Innis & Gelfand 1990). A variety of PCR conditions was tried, and good amplifications for the primers and kowhai DNA were obtained using the following parameters:

95°C for 60 seconds to separate DNA strands,

55°C for 60 seconds to allow primer annealing, and

72°C for 120 seconds for DNA copying.

Fig. 2.3 The approximate location of primers used in this study. The length of sequence between the two genes *atpB* and *rbcL* is approximately 1000 base pairs, and of this 647 base pairs were sequenced between the gene *atpB* and the internal primer *Be*, shown by the line in bold. Direction of amplification proceeds from 5 to 3' ends of the DNA strands, as indicated by the direction of the arrows.



The cycle was repeated 45 times. Tubes were then cooled to 4°C using the PCR machine soak file, and were removed to the fridge. A small amount of each sample was then run on an mini-gel with a DNA ladder to determine that a 1000 bp fragment had been retrieved.

The PCR sample was purified ready for direct sequencing using Magic PCR Mini Preps ™ mini-columns. One hundred microlitres of direct purification buffer was added to the template DNA (approximately 100µl). This was mixed for 1 minute with 1 ml of DNA purification resin and then passed through the mini-column using a syringe. The column was washed with 2 ml 80% isopropanol. Excess alcohol was removed by centrifugation for 20 seconds. Fifty microlitres of dH_2O was then added to the mini-column and the DNA allowed to resuspend for 1 minute. The sample was then collected by centrifugation for 20 seconds. Two microlitres of the purified product was then checked on a mini-gel prior to sequencing. Recovery of the PCR product using the mini-columns was consistent and high.

Five primers were used for sequencing. One was the external *atpB* primer, and four additional primers were designed in the intergene region between the genes *atpb* and *rbcL*. The amplification primer *Be* was designed from the alignment of sequences from a range of dicotyledons, and occurred at about 647 base pairs from the *atpB* gene (see Zurawski and Clegg 1987; Zurawski *et al.* 1984). The other three internal primers were designed during the study as sequence data from the *kowhai* became available, and were dispersed at intervals between the primers *BO1* and *Be* to enable sequencing in both directions across this region (Figure 2.3.).

The oligonucleotide sequences of the four internal sequencing primers are;

BE	5′	ATgTTgTATATgTAAATCC	3′
BG	5′	gAATTggTTggATggTACC	3′
BQ	5′	gTTAATTCAATAATAAATggg	3′
BP	5′	gTggTAggATTTATTCTCAT	3′

Direct Sequencing of PCR Products

A single-stranded sequencing procedure was used, limiting one primer in the amplifiction reaction (*eg.* Gyllenstein & Erlich 1988), using Taq DNA polymerase and ^{33}P as the radiolabel source. One microlitre of purified DNA template was added to a cocktail containing 10 ng of one primer, 4µl of cycling mix (buffer and Taq polymerase), 0.5µl of ^{33}P isotope, and made up to a volume of 30µl with dH_2O. This was mixed by flicking and briefly spun in the microcentrifuge a few times to ensure proper mixing, and four equal proportions were pipetted into each of four 0.5 ml PCR tubes containing 2µl of the four terminal dideoxynuucleotides. Termination reactions were run for 25 cycles at the following temperatures;

94°C for 30secs.

45-50°C for 30secs, depending on the binder-specificity.

70°C for 60secs.

Four microlitres of stop solution were added to each tube at the end of the reaction. Sequencing reactions were run on 15% acrylamide gels, 8M urea, then dried, and exposed to Kodak X-omat AR film (Eastman Kodak Co., Rochester, New York, USA).

Methods of Tree Reconstruction.

Sequences can aligned and compared using various multiple alignment programs such as Clustal V, Clustal W, and PREPARE (*eg.* Higgins *et al.* 1991; Thompson *et al.* 1994). This is obtained by inserting gaps, corresponding to insertions or deletions, into one or more of the sequences in order to place positions inferred to be homologous into the same column of the data matrix.

In addition to positional homology, analysis of sequence data also requires the determination of character polarities in order to produce a rooted phylogenetic tree. Character polarity is inferred using the method of outgroup comparison, and the location at which the outgroup joins the unrooted tree implies a root with respect to the ingroup taxa. The assignment of taxa to the outgroup constitutes an assumption that the remaining taxa are monophyletic, however, and if this assumption is wrong, the tree will be rooted incorrectly (Swofford *et al.* 1996).

Different phylogenetic analysis algorithms use different subsets of the information contained within aligned sequences, and have different optimality criteria for evaluating it and constructing evolutionary trees (see Swofford *et al.*, 1996; Nei 1991; and Penny *et al.* 1992 for reviews).

Hadamard Method.

The Hadamard conjugation model has three essential elements (Penny *et al.* 1992, 1993), namely, a tree, a mechanism for sequence change, and probabilities of changes (lengths) along branches of the tree. Hendy, Penny and Steel have worked out that an invertible relationships exists between patterns in the data, the paths between taxa and the edges in reconstucted trees. Using Hadamard matrices you can add and subtract paths to get values for edges (or patterns). Similarly you can add and subtract the patterns (edges) to get values for paths. If paths are corrected for multiple substitutions however, "symmetric correction" formulae must be used, as only these are invertible. Application of this method gives a "spectrum" which provides a quantitative measure of conflicting signals in the data which can support different phylogenetic hypotheses.

There are two principle programs for the Hadamard conjugation:

PREPARE - which reads the sequences in a variety of forms (interleaved, block, Nexus), and can calculate base composition, and frequencies of patterns in the sequences. Output files can be prepared for analysis by Hadtree, PAUP*, and PHYLIP.

HADTREE - reads the partition frequency data from Prepare, corrects for multiple changes (using Cavender's 1978 model), and searches for an optimal tree using the closest tree criterion (Hendy & Penny 1989). It also has the ability to use other optimality criteria, such as parsimony.

Maximum Parsimony.

Parsimony refers to the general notion that simplert hypotheses are preferable to more comlicated ones, and that *ad hoc* hypotheses should be avoided whenever possible. Methods for estimating trees under the criterion of parsimony equate "simplicity" with the explanation of attributes shared among taxa as due to their inheritance from a common ancestor. In general, parsimony methods operate by selecting trees that minimise the total tree length: the number of evolutionary steps (transformations of one character state to another) required to explain a given set of data. When character conflicts occur, however, *ad hoc* hypotheses cannot be avoided if the observed character distributions are to be explained, and assumptions of homoplasy (convergence, parallelism, or reversal) must be invoked (Swofford *et al.* 1996).

Maximum Likelihood.

Maximum likelihood methods of phylogenetic inference evaluate a hypothesis about evolutionary history in terms of the probability that a proposed model of the evolutionary process and the hypothesized history would give rise to the observed data (Swofford *et al.* 1996). In addition to its consistency properties, maximum likelihood is useful because it often yields estimates taht have a lower variance than other methods (*ie*, it is frequently the estimation method least affected by sampling error). It also compensates for superimposed changes in the data. To calculate the likelihood of a full tree, it is necessary to calculate the likelihoods of the occurrence of each state at each node in the tree, and then to combine the likelihood of each of its daughter trees (*ie*, descendant lineages).

Searching for Optimal Trees.

For data sets of small to moderate size (8-20 taxa depending on the criterion), exact methods that guarantee the discovery of all optimal trees may be used. For larger data ets, exact solutions require a prohibitve amount of computing time; consequently, approximate methods that do not guarantee optimality must be used (Swofford *et al.* 1996). The Branch-and-Bound method was first applied to evolutionary trees by Hendy and Penny (1982), and differs from exhaustive searches by quickly eliminating parts of the search tree that only contain suboptimal solutions. This method can be used to search for optimal trees under parsimony, maximum likelihood, and additive distance criteria in programs such as PAUP* (Swofford 1996).

Aligned sequences contain sites of variability, the patterns of which can be evaluated by clustering or phylogenetic algorithms. Different phylogenetic algorithms use different subsets of the information contained within aligned sequences, and have different optimality criteria for evaluating it and constructing evolutionary trees (Swofford *et al.*, 1996). This thesis uses observed nucleotide data of *Sophora*; the unknowns are the branching order and branch lengths of the tree. The signals in the data are examined in this chapter using several methods of analysis, including the Hadamard conjugation method for spectral analysis (Hendy & Penny 1993), as well as parsimony, neighbour-joining (Saitou & Nei 1987), and maximum likelihood within the PAUP* package (Swofford 1996). Divergence times of Edwardsia taxa are estimated by use of the molecular clock (Zuckerkandl & Pauling 1965).

The CTAB extraction procedure worked well with both fresh and silica dried leaf material from a number of different taxa, however in some species (*e.g. Sophora tetraptera* & *Sophora tomentosa*) only the use of fresh seedling tissue yielded clean DNA, and an additional clean-up step was necessary. High molecular weight chloroplast DNA was obtained, and most species provided suitable substrates for PCR amplification. For a few species, however, the incorporation of secondary metabolites, phenolics and other contaminating compounds, resulted in a yield of discoloured DNA, which subsequently proved impossible to amplify using the PCR. Chloroplast DNA from *Sophora tetraptera* and *Sophora tomentosa* were particularly "grubby" in this respect. Additional problems were encountered for *Sophora microphylla* var. *fulvida, S. microphylla* var. *longicarinata*, *S. toromiro* and *S. chrysophylla*, which did not amplify well under the series of PCR reaction trials, despite yielding relatively clean DNA.

The very sensitivity of the PCR makes the procedure vulnerable to the amplification of contaminating DNA (Kitchin *et al.* 1990). When this study was begun no prior sequencing of plants had been done in the laboratory area, so contamination from other plant groups was extremely unlikely. However, the risk of cross-contamination between *kowhai* samples was very real, and every effort was maintained to keep the working area hygienic in this respect.

Alignment of the *Sophora atpB-Be* sequences were made using the multiple alignment program Clustal V (Higgins & Bleasby 1991). The partial sequences of two further outgroup papilionoid taxa were obtained from Genbank datalibrary (*Pisum sativum, Vicia faba*), and were included in the alignment. Amplification of contaminating, non-legume DNA template was discounted by the consistency of the *Sophora* DNA sequences, and by alignment with these Genbank sequences. Cross-contamination between different *Sophora* samples was also rejected because the same sequence was obtained from separate preparations of the same DNA sample, and from separate amplifications of the same DNA sample. The number of base pair differences between taxa also confirmed that the correct sequences were obtained.

The 647 base pair region of cpDNA, between the gene *atpB* and intergene *rbcL* promoter region, *Be*, was aligned for 12 species and populations of *Sophora* and the outgroup genera *Carmichaelia* and *Clianthus* (Figure 3.1). The partial sequences of two additional outgroup legume taxa were also included in the alignment (*Pisum sativum*, *Vicia faba*), obtained from the Genbank sequence Database. These were used to confirm that the correct region was being obtained from sequencing, but were not included in most of the following phylogenetic analyses because they did not contribute further informative comparisons with the section Edwardsia.

Variable Sites in the *Sophora* Data Set.

Percentage similarity values for each pairwise comparison was obtained from the number of differences between each pair, divided by the total number (555); these are called Hamming distances. This value is then multiplied by 100 to give % similarity (Table 3.1.). The table shows that high levels of sequence similarity exist between all of the taxa investigated in this study, particularly among Edwardsia taxa (with values ranging from 97.6- 100%). No sequence differences were found between *Sophora prostrata* and the Gough Island population of *Sophora microphylla* (Table 3.2). Edwardsia taxa collectively share strongest sequence similarity to *Sophora tomentosa* (mean 95.6%), and interestingly, they are next most similar with the outgroup genus *Clianthus* than *Sophora japonica* (mean values 90.4% and 86.6%, respectively). This latter result appears to be in conflict with a monophyletic arrangement of taxa in the genus *Sophora* and is discussed again later. Convergence (or parallel evolution) among distantly related taxa can provide conflicting signals which prevent taxon from being placed into mutually exclusive groups. However, generally regions in the range of 1000 base pairs are preferred for

statistical significance, as the sampling error associated with obtaining short regions of sequence DNA are likely to be large. Caution must be exercised in the interpretation of phylogenetic relationships based on a small data set, especially when little information from other regions of chloroplast and/or nuclear genomic sequences is available.

There are 134 variable sites across the *Sophora* sequence data set, of which 61 are insertion/deletion events. Many of these events appear to be the result of "slip-strand mispairing" - where short repetitive regions of the DNA are gained or lost. Examples of this are the 6 bp insertion 'CCAGAA' in *Sophora japonica* at position 199-204 in the sequence data set, and the 3 bp deletion 'AAG' in *S. japonica* at position 455-457 in the data. The exact nucleotide sequence for each of these events are repeated in the region immediately downstream (Fig. 3.1.). Of particular interest is the 6 bp deletion of 'ATATAT' found in three of the Edwardsia taxa, *Sophora howinsula, S. tetraptera*, and the Canterbury population of *S. microphylla*, at position 231-236 in the data sequence (Fig. 3.1). This deletion event does not appear to be related to slip-strand mispairing, as flanking sequences immediately up- and downstream are dissimilar. Because a proper treatment is not obvious, sequence positions with gaps are usually omitted from analyses (Swofford *et al.* 1996).

The remainder of variable sites are substitution events, including both transitions (A/T, C/G) and transversions (AT/GC). Fifty-six of these substitutions are singletons, where one taxa differs from all the rest at that particular site. Singletons are useful for estimating rates of evolution, and are therefore used when compensating for multiple changes before selecting a tree. Such columns are not used by some tree selection criteria (such as parsimony and minimal evolution) when actually selecting the tree. When all non-parsimony sites (constant columns, insertion/deletion events and singletons) are removed from the *Sophora* data set, a subset of only 17 sites are left (Table 3.2). These parsimony sites amount to only 3% of the data sequenced for this study, and are therefore expected to provide low statistical support for following phylogenetic analyses.

Aligned sequences used for analysis.

columns 1 - 90

```
Canterbury  ?????????? ?????????? ????????AC AAGTAACGTT AATTTGTTGA CCAATAGTAT CTTGGCCCTT -AACTACTAG AGCATTGTAA
S. howinsu  ?????????? ?????????? ???????CAC AAGTAACGTT AATTTGTTGA CCAATAGTAT CTTGGCCCTT -AACTACTAG AGCATTGTAA
Chatham Is  ?????????? ?????????? ?????????? AAGTAACGTT AATTTGTTGA CCAATAGTAT CTTGGCCCTT -AACTACTAG AGCATTGTAA
Chile micr  ?????????? ?????????? ?????????? AAGTAACGTT AATTTGTTGA CCAATAGTAT CTTGGCCCTT -AACTACTAG AGCATTGTAA
S. tetrapt  ?????????? ?????????? ?????????? AAGTAACGTT AATTTGTTGA CCAATAGTAT CTTGGCCCTT -AACTACTAG AGCATTGTAA
S. prostra  ?????????? ?????????? ?????????C AAGTAACGTT AATTTGTTGA CCAATAGTAT CTTGGCCCTT -AACTACTAG AGCATTGTAA
Stevens Is  ?????????? ?????????? ?????????? AAGTAACGTT AATTTGTTGA CCAATAGTAT CTTGGCCCTT -AACTACTAG AGCATTGTAA
Gough Isla  ?????????? ?????????? ????????AC AAGTAACGTT AATTTGTTGA CCAATAGTAT CTTGGCCCTT -AACTACTAG AGCATTGTAA
Northland   ?????????? ?????????? ?????????? ?AGTAACGTT AATTTGTTGA CCAATAGTAT CTTGGCCCTT -AACTACTAG AGCATTGTAA
S. raivava  ?????????? ?????????? ?????????? ??GTAACGTT AATTTGTTGA CCAATAGTAT CTTGGCCCTT -AACTACTAG AGCATTGTAA
S. tomento  ?????????? ?????????? ?????????? ???TAACGTT AATTTGTTGA CCAATAGTAT CTTGACCCTT -AACTACCAG AGCATTGTAA
S. japonic  ?????????? ?????????? ?????????? ?????????? ????????GA CCAACAGTAT CTCGACCATT -AACTACCAG AGCGTTGTAA
Clianthus   ?????????? ?????????? ?????????? ?????????? ?????????? ?CAACAGGAT CTAGACCTTT -CACTACCAG AGCGTTGTAA
Carmichael  ?????????? ?????????? ?????????? ?????????? ?????????? ?CAACAGGAT CTAGACCTTT -CACTACCAG AGCATTGTAA
Vicia faba  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Pisum sati  GATTATTTCC TAATAATTGC TGTACTTCAC AAGTTACGTT AATTTGTTTG CCAACCGTAT CTCGACCTTT GAACTATCAG AGCGTTGTAA
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columns 91 - 180

```
Canterbury  ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
S. howinsu  ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
Chatham Is  ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
Chile micr  ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
S. tetrapt  ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
S. prostra  ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
Stevens Is  ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
Gough Isla  ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
Northland   ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
S. raivava  ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
S. tomento  ATATTAGGCA TCTTCCCTGG AGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCT GGTTTTTTTT TTCAAGCGCA
S. japonic  ATATTGGGCA TCTTCCCTGG GGGAAAAGCT ACATCCAGTA CCGGACCAAT TATTTGAGCG ATACGTCCCA GGTTTTTTTT TTCAAGCGCA
Clianthus   ATATTAGGCA TCTTCCCAGG TGGAAAAGCT ACATCGAGTA CCGGACCAAT TATTTGGGAG ATACGCCCCA GATTTTTGTT TTCAAGTGCA
Carmichael  ATATTAGGCA TCTTCCCTGG TGGAAAAGCT ACATCGAGTA CCGGACCAAT TATTTGGGAG ATACGCCCCA GATTTTTGTT TTCAAGTGCA
Vicia faba  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Pisum sati  ATATAAGGCA TCTTCCCTGG TGGAAAAACT ACATCGAGTA CCGGACCGAT TATTTGCGTG ATACGCCCCA GATTTTTGTT TTCAAGTACA
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columns 181 - 270

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Canterbury  GAAACTTGGG GACCAGAA-- ----GTGGTA GGATTTATTC TCATATTAAA ------CCA- ------TTTT TTTTCTAAAA TTTTGGAAAT
S. howinsu  GAAACTTGGG GACCAGAA-- ----GTGGTA GGATTTATTC TCATATTAAA ------CCA- ------TTTT TTTTCTAAAA TTTTGGAAAT
Chatham Is  GAAACTTGGG GACCAGAA-- ----GTGGTA GGATTTATTC TCATATTAAA ATATATCCA- -----TTTTT TTTTCTAAAA TTTTGGAAAT
Chile micr  GAAACTTGGG GACCAGAA-- ----GTGGTA GGATTTATTC TCATATTAAA ATATATCCA- -----TTTTT TTTTCTAAAA TTTTGGAAAT
S. tetrapt  GAAACTTGGG GACCAGAA-- ----GTGGTA GGATTTATTC TCATATTAAA ------CCA- ------TTTT TTTTCTAAAA TTTTGGAAAT
S. prostra  GAAACTTGGG GACCAGAA-- ----GTGGTA GGATTTATTC TCATATTAAA ATATATCCA- -----TTTTT TTTTCTAAAA TTTTGGAAAT
Stevens Is  GAAACTTGGG GACCAGAA-- ----GTGGTA GGATTTATTC TCATATTAAA ATATATCCA- -----TTTTT TTTTCTAAAA TTTTGGAAAT
Gough Isla  GAAACTTGGG GACCAGAA-- ----GTGGTA GGATTTATTC TCATATTAAA ATATATCCA- -----TTTTT TTTTCTAAAA TTTTGGAAAT
Northland   GAAACTTGGG GACCAGAA-- ----GTGGTA GGATTTATTC TCATATTAAA ATATATCCA- -----TTTTT TTTTCTAAAA TTTTGGAAAT
S. raivava  GAAACTTGGG GACCAGAA-- ----GTGGTA GGATTTATTC TCATATTAAA ATATATCCA- -----TTTTT TTTTCTAAAA TTTTGGAAAT
S. tomento  GAAACTTGGG TACCAGAACC AGAAGTGGTA GGATTTATTC TCATATTAAA ATATATCCA- -----TTTTT TTTTCTAAAA TTTTGGAAAT
S. japonic  GAAACCTCAG GACCAGAA-- ----GTGGTA GGATTTATTC TCATACTAAA ATATATCCA- -----TTTTT TTTTCGAAAA TTTT-GAAAT
Clianthus   GAAATCTCAG TATCAGA--- -------G-- TCAGTTATTC TCATATTAAA ATCTATCCGT TTTTTTTTTT TTTTCTAAAA TTT-GGAAAT
Carmichael  GAAATCTCAG TATCAGA--- -------G-- TCAGTTATTC TC-TATTAAA ATCTATCCGT TTTTTTTTTT TTTTCTAAAA TTT-GGAAAT
Vicia faba  ?????????? ?????????? ?????????A GGAGTTATTG TCATATTAAA AAATATCCA- ----TTTTTT TGAAAAAAAA AAATTGAAAT
Pisum sati  GAAACCTCAG TATCAGAA-- ----GGGGGA GGAGTTATTG TCAT?????? ?????????? ?????????? ?????????? ??????????
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columns 271 - 360

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Canterbury  CAAAAATAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
S. howinsu  CAAAAATAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
Chatham Is  CAAAAATAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA.---------- ---------- ---------- --ATAAAT--
Chile micr  CAAAAATAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
S. tetrapt  CAAAAATAGA A-TGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
S. prostra  CAAAAATAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
Stevens Is  CAAAAATAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
Gough Isla  CAAAAATAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
Northland   CAAAAATAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
S. raivava  CAAAAATAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
S. tomento  CAAAAAGAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
S. japonic  AAAAAAGA-- AATGTTCGAT AACAAAGCAA GTTGATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAATAA
Clianthus   CAAAAATAGA AATGTTCGAT AACAAAGCAA GTTAATCGGT TAATTCAATA ---------- ---------- ---------- --ATAAAT--
Carmichael  CTGGAAAAAA AATGTTCGA? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Vicia faba  TAAGAAAAAA -CTGTTTGAT AACAAAGCAA GTTGATCGGT TAATATTCTT AATTTTCTTA ATATTTAATA ATATATAAAA GAATAAAT--
Pisum sati  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
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columns 361 - 450

```
Canterbury  ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAATTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
S. howinsu  ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAATTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
Chatham Is  ------GGGA GTT-AGTATT CTATTTTGTT GGTACCATCC AACCAATTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
Chile micr  ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAATTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
S. tetrapt  ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAATTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
S. prostra  ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAATTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
Stevens Is  ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAATTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
Gough Isla  ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAATTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
Northland   ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAATTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
S. raivava  ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAATTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
S. tomento  ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAATTCA A----TTGTT TACGTATTCA ATTTCAATGA TTTAAGTTTC
S. japonic  GAAACTGGGA GTTCAGTATT CTATTTTCTT GGTACCATCC AACCAATACA ATTCATTGTT TACTTATTCA ATTTAAATGA TTGAATTTTC
Clianthus   ------GGGA GTT-AGTATT CTATTTTCTT GGTACCATCC AACCAAGTCA A----TTGTT TACTTATTCA ATTTCAATGA TTTAATTTTC
Carmichael  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Vicia faba  ------GTAA GTT-AATAAT CGATTTTCTT GGTACCATCC AACCAATTCA AT---TGTTT ------TTAA ATTTCAATGA GTGAATTTTC
Pisum sati  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
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columns 451 - 540

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Canterbury  AAGTTCAACC AAGTAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA A-TCTTTCAT TTGTCTATTA TTATAGACAA TTATAGACAA
S. howinsu  AAGTTCAACC AAGTAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA A-TCTTTCAT TTGTCTATTA TTATAGACAA TTATAGACAA
Chatham Is  AAGTTCAACC AAGTAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA A-TCTTTCAT TTGTCTATTA TTATAGACAA TTATAGACAA
Chile micr  AAGTTCAACC AAGTAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAG A-TCTTTCAT TTGTCTATTA TTATAGACAA TTATAGACAA
S. tetrapt  AAGTTCAACC AAGTAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA A-TCTTTCAT TTGTCTATTA TTATAGACAA TTATAGACAA
S. prostra  AAGTTCAACC AAGTAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA A-TCTTTCAT TTGTCTATTA TTATAGACAA TTATAGACAA
Stevens Is  AAGTTCAACC AAGTAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA A-TCTTTCAT TTGTCTATTA TTATAGACAA TTATAGACAA
Gough Isla  AAGTTCAACC AAGTAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA A-TCTTTCAT TTGTCTATTA TTATAGACAA TTATAGACAA
Northland   AAGTTCAACC AAGTAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA A-TCTTTCAT TTGTCTATTA TTATAGACAA TTATAGACAA
S. raivava  AAGTTCAACC AAGTAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA A-TCTTTCAT TTGTCTATTA TTATAGACAA TTATAGACAA
S. tomento  TAGTTCAACC AAG-AAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA AG-CTTTCAG TTGTCTATTA TTATAGACAA GTATAGACAA
S. japonic  AAGTTCAACC AAG----TC- ---ATTTTGA AA-ATATCAC CTGGGATGAA A-TCGTTC-G AAGTCTATT- -----GACTA TTATAGACAA
Clianthus   AAGTTCAACC AA-TAAGTC- ---AGTTTTA AA-ATATAAC ATTGGATGAA -GTCTTTCAT GTGTCTATTA TTATAGACAA TTATAGACAA
Carmichael  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????TT TTTTTTATGA TTCTAGACAA
Vicia faba  AAGGTCAACC CAGTCATTAT GAAAATTTTA ATTGGATGAA ATCTTTTGAA AGTCTTTCAT TTGTTTATCA TTCTAG---A TTATAGACAA
Pisum sati  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
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      columns 541 - 630
Canterbury  TACCATCTAT ATTA-CTATA TTAAGGGAAT TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATTTC ATTAGCCCTT ATGTCATATT
S. howinsu  TACCATCTAT ATTA-CTATA TTAAGGGAAT TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATTTC ATTAGCCCTT ATTTCATATT
Chatham Is  TACCATCTAT ATTA-CTATA TTAAGGGAAT TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATTTC ATTAGCCCTT ATTTCATATT
Chile micr  TACCATCTAT ATTA-CTATA TTAAGGGAAC TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATTTC ATTAGCCCTT ATGTCATATT
S. tetrapt  TACCATCTAT ATTA-CTATA TTAAGGGAAT TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATTTC ATTAGCCCTT ATCTCATATT
S. prostra  TACCATCTAT ATTA-CTATA TTAAGGGAAG TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATTTC ATTAGCCCTT ATGTCATATT
Stevens Is  TACCATCTAT ATTA-CTATA TTAAGGGAAT TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATTTC ATTAGCCCTT ATGTCATATT
Gough Isla  TACCATCTAT ATTA-CTATA TTAAGGGAAG TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATTTC ATTAGCCCTT ATGTCATATT
Northland   TACCATCTAT ATTA-CTATA TTAAGGGAAC TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATGTC ATTAGCCCTT ATGTCATATT
S. raivava  TACCATCTAT ATTA-CTATA TTAAGGGAAG TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATTTC ATTAGCCCTT ATATCATATT
S. tomento  TACCATCTAT AGTA-CTATA TTAAGGGAAG TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATCTC ATAAGCCCTT ATCTCATATT
S. japonic  TACCATCTAT ATTA-C---- TCTATGGAAT TCGAACCTGA ACTTTATTTA CGATTCCGTT TTTCTATCTC ATTGGCCCTT ATTTCATATT
Clianthus   TACCATCTAT ATTA-CTATA TTAAGGGAAT TCGAACTTTC ACTTTATTTC CGAGTCAGTT TTTCTATGTC ATTGGCCCTT ATTTCATATT
Carmichael  TACTATCTAT ATTA------ TCTATGTAAT TCGAACTTTC ACTTTATTTC CGAGTCCGTT TTTCTATGTC ATTGGCCCTT ATTTCATATT
Vicia faba  TACTATCTAT ATTATCTATG TAATTCGAAC CTGAACTTT- -GTTTGTTTA TGATTCCGTT TTTCTATCTC ATTGGTCCTT CTTTTTTCTT
Pisum sati  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????

      columns 631 - 692
Canterbury  TTATCAGCAT ACGATTTAAA ACCTAGTATA TTTTTTTTAT CTATTTATTT TTTTTTTCGT GG
S. howinsu  TTATCAGCAT ACGATTTAT- ACCTAGTA?? ?????????? ?????????? ?????????? ??
Chatham Is  TTATCAGCAT ACGATTTATT ACCTAGTATA ?????????? ?????????? ?????????? ??
Chile micr  TTATCAGCAT ACGATTTAT- ACCTAGTATA TTTTTTTTAT CTATTTATTT TTTTTT-CGT GG.
S. tetrapt  TTATCAGCAT ACGATTTAA- ACCTAGTATA TTTTTTTTAT CTATTTATTT TTTTTTTCG? ??
S. prostra  TTATCAGCAT ACGATTTAT- ACCTA????? ?????????? ?????????? ?????????? ??
Stevens Is  TTATCAGCAT ACGATTTAT- ACCTAGTATA TTTTTTTTAT CTATTTATTT TTTTTT-CGT GG
Gough Isla  TTATCAGCAT ACGATTTAT- ACCTAGTATA TTTTTTTTAT CTATTTATTT TTTTTT-CGT GG
Northland   TTATCAGCAT ACGATTTAT? ?????????? ?????????? ?????????? ?????????? ??
S. raivava  TTATCAGCAT ACGATTTAT- ACCTAGTATA TTTTTTTTAT -TATTTATTT TTTTT--CGT ??
S. tomento  TTATCAGCAT ACGACTTAT- ACCTAGTATA TTTTTTTT?? ?????????? ?????????? ??
S. japonic  TCATCAGCAT ACGATTTAT- ACCTAGCATA TTTTTTTTAC C-----TTTT TCTTTT-CGT GG
Clianthus   TCATCAGCAT ACGATATTAT ACCTAGCATA TTTTTTTTAC CGA---TTTT TCTTTT-CGT ??
Carmichael  TCATCAGCAT ACGATATTAT ACCTAGCATA TTTTTTTTAC CGA---TTTT TCTTTT-CGT ??
Vicia faba  TCCTCAGCAT AGGAT-TTAT ACTATGCAGA TTCTTTTTAC CAA---TTTT TCTTTTTATT GG
Pisum sati  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??
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Fig. 3.1. Aligned sequence data for eleven species of *Sophora*, *Clianthus puniceus,* and *Carmichaelia arborea* (sequenced in this study), and partial sequence for two additional legume outgroup taxa, *Pisum sativum* and *Vicia faba* (obtained from Genbank). "?" denotes where sequence data was either not available on Genbank (*Vicia* and *Pisum*) or was too close to the primers to be read.

Table 3.1. Percentage Sequence Similarity (based on Hamming Distances). The total number of sequence differences (base substitutions + insertion/deletion events) in pairwise comparisons are given in the top right of the matrix, while the percentage sequence similarity in pairwise comparisons (calculated as # differences divided by total number of base pairs) are given in the lower left of the matrix.

Taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1. Canterbury	**0**	1	8	9	3	8	7	8	10	9	30	79	53
2. *S. howinsula*	99.8	**0**	7	10	3	9	8	9	11	9	28	77	53
3. Chatham Is.	98.5	98.7	**0**	3	10	2	1	2	4	2	24	77	53
4. Chile	98.4	98.0	99.5	**0**	12	2	2	2	1	3	22	72	53
5. *S. tetraptera*	99.5	99.5	98.0	97.8	**0**	11	10	11	13	11	30	79	54
6. *S. prostrata*	98.5	98.4	99.6	99.6	98.0	**0**	1	0	3	1	21	72	53
7. Stevens Is.	98.7	98.5	99.8	99.6	98.2	99.8	**0**	1	3	2	22	71	52
8. Gough Is.	98.5	98.4	99.6	99.6	98.0	100	99.8	**0**	3	1	21	72	53
9. Northland	98.0	98.0	99.2	99.8	97.6	99.5	99.5	99.5	**0**	4	22	72	52
10. *S.raivavaeensis*	98.4	98.4	99.6	99.5	98.0	99.8	99.6	99.8	99.2	**0**	21	72	53
11. *S. tomentosa*	94.6	95.0	95.6	96.0	94.6	96.2	96.0	96.2	96.0	96.2	**0**	85	65
12. *S. japonica*	85.7	86.0	86.0	87.0	85.7	87.0	87.2	87.0	87.0	87.0	84.7	**0**	101
13. *Clianthus*	90.5	90.5	90.5	90.5	90.0	90.5	90.6	90.5	90.6	90.5	88.0	82.0	**0**

Table. 3.2. Removal of Constant Columns in the *Sophora* data set, leaving a subset of 17 parsimonious substitution sites. Both sites of deletion between three of the Edwardsia taxa are also shown.

Taxon	Position in the Data Set - Figure 3.1.																	
	55	65	78	84	160	186	188-189	191	201-206	216	277	510	570	608	614	623	632	649
S. microphylla (Canterbury)	T	G	T	A	T	T	GG	G	- - - - - -	-	T	T	T	T	A	**G**	T	**A**
S. howinsula	T	G	T	A	T	T	GG	G	- - - - - -	-	T	T	T	T	A	T	T	T
S. microphylla (Chatham Is.)	T	G	T	A	T	T	GG	G	ATATAT	T	T	T	T	T	A	T	T	T
S. microphylla (Chile)	T	G	T	A	T	T	GG	G	- - - - - -	T	T	T	**C**	T	A	**G**	T	T
S. tetraptera	T	G	T	A	T	T	GG	G	ATATAT	-	T	T	T	T	A	**C**	T	**A**
S. prostrata	T	G	T	A	T	T	GG	G	ATATAT	T	T	T	**G**	T	A	**G**	T	T
S. microphylla (Stevens Is.)	T	G	T	A	T	T	GG	G	ATATAT	T	T	T	T	T	A	**G**	T	T
S. microphylla (Gough Is.)	T	G	T	A	T	T	GG	G	ATATAT	T	T	T	**G**	T	A	**G**	T	T
S. microphylla (Northland)	T	G	T	A	T	T	GG	G	ATATAT	T	T	T	**C**	**G**	A	**G**	T	T
S. raivavaeensis	T	G	T	A	T	T	GG	G	ATATAT	T	T	T	**G**	T	A	A	T	T
S. tomentosa	T	**A**	**C**	A	T	T	GG	**T**	ATATAT	T	**G**	**G**	**G**	**C**	A	**C**	T	T
S. japonica	**C**	**A**	**C**	**G**	**A**	**C**	**CA**	G	ATATAT	T	**G**	**G**	T	**C**	**G**	T	**C**	T
Clianthus puniceus	**C**	**A**	**C**	**G**	**A**	**C**	**CA**	**T**	ATCTAT	T	T	T	T	**G**	**G**	T	**C**	A

Transitions And Transversions.

Nucleotide substitutions in the form of transitions (A/T and G/C) and transversions (AT/GC) occur during evolution. At earlier stages of evolutionary divergence, transitions are usually more frequent, but there are exceptions. The numbers of transversions have been used to estimate the degree of divergence between more distantly related taxa (*e*.g. Wilson et al. 1987), because their slower accumulation rate means they may be less likely to be obscured by multiple substitutions than transitions. However, the *Sophora* data presented here shows a greater accumulation of transversions in sequences, referenced to *Sophora japonica*, and the ratio of transitions to transversions is typically about 1:3. Further, two of the transversion substitution sites (positions 570 and 623 - see Table 3.2) seem to be extremely variable between all *Sophora* taxa sequenced, and it thus seems possible that they may have accumulated "multiple-hits" during evolutionary divergence of sequences. Table 3.3. provides a summary of the transversions between sequences in the data.

Phylogenetic Analysis of DNA Sequences

Phylogenetic analysis seeks to infer the history most consistent with a set of data by quantifying the signals supporting different sets of relationships. For example, Table 3.2 shows that the signals at base position 623 provide support for the Chilean population of *Sophora microphylla* grouping with *Sophora prostrata* and all other populations of *S. microphylla* except the Chatham Islands. At base position 570 however, signals support a grouping of only Chilean and Northland populations, and in this example these two would have the strongest relationship. Similarly, the signals at base position 649 support a relationship between *Sophora tetraptera* and the Canterbury population of *S. microphylla*, while at base position 570, signals group these two taxa with *S. howinsula*, the Chatham and Stevens Island populations of *S. microphylla*. Quantifying the signals supporting different sets of relationships in this way indicates the support, and hence reliability, for phylogenetic relationships. Discussion of the Hadamard conjugation in relation to other methods can be found in Penny *et al.* (1991, 1992).

Spectral Analysis of DNA Sequences.

The Hadamard conjugation uses a discrete Fourier transform to adjust for unobserved nucleotide changes in DNA sequences (Hendy & Penny 1993). Application of this method gives a "spectrum" which provides a quantitative measure of conflicting signals in the data which can support different phylogenetic hypotheses. Two-character state analyses divide the signals into

bipartitions, segregating for example taxa having a purine (*A* or *G*) from those having pyrimidines (*C* or *T*) at a specific site, and taxa can be grouped into bipartitions with 0, 1, 2, ..., n taxa. The number of occurrences for each bipartition are summed for the sequence, and the frequency of the bipartition is calculated by dividing by the total sum of bipartitions in the data set, including the bipartition which contains all the taxa (*i.e.* constant sites). The frequencies of bipartitions are called signals and the collection of signals are referred to as a spectrum (Hendy & Penny 1993). Individual signals in the spectrum may be referred to as a spectral signal. Bipartitions with only one taxon correspond to external branches on a tree - the branches leading only to that taxon. The other bipartitions (excluding the one with all the taxa in it) are potential internal branches (or edges) in the phylogenetic trees. Taxa are numbered from 1-13 according to their position in the data set, for example *S. microphylla* (Canterbury) will have index 1 (2^{1-1}) and *S. tetraptera* index 16 (2^{5-1}). The "strongest signal" has the highest frequency, and means that a relatively large number of sites in the sequence support the grouping of the taxa in this bipartition. If this signal is in the tree, its branch would have the largest internal length. Each bipartition is uniquely identified by the sum of the indices of the taxa it contains (see Fig. 3.2).

Partially resolved relationships using the Hadamard Conjugation

The 10 strongest bipartition signals in the spectrum are identified (Fig. 3.2) and correspond to:

2047 *Sophora japonica* and *Clianthus*;

1023 *S. japonica, S. tomentosa* and *Clianthus*;

264 Chilean and Northland *S. microphylla* populations;

6 Chatham Island *S. microphylla* and *S. howinsula*;

160 Gough Island *S. microphylla* and *S. prostrata*;

672 Gough Island *S. microphylla*, *S. prostrata* and *S. raivavaeensis*;

329 Canterbury, Chilean, Stevens Island and Northland *S. microphylla* populations;

1001 Chatham Island *S. microphylla*, *S. howinsula*, *S. tetraptera, S. tomentosa, S. japonica* and *Clianthus*;

1017 Chatham Island *S. microphylla*, *S. howinsula*, *S. tomentosa, S. japonica* and *Clianthus*;

328 Chilean, Stevens Island and Northland populations of *S. microphylla*.

Table. 3.3. Nucleotide changes in the *Sophora* data set; the number of transversions are recorded in the upper right of the matrix, while the lower left shows the total number of changes. (transversions weighted 1.0).

1	Canterbury	**0**	2	2	1	2	2	1	2	2	2	12	30	20
2	S. howinsu	2	**0**	0	1	2	2	1	2	2	2	10	28	20
3	Chatham Is	2	0	**0**	1	2	2	1	2	2	2	10	28	20
4	Chile micr	3	3	3	**0**	3	1	0	1	1	1	11	29	21
5	S. tetrapt	2	3	3	5	**0**	4	3	4	4	4	12	30	20
6	S. prostra	2	2	2	2	4	**0**	1	0	2	0	10	30	22
7	Stevens Is	1	1	1	2	3	1	**0**	1	1	1	11	29	21
8	Gough Is	2	2	2	2	4	0	1	**0**	2	0	10	30	22
9	Northland	3	3	3	2	5	2	2	2	**0**	2	12	30	20
10	S. raivava	3	2	2	3	4	1	2	1	3	**0**	10	30	22
11	S. tomento	16	15	15	16	16	14	15	14	15	14	**0**	36	28
12	S. japonic	51	49	49	52	52	51	50	51	51	51	57	**0**	42
13	Clianthus	38	38	38	41	39	40	39	40	39	40	46	62	**0**
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**

Fig. 3.2. Taxa included in each bipartition are indicated by an asterix, and each bipartition is identified by it's unique number. The ten most well supported bipartitions are identified;

	1 1 2 4 6 8 0 2		1 1 2 4 6 8 0 2		1 1 2 4 6 8 0 2
2047	..........**	**1023**	.........***	**264**	...*....*....
6	.**..........	**160**	.....*.*.....	**672**	.....*.*.*...
2041	.**.......**.	17	*...*........	1696	.....*.*.**..
489	*..*.****....	1536	.........**..	**329**	*..*..*.*....
1001	.**.*.....***	511	.........****	351	.....*.*.****
3071	..........*.*	1000	...*.*****...	**1017**	.**.......***
488	...*.****....	22	.**.*........	1007	....*.....***
328	...*..*.*....	936	...*.*.***...	87	***.*.*......
3078	.**.......**.	2054	.**........*.	2025	.**.*......**
856	...**.*.**...	1006	*...*.....***	1960	...*.*.****..
424	...*.*.**....	2024	***.*......**	1206	.**.**.*..*..
65	*.....*......	505	.**......****	23	***.*........
345	*..**.*.*....	678	.**..*.*.*...	518	.**......*...
2046	*..........**	1552	....*....**..	767	........*.***
1712	....**.*.**..	1031	***.......*..	4079	....*.......*
3055	....*.....*.*	1040	....*.....*..	225	*....***.....

Tree partitions are:

2047 1023 264 6 160 672 329 1001 1017 328

The ten compatible bipartitions that form the optimal tree are identified in bold, and listed at the bottom of the preceding figure. The support for and against different edges are shown in graphical format in Figure 3.3, where the 10 strongest signals are in bold.

On the basis of the tree reconstruction, *Sophora japonica* appears to be the oldest member of the genus and approximately coincides with the "root" of the *Sophora* tree. *Sophora tomentosa* appears to have diverged more recently from the remaining taxa in the data set, and the divergences within the Edwardsia group more recent again. Generally there is low support for edges in Edwardsia taxa, compared with those for *S. tomentosa, S. japonica* and *Clianthus* (Fig. 3.3). This results in the phylogeny of the Edwardsia being incompletely resolved, although a few relationships seem to be more clear (Figure 3.4). The Chatham Island *Sophora microphylla* and Lord Howe Island *Sophora howinsula* appear to be closely related, and form the first external branch for New Zealand taxa. The placement of *S. tetraptera* between this pair of taxa and the rest of the tree is interesting from the standpoint that the Chatham Island taxa is more closely related to *S. howinsula and S. tetraptera* than to the other populations of *S. microphylla* (Fig. 3.4). The rest of the *Sophora microphylla* populations appear to form an essentially homogeneous group which includes *S. prostrata* and *S. raivavaeensis*. Within this general grouping two closer sets of relationships are suggested: between the Chilean and Northland populations of *Sophora microphylla*, with moderate edge support in the Lento-plot (Lento *et al.* 1993); and between the three taxa *Sophora prostrata*, *S. raivavaeensis*, and Gough Island *S. microphylla*, which also form a external branch with low to moderate edge support (Figs. 3.3 &3.4).

If we look at the bipartitions for which there is support, but which are not in the optimal tree, then we find 489 (1,4,6,7,8,9 = nearly all the *Sophora microphylla* populations plus *Sophora prostrata*). It means that there is at least one site that is supporting this, and may well mean that *S. microphylla* should come together but duplicate changes elsewhere hide it (this is discussed further).

Fig. 3.3. Spectral Analysis Graph (Lento-plot) showing the support for and against bipartitions in the data set. Positive values are support for bipartitions, negative values are the strength of contradictions normalized so that the sum of the support is equal to the sum of contradictions. The bipartitions in the optimal tree (selected by closest tree) are in black. (The last bipartition -3055- is not represented on the graph).

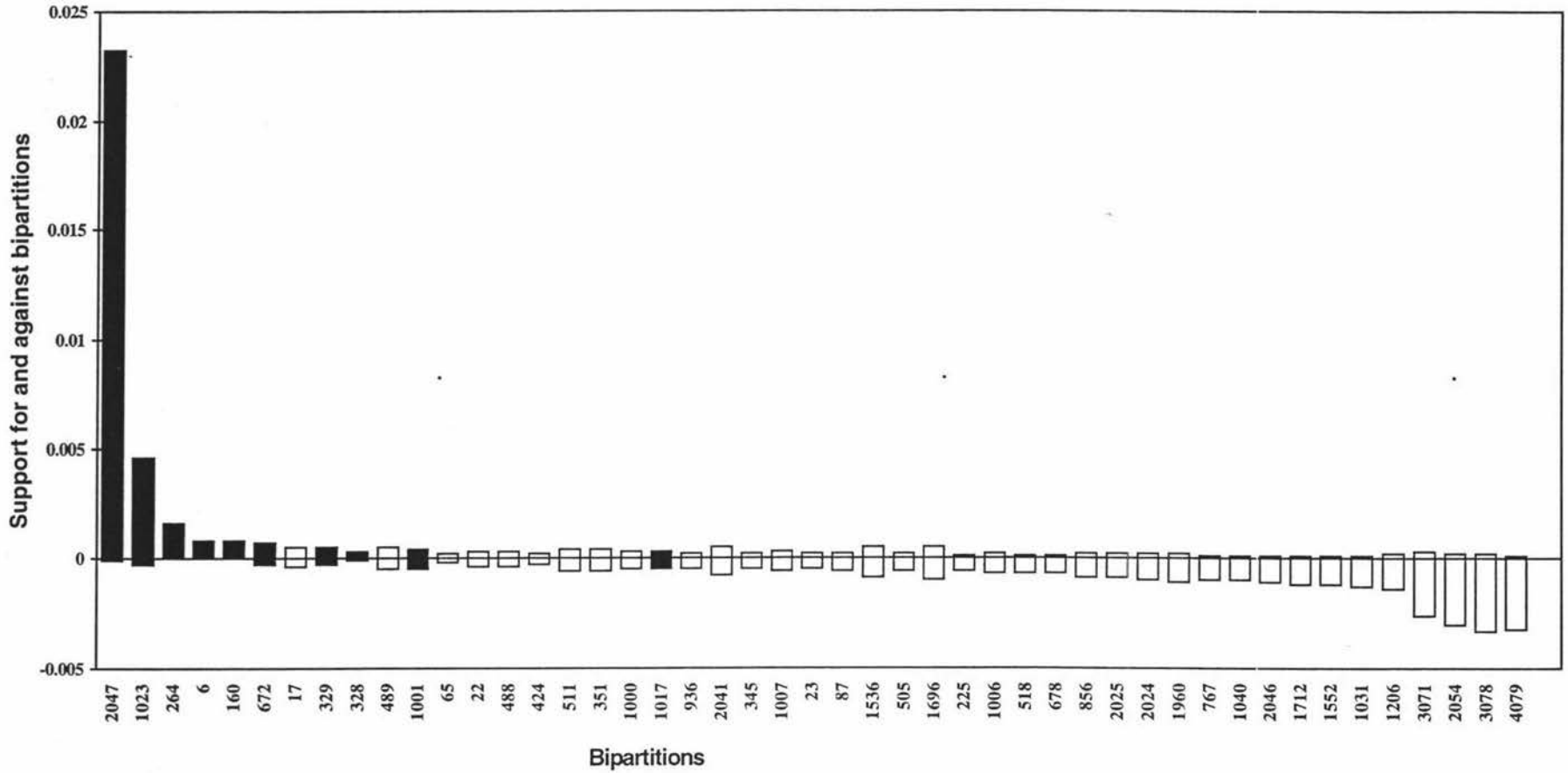
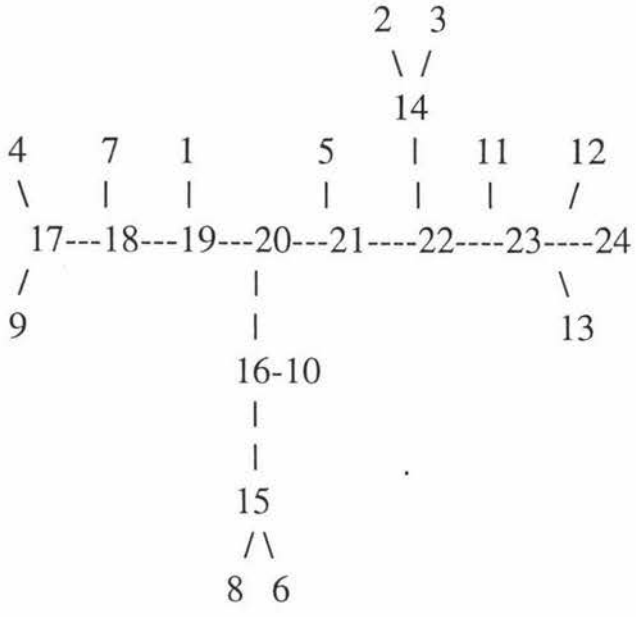


Fig. 3.4. The unrooted tree of phylogenetic relationships estimated by closest tree. Numbers 14-24 correspond to internal nodes in the tree, and Numbers 1-13 correspond to the following taxa; (**1**.) Canterbury *S. microphylla*; (**2**.) *Sophora howinsula*; (**3**.) Chatham Islands *S. microphylla*; (**4**.) Chile *S. microphylla*; (**5**.) *Sophora tetraptera*; (**6**.) *Sophora prostrata*; (**7**.) Stevens Island *S. microphylla*; (**8**.) Gough Island *S. microphylla*; (**9**.) *Northland S. microphylla*; (**10**.) *Sophora raivavaeensis*; (**11**.) *Sophora tomentosa*; (**12**.) *Sophora japonica*; (**13**.) *Clianthus puniceus*.



Maximum Parsimony and Distance-Based Analyses.

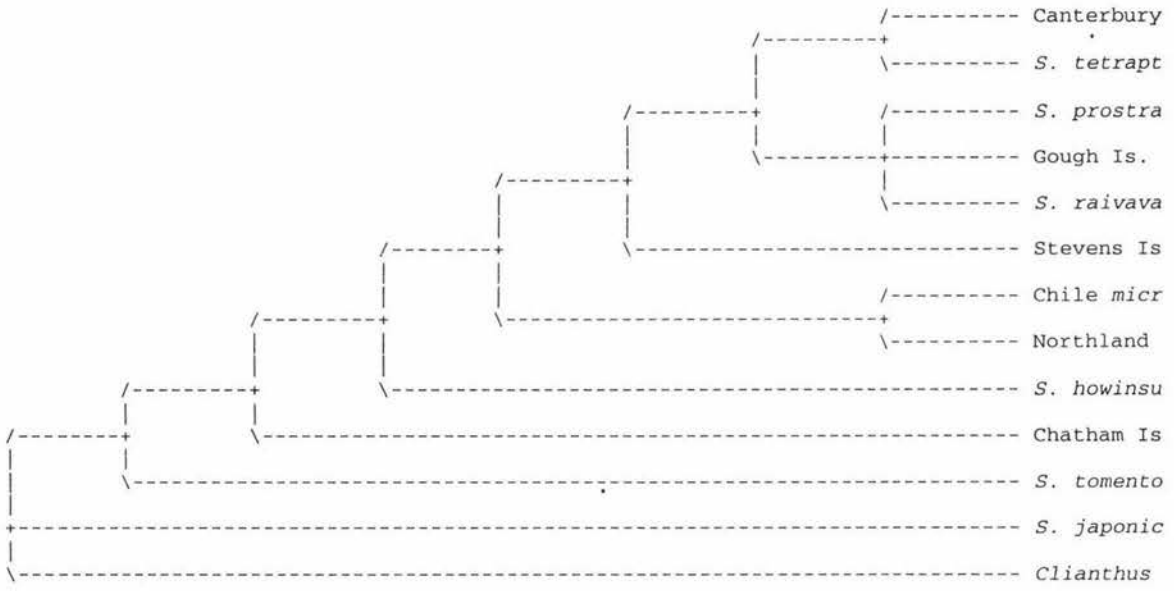
To confirm that aspects of the Hadamard conjugation were not a cause of lack of resolution among the Edwardsia sequences, other phylogenetic methods of analysis were applied to the data set using the computer analysis package PAUP* (Swofford 1996). These included maximum parsimony (Swofford *et al.* 1996), minimum evolution and neighbour-joining (Saitou & Nei 1987), and distance based methods. A variety of methods for correcting for multiple changes were tried, including Kimura 2-Parameter and Logdet. The different trees resulting from these analyses were then compared.

Maximum parsimony produced a large number of different trees in heuristic and branch-and-bound searches, and only two of the 45 trees are reproduced here as examples (Fig. 3.5). These trees differed from the Hadamard tree in the placement of *Sophora howinsula* and Chatham Island *S. microphylla* on separate external branches (Fig. 3.5). The trees also showed considerable variation in the position of certain branches and taxa. For example, Tree A places the Stevens Island *Sophora microphylla* on a external branch between the Chilean/Northland branch and the Gough Island/*S.raivavaeensis*/ *S. prostrata* branch, while Tree B places the same taxon outside both branching sets, after *Sophora howinsula* (Fig. 3.5). Tree B also nests the Gough Island/ *S.raivavaeensis*/*S. prostrata* branch within the Chilean/Northland branch.

Neighbour-joining analyses produced 3 different trees with uncorrected, and corrected under the Kimura 2-P and Logdet models (Lockhart *et al.* 1994). The uncorrected (or observed) tree was found to be similar to the Hadamard tree, and hence only the Kimura 2-P and Logdet trees are shown here (Figs. 3.6 & 3.7). The Kimura 2-P tree differs from both the Hadamard and Logdet trees in the placement of *Sophora tetraptera* with the Canterbury population of *Sophora microphylla*, which now branch from the same trunk node as the *S. raivavaeensis*/*S. prostrata*/Gough branch (Figs. 3.4 & 3.6). The Logdet tree also differs slightly from the Hadamard tree in that the *S. howinsula*/Chatham branch no longer has an internal node at the position corresponding to 14 (in Fig. 3.4), but now both taxa arise from the node corresponding to 22 (see Figs. 3.4 & 3.7).

Fig. 3.5. Maximum parsimony tree reconstruction for the 73 bases of *atpB-Be* sequence from 12 *Sophora* and *Clianthus*, using heuristic search settings (in PAUP*). **A**.) Tree number 5 of 45 possible trees ; **B**.) Tree number 19 of 45 possible trees.

Tree **A**.



Tree **B**.

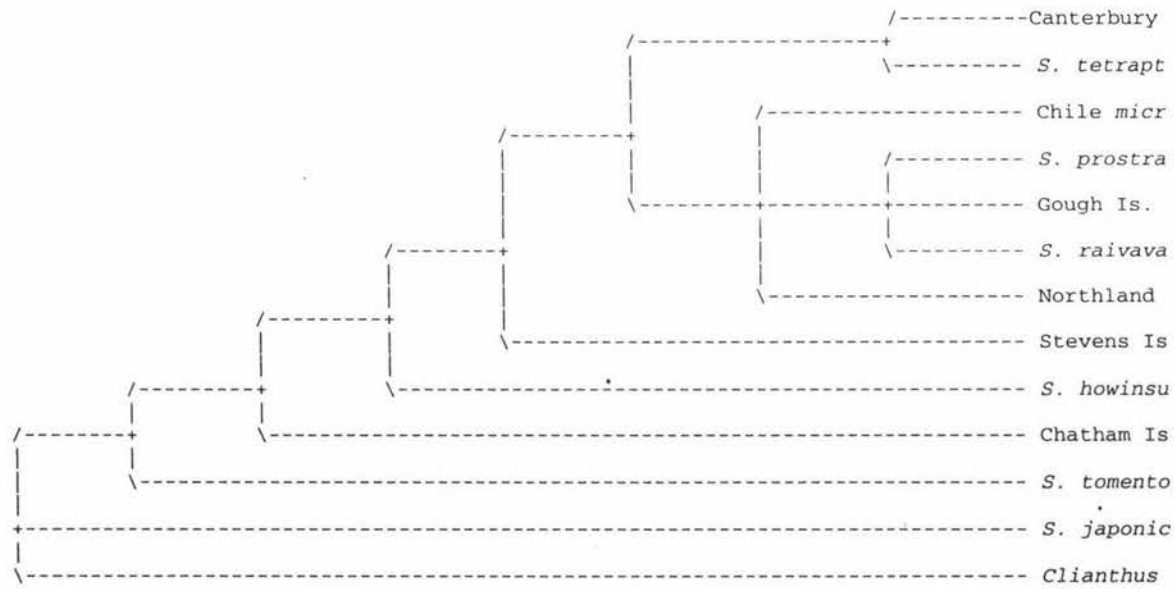


Fig. 3.6. Neighbour-joining tree for the 73 bases of *atpB-Be* cpDNA sequence from 12 *Sophora* and *Clianthus*, using the Kimura 2-parameter distance correction (PAUP* was used). Lengths of branches are proportional to the probability of change along that branch. Note that "+" denotes the position of an internal node, and --//-- shows where partial length was removed from a branch in order to fit the tree to the page.

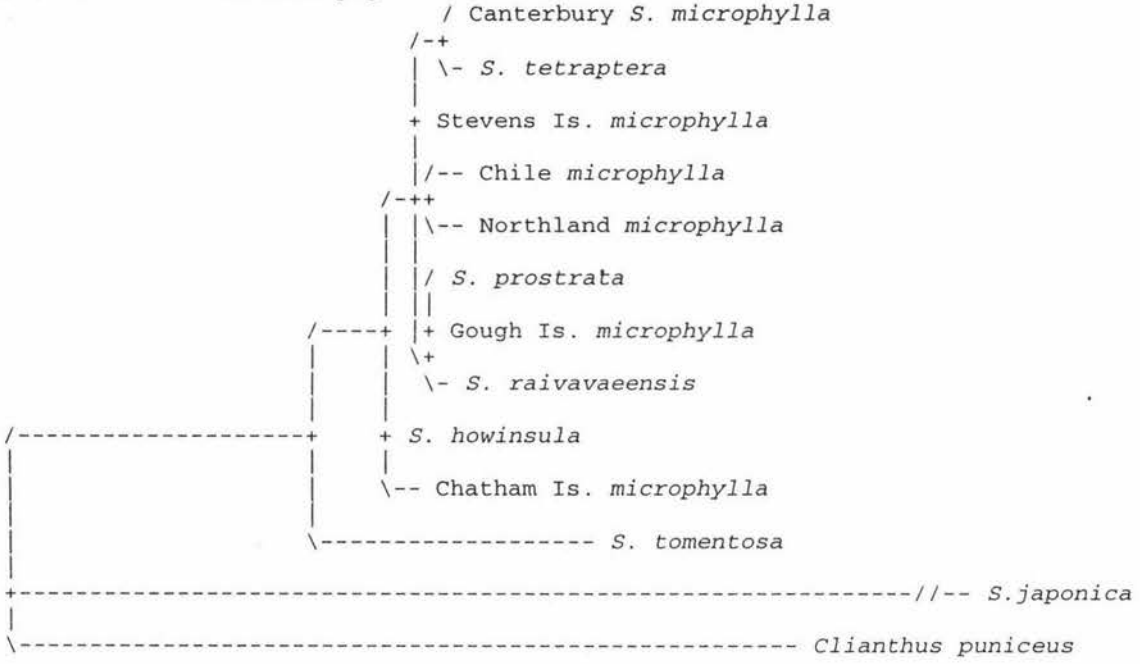
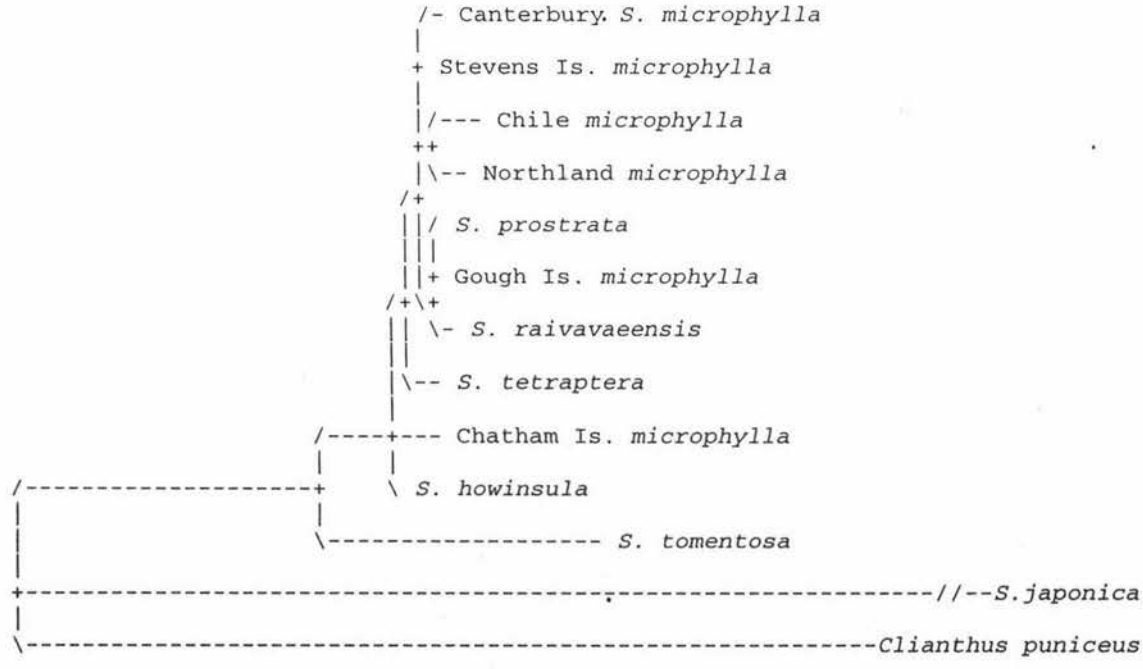


Fig. 3.7. Neighbour-joining analysis using the Logdet/paralinear distance correction measure. The symbols "+" and "--//--" are both explained in figure 3.6.



Overall the trees show that the New Zealand and South Pacific *Sophora* (plus Gough Island) are definitely closely related, but it is not easy to resolve their exact relationships. There is a small signal that unites most of the microphylla group but there are some parallel mutations that tend to mask this signal.

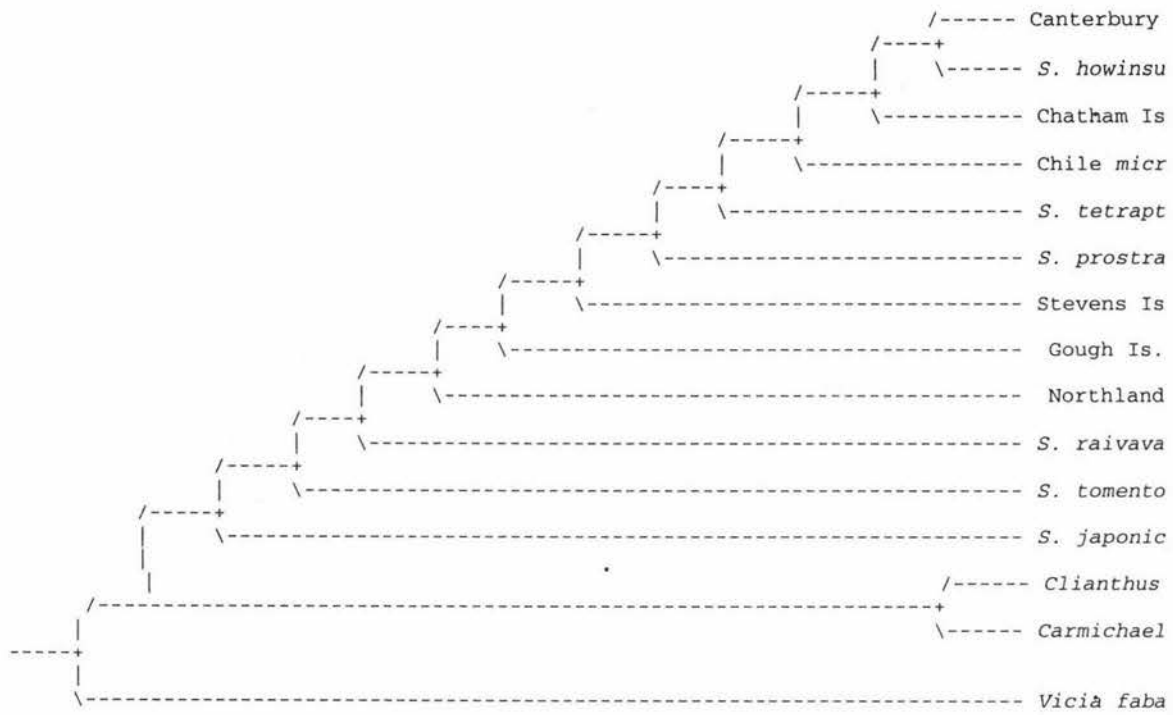
Estimating the Proportion of Invariable sites.

The correction for unobserved changes assumes that all sites are equally free to vary. This is not the case for functional genes (Fitch & Margoliash 1967, Shoemaker & Fitch 1989), and as shown by this data set, more than half of the *atpB-Be* cpDNA region is conserved across the genus *Sophora*. Assuming that all sites are free to vary will mean that corrections for unobserved changes will underestimate the true number of substitutions. This is because the frequency of change per site is lower if averaged across the whole sequence. If only a proportion of the sites can change then the actual frequency of substitutions at variable sites is higher, and so the probability of multiple changes should be higher. Lengths of some potential internal edges in the tree will then be longer and may result in greater resolution. The proportion of invariable sites was estimated to be 77% using Phylip with maximum likelihood and estimating the optimal fit between the data and the model, and this figure was applied in all further phylogenetic analysis.

Maximum Likelihood Analysis.

Maximum likelihood is useful because it is frequently the estimation least affected by sampling error (Lockhart *et al.* 1996). Assuming some probabilistic mechanism, it tries to fit all the patterns in sequence data to a tree (*e*.g. parsimony sites to internal edges; external patterns to external edges). However, it also takes a prohibitive length of time to conduct searches, and is generally not recommended for analysis of more than 11 taxa. Maximum likelihood analysis was performed on the *Sophora* sequence data, using the Hasegawa-Kishino-Yano (1985) model of evolution and branch-and-bound searching criteria. Enforcing the molecular clock did not alter the tree topology, so that only one tree was produced (after more than 20 hours of search time), and this is shown in figure 3.8. The tree differs from all other trees obtained previously by grouping *Sophora howinsula* with the Canterbury *Sophora microphylla* population (instead of the Chatham Island population), and all other Edwardsia taxa form external branches (Fig. 3.8). As has already been mentioned, no sequence differences were found between *Sophora prostrata* and the Gough Island

Fig. 3.8. Maximum likelihood tree for the 73 bases of *atpB-Be* cpDNA sequence from 12 *Sophora* and *Clianthus* using the Hasegwa-Kishino-Yano (1985) model of evolution. *Carmichaelia* and *Vicia faba* were also included. Branch and bound searching criteria were used, and the molecular clock was enforced.



population of *S. microphylla*, and yet the maximum likelihood analysis separates these two taxa with the Stevens Island population falling between them.

Bootstrapping and Support for Phylogenetic Trees.

Bootstrapping is a way of testing whether there is a relatively large number of patterns in the data which support particular relationships. Using bootstrapping will warn if the sample is too short to allow a conclusion. Bootstrapping was applied to each of the following analyses; maximum parsimony, neighbour-joining (uncorrected, Kimura 2-P, and Logdet), and maximum likelihood. Two examples of the outputs from bootstrapping are given in Table 3.5 and Figure 3.9, where 100 replicates were performed under an uncorrected, neighbour-joining model. The majority rule consensus tree shown is typical for all of the bootstrap analyses except Maximum Likelihood, and all trees generally gave very low edge supports between Edwardsia taxa (less than 50% - see Table 3.4. & Fig. 3.9). Comparisons between the methods were made and are summarised in Table 3.4. Maximum likelihood analysis resulted in a totally different tree topology to all other analyses (compare Figs. 3.4 - 3.8), thus direct comparisons of support for various branches were not able to be made with other bootstrap analyses. Maximum Likelihood bootstrapping gave 33% support for all internal branches (using 100 replicates), including internal branches between *Sophora japonica* and *Sophora tomentosa*, and *S. tomentosa* and the Edwardsia taxa, which yielded between 69-100% support under other analyses. The Maximum Likelihood bootstrap consensus tree is not reproduced here.

The bootstrap partitions (Table 3.5) are useful for evaluating the support for different sets of relationships not produced by optimized-searching procedures. For example, 6% of the bootstrap replicates (in uncorrected neighbour-joining bootstrapping) support a relationship between the Northland, Gough Island and Chilean populations of *S. microphylla*, and 5% of the replicates also include *Sophora prostrata* with this group. This is not reported in any of the produced trees, which tend to separate these into two branches.

Fig. 3.9. Maximum likelihood tree for the106 bases of *atpB-Be* cpDNA sequence from 12 *Sophora, Clianthus, Carmichaelia* and *Vicia*, using the Hasegwa-Kishino-Yano (1985) model of evolution. Branch and bound searching criteria were used, and the molecular clock was enforced.

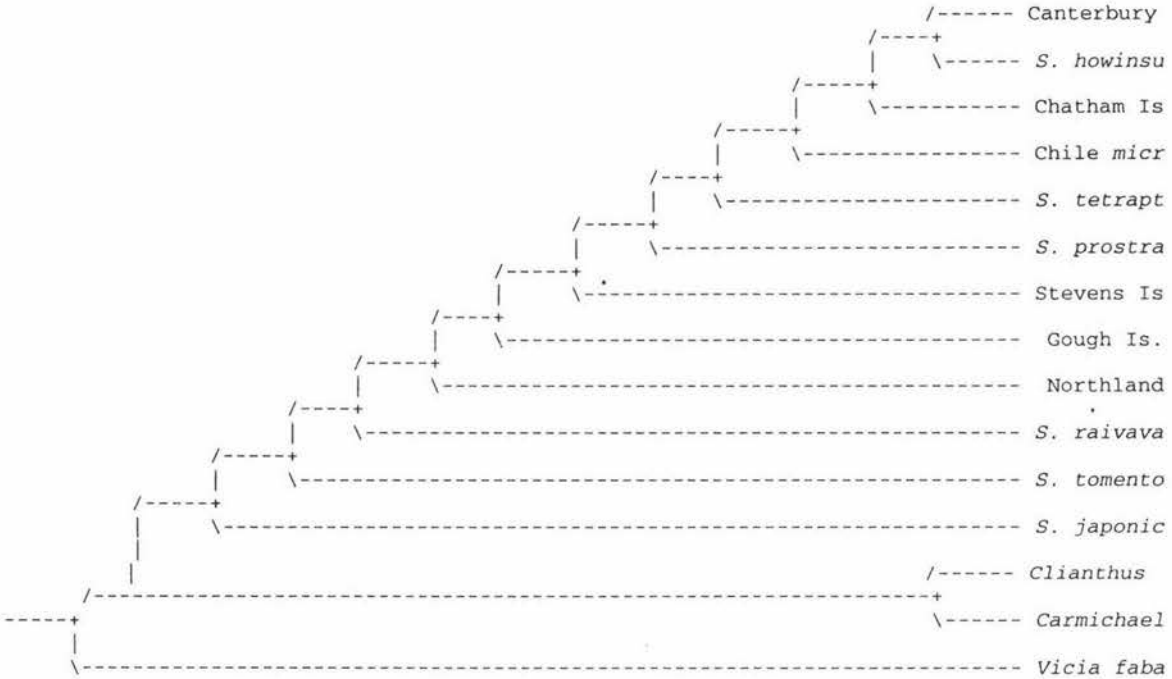


Fig. 3.10. Neighbour-joining (minimum evolution - uncorrected) bootstrap consensus tree for the 73 bases of atpB-Be cpDNA sequence from 12 *Sophora* and *Clianthus*. Values on internal edges are percentages and correspond to the number of times that edge occurred in 100 bootstrap replications. Only two of the internal edges occur in more than 80% of the bootstrapped trees. The tree is unrooted.

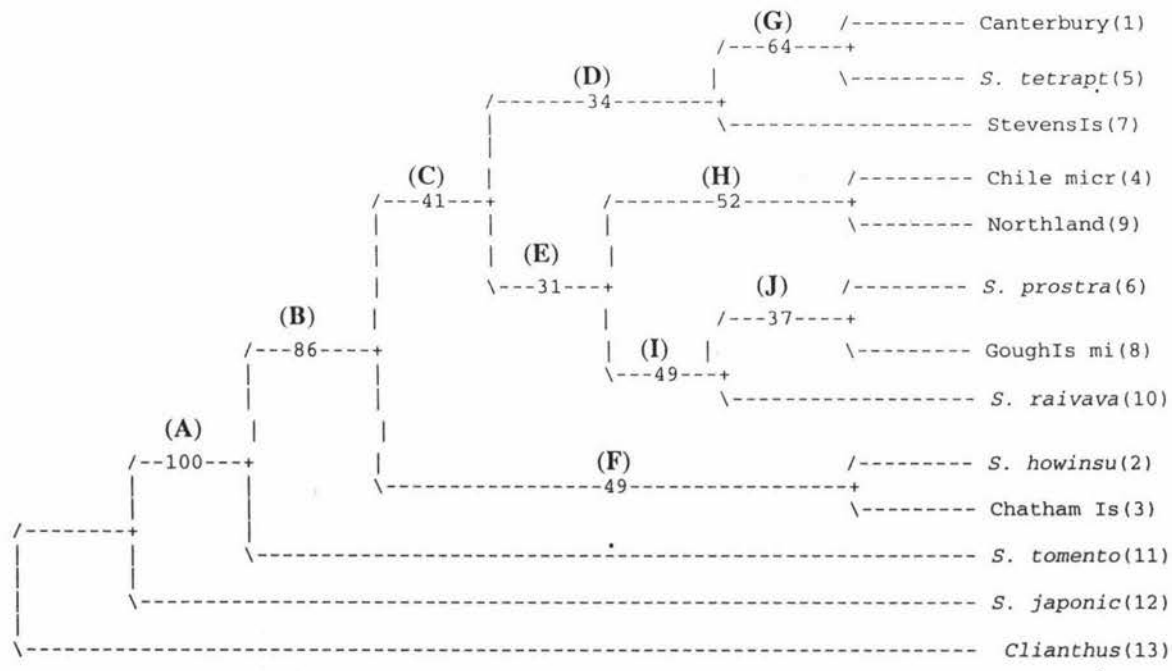


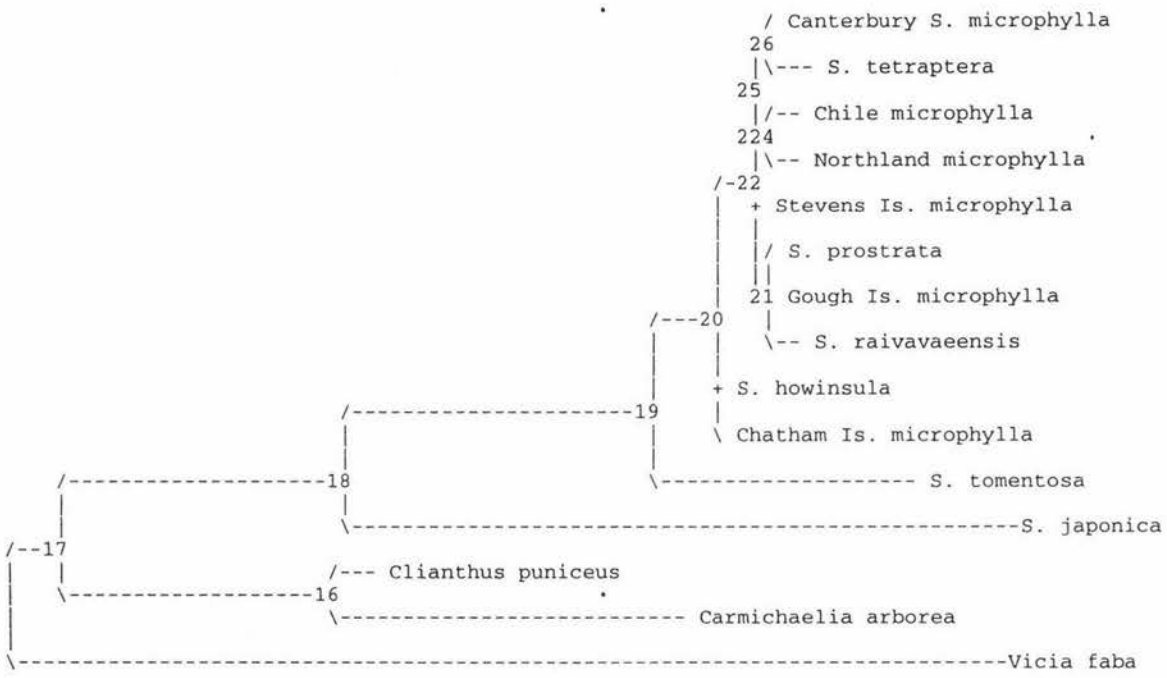
Table 3.4 Comparing bootstrap supports for internal branches between different phylogenetic models and distance correction measures. All values are percentages (%). The alphabetical list of branches corresponds to a particular branch on the tree, as shown in the above figure 3.9.

Branch	Maximum Parsimony	Neighbour-joining (uncorrected)	Neighbour-J Kimura 2-P (corrected)	Neighbour-J Logdet (corrected)
A	99	100	100	94
B	75	86	71	69
C	17	41	17	13
D	9	34	20	12
E	14	31	10	8
F	23	49	26	16
G	46	64	60	44
H	48	52	38	40
I	26	49	21	23
J	20	37	28	31

Table 3.5 . Neighbour-joining (uncorrected) bootstrap partitions found in one or more trees and frequency of occurrence:

Frequency		Frequency	
111111 123456789012346		111111 123456789012346	
..........****	99.00	...*....*.*****	4.00
..........**.	78.00	...*..*........	4.00
..........*****	75.00	.***.*.***.....	3.00
..........*..*	55.00	.*.......*.....	3.00
...*....*......	48.00	..**....*......	3.00
.***.**********	46.00	........*..****	3.00
...........***	45.00	.***.....******	3.00
.....*.*.*.....	26.00	.*.*.******....	3.00
.**............	23.00	..*...*...*****	3.00
............**	21.00	...*...**.*****	3.00
.....*.*.......	20.00	..**.*.***.....	3.00
.**.......*****	17.00	.**.....*.*****	3.00
..*.......*****	15.00	.***..*.*......	3.00
.***.*****.....	14.00	.***.**..******	3.00
...*.*.***.....	14.00	.....*.*..*****	3.00
.....*...*.....	12.00	.....**..*.....	3.00
.***.******....	9.00	.*.*.*****.....	3.00
.*........*****	9.00	..*.*..........	3.00
...*.*****.....	9.00	.........**....	3.00
.........******	9.00	.****...*.*****	3.00
.......*.*.....	8.00	.***.**.*******	3.00
....*.....*****	8.00	..*...*........	3.00
.....*.*.**....	8.00	..*......******	3.00
...*..*.*......	7.00	...*.*.**.*****	3.00
.**......******	7.00	.*.*....*......	3.00
...*.*..*......	7.00	.*.*.****......	3.00
.***.*.********	7.00	.*.*.*.********	3.00
....*......****	6.00	...*.****......	3.00
...*...**......	6.00	.**..**.*******	2.00
.**......*.....	6.00	.***.***.******	2.00
.********.....	6.00	.*....*........	2.00
.....**........	6.00	...*....*..****	2.00
...*.*..**.....	6.00	.....*.***.....	2.00
........*.*****	6.00	.**..**..******	2.00
....*.....*....	6.00	..******..*****	2.00
.....*.*.******	6.00	.....**.**.....	2.00
.....***.*.....	5.00	.**..**..*.....	2.00
.**.*.....*****	5.00	.*....*...*****	2.00
...*.*.**......	5.00	...*.*.*.*.....	2.00
.**........****	5.00	.*..*.....*****	2.00
.*.*..*.*......	5.00	.***..*********	2.00
...*.*.****....	4.00	..**.*****.....	2.00
...*.*.********	4.00	...******.....	2.00
.***..*..******	4.00	....**.*.*.....	2.00
.**.*....******	4.00	.....*.****....	2.00
...*...*.......	4.00	.**...*...*****	2.00
...*.**........	4.00	.*.........****	2.00
.....*...******	4.00	..**..*.*......	2.00
.....*....*....	4.00	..**....*.*****	2.00
..*........****	4.00	.***..***.*****	2.00

Fig. 3.11. Maximum parsimony tree for 106 bases of *atpB-Be* cpDNA for 12 *Sophora*, *Clianthus puniceus*, *Carmichaelia arborea*, and *Vicia faba*.



Estimating Divergence Times for New Zealand *Sophora*.

Using the principle of the molecular clock, there have been a number of attempts to estimate divergence times of major plant taxa. The relationship between substitution rate and time is demonstrated by the greater number of changes between older lineages of *Sophora* than in the comparatively younger section Edwardsia (Fig. 3.1. & 3.6). This thesis estimates the time of evolution in Edwardsia by calibrating them against the rate of evolution in *Sophora tomentosa* (whose first known fossils occur 30Ma - Sousa & Delgado 1993).

The last common ancestor of *Sophora tomentosa* and *Sophora japonica* will be older than 30 million years ago. By comparing the number of differences between *S. tomentosa* and the average of the Edwardsia group we get an evolutionary rate of one change per 4 million years taking 14-15 changes over 60 million years, 30 for the tomentosa lineage and 30 for Edwardsia. Neighbour-joining distance analyses (Figs. 3.6 & 3.7), including taxa from the genera *Carmichaelia* and *Vicia* (Fig. 3.10), suggest a heterogeneous rate of evolution between different lineages within *Sophora*. Branch lengths for Edwardsia are shorter than those for *S. tomentosa* and *S. japonica* (Fig. 3.10), and this adds uncertainty about the reliability of calibrating the rate of evolution in Edwardsia from the rates in other *Sophora* lineages. The Edwardsia appear to be evolving at only about half the rate of *S. tomentosa*, a better estimate may be one change per 6 million years (5 changes of 30 million years in Edwardsia, 10 in tomentosa). In the absence of any additional information, this estimate will be used in the remainder of the discussion.

Summary of Results.

The sequence region examined in this thesis is part of the intergene region between *atpB* and *rbcL* genes in the chloroplast genome. Non-coding regions are generally thought to evolve more rapidly than translated gene regions, because they are not under the same functional constraints. It was expected that the rate of evolution in the *atpB-rbcL* would be great enough to provide resolution between species and populations, without accumulating too many multiple hits - which obscure the real amount of evolutionary change between taxa. This sequencing region has been useful for resolving relationships between different families and genera (for example, Savolainen *et al.* 1994), and this study confirms its utility at this level of relationship. Analysis of the data demonstrated good resolution between different sections of the genus *Sophora*, and with *Clianthus, Carmichaelia*, and *Vicia* (Fig. 3.8). The region had not previously been used in

phylogenetic investigations at the level of species and genera, however, and this study demonstrates that it does not evolve rapidly enough for comparisons between very closely related taxa.

Rate of Molecular Evolution in Southern Hemisphere Sophora.

The *Sophora* data set is extremely conserved in this sequencing region, and the proportion of invariant sites was estimated to be greater than 70%. Detailed examination of this region reveals conserved promoter and ribosome binding sites; as a consequence of these conserved features, the total rate of nucleotide substitution has been estimated to be about equivalent to that of the *rbcL* coding region (Zurawski and Clegg 1987; Zurawski *et al.* 1984). Significantly, two positions in the aligned sequences appeared to be hyper-variable, and nucleotide base differences were observed between most taxa. These may have accumulated multiple substitutions - and algorithmic corrections for these unobserved changes may not result in selection of the correct tree.

Inability to completely resolve the phylogeny for Edwardsia taxa.

The percent sequence divergence between taxa differed from 13-15% in *Sophora japonica* with respect to other taxa, to 0-2% between all the Edwardsia taxa (Table 3.1). Only four parsimony sites were found to occur between Edwardsia, which amounts to less than 1% of each of these sequences obtained. An optimal sequence divergence in phylogenetic studies occurs because, as sequences diverge, the proportion of highly informative "single-hit" sites reaches a maximum, then decreases upon further sequence divergence, whereupon the less informative "multiple-hit" sites become predominant. Ritland and Eckenwalder (1992) suggest that the true optimal sequence divergence between taxa is probably lower than 25%, perhaps at approximately 10% divergence.

This conservatism in evolutionary rate, as well as the occurrence of two extremely variable sites, pose problems for recovering the correct branching order and edge lengths in the phylogenetic tree.

While none of the signals between *kowhai* are in conflict, their small number of patterns from which to draw phylogenetic conclusions prevents the taxa being placed into mutually exclusive groups. Four bipartitions in the *kowhai* data seem to be more robust, in that they have

moderately strong spectral signals and/or the taxa consistently grouped together (Figs. 3.3, 3.4,3.5,3.6,3.7). These taxa are Chilean and Northland populations of *S. microphylla* (which differ by 1 nucleotide substitution), Chatham Island *S. microphylla* and *S. howinsula* (no nucleotide differences), Gough Island *S. microphylla* and *S. prostrata* (no nucleotide differences), and the latter two with *S. raivavaeensis* (1 difference).

The use of different tree construction methods, and different optimality criteria, resulted in the production of a number of phylogenetic interpretations of the data set. Trees differed in the placement of various branches and taxa, but the general placement is with *Sophora tetraptera*, *S. howinsula* and the Chatham population of *S. microphylla* at the base of the southern Edwardsia tree, with the rest of the group forming an essentially homogeneous complex with short internal branch lengths. The deletion event observed between *S. tetraptera, S. howinsula* and the Canterbury population of *S. microphylla* provides indirect support for a close relationship between these three taxa, although the deletion may have occurred independently in the latter. Alternatively, the second tree places the Pacific Island *Sophora raivavaeensis* at the base of the southern Edwardsia tree (see Fig. 3.8), with the Lord Howe and New Zealand taxa falling inside this branch. There was a small amount of bootstrap support for this hypothesis (8-9%). Also, the similar morphologies between this taxa, *S. tetraptera* and *S. howinsula* may be further evidence in support of this tree.

The *Sophora* data presented here are particularly useful for demonstrating the variability of molecular regions in relation to increasing evolutionary time, since the number of nucleotide substitutions accumulate with taxonomic distance. *Sophora japonica* and *Sophora tomentosa* have accumulated a greater number of substitutions than the Edwardsia species and populations, which was expected on the basis of their earlier known fossil records (middle Eocene and Oligocene, respectively), and presumably older divergence times. The Edwardsia taxa were found to be extremely genetically similar (between 98 and 100% sequence similarity). It is concluded that this chloroplast sequencing region is not useful for investigations between very closely related taxa. Future studies of recently evolved groups, such as subspecies and populations, should make use of larger data sets (in the region of 1000 bp), and faster evolving regions of plastid or nuclear DNA.

Incompletely Resolved Phylogeny for Edwardsia species of *Sophora*.

The phylogeny was not fully resolved for all Edwardsia taxa sampled in this study, and generally there was low statistical support (8-64%) for edge lengths in tree reconstructions. Support for edge lengths between the comparatively older species *Sophora japonica* and *Sophora tomentosa* were significantly greater (94-100% and 69-86%, respectively), and thus lack of resolution appears to be attributable to a recent evolution of southern Edwardsia taxa. A few of the relationships seemed a little more robust, in that they have moderately strong spectral signals and/or the taxa consistently grouped together. For instance, Chilean and Northland populations of *S. microphylla* (which differ by 1 nucleotide substitution), Chatham Island *S. microphylla* and *S. howinsula* (no nucleotide differences), and *Canterbury S. microphylla* and *S. tetraptera* (1 nucleotide substitution). However, most trees tended to group *Sophora prostrata* most closely with the Gough Island population of *S. microphylla* and with *S. raivavaeensis*. As this species is endemic to New Zealand, it was expected that it would be most closely related to a New Zealand population of *S. microphylla*, or *S. tetraptera*. This seemingly anomalous result therefore casts some doubt about the reliability of phylogenetic analyses of this data set, although the trees are useful in suggesting relationships that can be tested in future studies.

The occurrence of the 6 base pair deletion in *Sophora tetraptera, S. howinsula*, and the Canterbury population of *S. microphylla* (231-236bp position in the data set), provides additional phylogenetic support for a close relationship between these taxa. The deletion is not present in ancestral *Sophora japonica* or *S. tomentosa*, and is thus derived in these three species. A close relationship between *S. howinsula* and *S. tetraptera* is supported on the basis of their similar morphology. The presence of this deletion in the Canterbury *S. microphylla* may be independently derived, or it may signify a close evolutionary relationship between this and the other two mentioned taxa.

A Recent Diversification of New Zealand Edwardsia

The percentage similarity and the short internal edge lengths for comparisons among the Edwardsia taxa (Figs. 3.1, 3.6,3.7) suggest that they have diverged relatively recently. The apparent readiness of New Zealand taxa to hybridize may be further evidence of a recent origin and diversification.

Molecular clock estimates the deepest divergence in the group between *Sophora howinsula* and the Chatham Island population of *Sophora microphylla*. The bulk of the rest of the taxa grouped within the essentially homogeneous *Sophora microphylla* complex, which (on the basis of tree reconstructions) appear to be evolved more recently. *Sophora tetraptera* is often considered to be the most ancestral New Zealand taxon, however phylogenetic analyses consistently place *Sophora tetraptera* in the "ingroup" relative to the Chatham Island microphylla.

The polymorphic *Sophora microphylla* make up the greatest numbers of taxa in the Southern Hemisphere, and the phylogeny generally seems consistent with a New Zealand radiation and dispersal of the species. The basal placement of the Chatham Island population raises the possibility that New Zealand may not be the ultimate island of origin for *Sophora microphylla*, but additional conclusions are difficult to draw. The species appears to be undergoing adaptive radiation, diversifying with geographical isolation on the mainland, offshore and oceanic islands, and many of the populations may be of fairly recent origin. The close relationship suggested between the Chilean and Northland populations of *Sophora microphylla* supports a dispersal from New Zealand to Chile. Markham and Godley's (1978) studies found a close relationship existed between the Chilean and Gough Island populations of *S. microphylla*, and suggested that the Gough Island population originated from Chile (consistent with the direction of the Circum-

antarctic current). While evidence for this was not reproduced in phylogenetic tree-reconstructions, there is some bootstrap support (6%) for a close relationship between these two and the Northland populations of *S. microphylla*.

A few relationships suggested by phylogenetic analysis contradict previous chemotaxonomic studies, particularly the nesting of *Sophora prostrata* and *Sophora raivavaeensis* within the homogeneous *Sophora microphylla* complex. This tends to suggest that they are not good taxonomic species, but rather "ecotypes" of the variable *S. microphylla* complex. Further studies, utilizing faster evolving regions of cpDNA and/or nuclear DNA, are necessary to determine whether these two taxa are sufficiently divergent to retain specific status, or whether they would be better relegated to subspecies. Forcing specific bipartitions to be included in a tree would be one way of determining the likelihood of alternative groupings of taxa. Continued development and refinement of analysis programs are expanding their utility, enabling more detailed questions to be asked of the data. Of particular use will be the ability to obtain and compare suboptimal trees so that potential phylogenetic relationships can be analyzed in more detail.

The very high genetic similarity between Edwardsia sequences supports the hypothesis that these taxa have diversified comparatively recently in the Southern Hemisphere. The variable New Zealand species - *Sophora microphylla* - appears to be undergoing adaptive radiation, diversifying into a broad diversity of habitats on offshore and oceanic islands. A few of the populations appear to have undergone some genetic differentiation, but they probably have not yet been isolated for long periods of evolutionary time. On the basis of phylogenetic analysis, the New Zealand endemic *Sophora prostrata* does not appear to be genetically distinct from this *Sophora microphylla* complex, although further conclusions are difficult to draw.

A few authors have suggested that some of the other Southern Hemisphere taxa currently recognized as separate species may also be further subspecies or populations of this *Sophora microphylla* complex; for example, *S. toromiro* of Easter Island (Godley 1985), *S. fernandeziana* and *S. masafuerana* of Juan Fernandez, and *S. macrocarpa* of Chile (Murray 1986).

The Hawaiian *Sophora chrysophylla* (Edwardsia) has similarly radiated relatively recently into a diverse assemblage of subspecies and varieties, since the oldest island - Kaui - formed about 5

million years ago (Lowrey 1995), and taxonomic classification in this radiation is also complex (see Chock 1956). These two species together represent the two major ongoing radiations within the section Edwardsia. Hence the greater part of this section appears to be of a relatively recent origin.

Estimating Times of Divergence for Edwardsia.

On the basis of fossil evidence, and now genetic distances, the genus *Sophora* appears to be relatively recent in the New Zealand flora and vegetation. The earliest reliable pollen records are from Northland, dating from the Hautawan to Castlecliffian stages (Early Pleistocene - 1.6 Ma), and provide a minimum occupation date for species in New Zealand (Table 1.3. Raine, pers. com.). However, the record may actually underestimate the length of time the genus has been in New Zealand due to factors relating to pollen deposition and identification (see Chapter One).

Assuming a perfect molecular clock, estimates based on the rate of substitution in Edwardsia taxa relative to *Sophora tomentosa* suggest a late Miocene/Pliocene origin of New Zealand species, from between 9.6 and 8.9 million years ago. Distance-based phylogenies (such as neighbour-joining - Figs. 3.6 & 3.7) suggest a heterogeneous rate of evolution between older and younger parts of the genus *Sophora*, however, and this adds considerable uncertainty to the reliability of calibrating divergence times for Edwardsia by the rate of evolution in *Sophora tomentosa* and *S. japonica*. Maximum likelihood methods are generally more appropriate when evolutionary rates differ among lineages (Felsenstein 1978; Saitou and Imanishi 1989). However, as previously mentioned, the time taken for maximum-likelihood tree searches was prohibitively long, and the resulting tree (Fig. 3.8) separated *S. prostrata* and Gough Island *S. microphylla* even though no base pair differences were found between these two sequences. This suggests that the optimal tree was not found under this method for the data set.

Time and Island of Origin

The only direct estimation available for the timing of immigration of species into a country is the fossil record, and the New Zealand *Sophora* pollen record is not particularly informative in this case, as individual species are not identified in the available records. The question of which Edwardsia species gave rise to *Sophora tetraptera* remain unanswered from this study, and

further work including Chilean, Reunion, and Hawaiian members are required to determine the ultimate origin for New Zealand species.

Estimates of divergence times using the *atpB-Be* molecular clock suggest the deepest divergence between southern Edwardsia taxa occurred in the Miocene (approximately 9 million years ago [Ma.]). On the basis of the known fossil record, the genus *Sophora* does not appear to be common in New Zealand until the Pleistocene (2 Ma.), although it seems highly possible that it may have reached here in earlier times. Molecular data offer no information concerning the geographical place origin of taxa, so that suggestions can only be hypothesized from biogeographical and ecological knowledge, combined with phylogenetic information about their closest living relatives and their distribution.

Hypothesis One: Transoceanic dispersal of Sophora to New Zealand.

According to the fossil record, the Tribe Sophoreae were not particularly abundant or diverse on Gondwanaland before the middle Eocene. On the basis of cytological and morphological studies in the genus *Sophora*, the most ancestral, living member is *Sophora japonica* (Styphnolobium), which first appears in the fossil record in the middle Eocene (50 Ma.) in western Tennessee (Chappill 1995). *Sophora tomentosa* (section Sophora) first appears in the fossil record during the Oligocene (30 Ma.) in Mexico. Phylogenetic reconstruction of the *Sophora* sequence data places *S. japonica* at the approximate "root" of the tree, with the sections Sophora and Edwardsia appearing to be younger, respectively. Therefore the New Zealand species would not be expected to have originated until well after the Oligocene, and explanations invoking long-distance dispersal of buoyant seeds must account for their present biogeographical distribution.

Hypothesis Two: Miocene dispersal of Sophora tetraptera to New Zealand.

Sophora tetraptera is often considered to be the most ancestral New Zealand taxon, which arrived by drift-dispersal of buoyant seeds. The species shares a close morphological similarity with *Sophora howinsula*, from Lord Howe Island, and phylogenetic analysis supports a close evolutionary relationship between these two taxa.

Evidence from the fossil record, and now phylogenetic estimates, suggest that *Sophora tetraptera* arrived in New Zealand relatively recently. Molecular clock estimates suggest a late Miocene divergence from other Edwardsia (about 9 million years ago), which considerably predates its

appearance in the New Zealand fossil record. No information is currently available to suggest the ultimate island of origin for this species, and a more extensive sampling of the Edwardsia taxa is required to determine its closest living relatives. Former predictions based on chemotaxonomy and morphology placed *Sophora tetraptera* at the ancestral base of New Zealand taxa, however, phylogenetic analyses consistently grouped the Chatham Island taxa with or outside *S. tetraptera* and *S. howinsula*.

The Pacific island *Sophora raivavaeensis* also appears to be morphologically similar to *Sophora tetraptera*, and there was a small amount of support from bootstrapping and maximum likelihood for it being ancestral to the rest of the southern taxa sequenced in this study. Future phylogenetic work should investigate the entire range of species within the section, so that hypotheses regarding the ultimate origin of New Zealand taxa can be more fully tested. Owing to their perhaps recent diversification, analyses were unable to completely resolve the phylogeny of the group, and the question of the most ancestral taxon remains unanswered.

Hypothesis Three: New Zealand Origin and Diversification of Sophora microphylla.

Several workers consider that *Sophora microphylla* originated in New Zealand and has subsequently dispersed to its other locations in the Pacific, Chile, and Gough Island in the Atlantic Ocean by drift dispersal of seeds. Under this hypothesis, it was expected that the south-temperate islands would be colonized in a general west to east pattern, following the course of the Westerly Wind Drift. If this assumption is correct, then the age of *Sophora microphylla* populations would follow an approximately linear pattern across the Pacific and Antarctic Oceans (Easter Island, Juan Fernandez, Chile, Gough Island). Phylogenetic analyses were not able to resolve the times of origin of different *Sophora microphylla* populations, and thus this study is unable to test this or other hypotheses suggested by Markham and Godley (1972).

Phylogenetic analysis tended to support a New Zealand radiation and dispersal since the early Pliocene, but were not able to directly resolve the origin of *Sophora microphylla* in New Zealand. Spectral analysis of New Zealand taxa placed the Chatham Island population outside *Sophora tetraptera*, which could be interpreted as a dispersal of S. microphylla ancestors to New Zealand. Further, the 6 bp deletion found in *S. howinsula, S. tetraptera* and the Canterbury population of *S. microphylla* suggests a close evolutionary relationship between these taxa,

unless the deletion is considered to be independently derived in the Canterbury taxa. No conclusions were able to be drawn regarding the exact step-wise colonization of Pacific and Atlantic oceanic islands and Chile. *Sophora prostrata* and *Sophora raivavaeensis* consistently group within the *Sophora microphylla* complex, which appears to comprise an essentially homogenous group. This is consistent with a relatively recent diversification within *Sophora microphylla*, and under this hypothesis it is concluded that not enough time has elapsed yet for genetic differentiation between individual populations.

Hypothesis Four: Pliocene Origin of Sophora prostrata.

The endemic shrub - *Sophora prostrata* - has non-buoyant seeds, and must be considered to be derived from a buoyant New Zealand ancestor. Under the climate hypothesis, it was suggested that *Sophora prostrata* diverged recently in New Zealand, perhaps in response to climatic and geologic changes during the Pliocene. On the basis of phylogenetic analyses, it appears more likely that *Sophora prostrata* is derived from a *Sophora microphylla* ancestor (rather than hybridizing and giving rise to *S. microphylla*), and it may not yet be sufficiently genetically distinct from other populations of *S. microphylla* to be regarded as a separate species. The suggestion is in conflict with previous chemotaxonomic studies, which were able to distinguish between these two species based on seed and leaf constituents (Briggs & Rickett 1937; Markham & Godley 1972).

Variation of Evolutionary Rate.

The different plant genomes have vastly different rates of evolution. Some sequences, such as the coding regions of ribosomal DNA, are evolutionarily conservative and variation is phylogenetically informative. Genomic DNA may be single copy, mid-repetitive or highly repetitive. Sequences can be coding, non-coding or regulatory, and these have different functional constraints and hence different rates of evolution and levels of variation.

Substitution or mutation rates probably do not remain constant throughout a phylogeny and through branches within a phylogeny. This study suggests that heterogeneous evolutionary rates occur within the genus *Sophora*, between comparatively much older and younger lineages in the tree. Neighbour-joining methods, and other distance-based methods, suggest the possibility of a "slowing" of evolutionary rate between Edwardsia taxa, particularly in respect to *Sophora*

tomentosa. Differential rates of evolution between *Clianthus* and *Carmichaelia* were also suspected by observed differences in branch lengths under these phylogenetic models. In general, it is impossible to decide whether a branch is short because it represents a short period of evolutionary time, or because a slow-down in evolutionary rate has occurred, unless a perfect molecular clock is assumed. Phylogenetic reconstruction can be difficult when rates of evolution differ greatly among lineages, because the effect of such rate variation upon the expected estimate of branch length (or evolutionary distance) is difficult to assess (Sanderson & Donoghue 1996). It would be useful to incorporate any rate variation into the phylogeny-building algorithms, both to remove bias from the estimate of average rate and to characterize further the evolutionary relationships among the sequences. Methods to estimate temporal variation of rate are needed, as such variation is difficult to estimate using present models which incorporate just one rate of mutation (*e*.g. transition and transversion rates assumed equal).

Hypotheses for Testing the Origins of New Zealand *Sophora*.

The phylogenetic relationships of New Zealand *Sophora* are largely unresolved by the current sequence data, but they support the possibility of a longer history in New Zealand than indicated by the fossil record. Analysis of the sequence data also suggests a rapid divergence of Edwardsia with an adaptive radiation still in progress in the *Sophora microphylla* complex. Whether the short edge lengths in trees represents a change in evolutionary rate or short periods of evolutionary time requires additional sequence information. Strategies to test the four hypotheses are discussed in Chapter Four. Regardless of which hypotheses receive the most support, the evolutionary history of the New Zealand, and probably other Edwardsia taxa, are much more complex than previously imagined, and offer many opportunities for more detailed investigations of evolution and dispersal in this group.

Testing the Hypotheses: What Other Plants to Sample?

Where did the New Zealand species originally disperse from? Suggestions can be made from the present distribution of the Edwardsia, using knowledge of the principle ocean currents and dispersal abilities of the species. Molecular data provide the most reliable indicator of the degree of phylogenetic divergence (*e*.g. Wayne *et al.* 1989). Studies including all 17-20 species and subspecies in the section Edwardsia, from Chile, Hawai'i, Reunion, Marquesas, Rapa, other

Pacific islands, Lord Howe Island, New Zealand, and Chatham Islands is essential for testing hypotheses about the phylogeny of the group. Molecular data can also be used to investigate questions relating to the deeper relationships within the genus *Sophora*. For example, which of the other eight sections of *Sophora* are most closely related to the Edwardsia? These sorts of questions are most accessible through the use of molecular hypotheses.

Testing the Hypotheses: What Other Genes to Sample?

Maternal inheritance, lack of conversion and intragenic recombination, and the great number of plastids in leaf cells (Clegg *et al.* 1986) make cpDNA a simpler system than the nuclear genome to investigate at the DNA sequence level. Faster evolving gene regions are required to test hypotheses about divergence times between the closely related *Sophora* species and subspecies however. Studies using Restriction Fragment Length Polymorphism (RFLP), such as those by Echt & McCoy (1989) and Apuya and co-workers (1988), have found that these provide useful genetic markers at the species level in plants. Hypervariable sequence analysis (DNA fingerprinting) could greatly reduce the labour and expense of detailed RFLP studies (Rieseberg & Brunsfeld 1992). Hypervariable loci are regions of the nuclear genome that contain tandem repeats of short DNA segments (minisatellites). At present the use of cpDNA in intraspecific (population) studies has not reached the same potential as mtDNA has in similar animal studies, but recent work is encouraging (Rogstad *et al.* 1988; Avise 1994; Hillis *et al.* 1996).

One approach to reconstructing phylogenies among genera and at higher taxonomic levels has been the analysis of the distribution of major structural rearrangements in the chloroplast genome (see Palmer *et al.* 1987; Downie & Palmer 1992; Doyle *et al.* 1996). Recent work has found that a large (50-kb) inversion occurs in most taxa of the subfamily Papilionoideae, relative to the gene order found most commonly among land plants (Doyle et al. 1996). Two papilionoid tribes, Swartzieae and Sophoreae, were heterogeneous for this inversion, which is consistent with a number of lines of evidence suggesting the polyphyly of these tribes (Doyle *et al.* 1996). This study by Doyle and co-workers sampled four different genera within the Sophora group (tribe Sophoreae), but did not use the genus *Sophora* - which could be included in further work.

The Potential of *Sophora microphylla* For Population Studies.

One of the great problems of biology is how a continuous process of evolution can produce the morphologically discontinuous groups known as species. Despite its title, *On the Origin of Species* made few inroads on this problem. The determination of genetic variation among individuals, populations and species is central to the study of evolution. The *Sophora microphylla* complex provides a group of organisms which have recently speciated, and studies of genetic variation within and between populations of this ecologically and geographically distinct species may be informative in this respect. The Hawaiian species, *Sophora chrysophylla*, appears to have similarly radiation there - and taxonomic classification is extremely complex (Chock 1956).

Rattenbury (1962) describes the situation in which there is an alternating fluctuation in the proportions of allelic combinations (and therefore of characters) in a group of interbreeding organisms, which corresponds with an approximately simultaneous alternation in environmental conditions such as, for example, the climatic fluctuations which occurred in the Pleistocene (see also Ogden 1989). This diversity may be critical in allowing communities to maintain functional integrity during prolonged drought or other adverse climatic conditions. If the current distribution of phenotypes and genotypes in *Sophora microphylla* is due in part to post Pleistocene dispersal then sequence analysis of the most variable region of cpDNA would be suitable for studying this. Some chloroplast sequences, such as minisatellites, are much more variable than the cpDNA region used in this study- and provide genetic markers that can be used to gain insights into the clonal biology of plants.

Time of Origin of Divaricating plants in New Zealand.

Cooper and Millener (1993), suggested that the time of origin of a divaricating plant and a non-divaricating relative would provide a useful test between the climate and moa-browsing hypotheses for the origin of the divaricating plant growth form in New Zealand. The New Zealand *Sophora* appeared to be a useful genus for testing hypotheses, because they comprise a tree, a divaricating shrub, and a tree with a divaricating juvenile. However, the fossil record, and now divergence estimates based on sequence analysis suggest that they were not present in New Zealand before the Pliocene, and this precludes their usefulness as a test between the moa-browsing and climate hypotheses.

Future tests between the two hypotheses should concentrate on plant genera whose ancestors are known to have been present during the Oligocene and Miocene, the period of "adaptive radiation" in the *moa* (Cooper *et al.* 1992). Some useful genera may include *Coprosma* (with 28 divaricating species and 21 non-divaricating species), and *Olearia* (with 24 divaricating and 7 non-divaricating species), both of which are found in pollen records dating back to the Oligocene (37-25 Ma.). The pair-wise dating of times of origin of a divaricating and non-divaricating species from several different genera can be used to test whether divarication arose in the New Zealand flora in response to one or more factors of the biotic and/or abiotic environment.

Testing the times of origin of a wide range divaricating species can also be used to test whether divarication arose 'simultaneously' in the New Zealand flora, or whether the appearance of this growth form in widely different genera and families arose less steadily over a longer geological and biological period of time. We might expect variations in the time of origin of this growth form between the genera *Coprosma* and *Sophora*, for example, as the former have been present in New Zealand for much longer periods of geological time, and have a much greater number of divaricating species. If this plant growth form appears sporadically over a long period of time, then it may be difficult to accept the influence of only one factor in their selection.

Conservation of *Sophora microphylla*.

Much conservation work has been focused on the Easter Island *Sophora toromiro*, which was thought to be extinct since 1956 (*e*.g. Lucas & Synge 1978; Schlatzer 1965; Weimark 1984; Lobin & Barthlott 1988; see also Christensen & Schlatzer 1993). The plant was rescued from extinction however, and plants grown from seeds collected on Easter Island by Thor Heyerdahl in 1955, Carlos Munoz Pizarro in 1935, and Professor Macmillan Brown in 1942, have been found growing in a number of Botanic Gardens around the world (Goteborg - Sweden; Vine del Mar - Chile; University of Bonn - Germany; Christchurch - New Zealand). Several attempts have been made to reintroduce the species back to Easter Island (Schlatzer 1965; Lobin & Barthlott 1988). However, Godley (1989) does not consider *Sophora toromiro* to be a distinct species, but rather a geographical race of the polymorphic New Zealand species, *Sophora microphylla*. Thus the genetic distinctiveness of island populations clearly have important implications for conservation priorities (see Avise 1989b).

The New Zealand populations of *Sophora microphylla* appear to be undergoing adaptive radiation, and this study confirms that some populations have differentiated genetically. Of particular interest is the phylogenetic separation of *Sophora microphylla* var. *chathamensis* from the rest of the *Sophora microphylla* complex. Further studies are required, using different genes and genomes, to determine whether the Chatham Islands population actually deserves subspecific status. The New Zealand *Sophora* have good potential for soil and bird conservation, and could be replanted in many areas along stream banks, retaking the role now performed by willows. Syrett *et al.* (1995) have discovered that *kowhai* is also a potential possible alternative host plant for the stem-mining weevil (*Pirapion immune*), which was being investigated as a possible biological control agent for European broom, *Cystisus scoparius* (L).

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