Herbivory in Antarctic fossil forests and comparisons with modern analogues in Chile

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The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

During the Tertiary (~ 50 million years ago) forests were present in Antarctica, but fossil evidence of insect life in the forests is rare. Extensive fossil floras from Antarctica contain evidence of insect herbivory on the leaves; these provide indirect evidence of past insect life. Such preservation of the behaviour of insects (insect trace fossil) can be used to examine the diversity of insects that lived in the forests of Antarctica in the past.

Palaeogene (65 Ma - 35 Ma) fossil floras from two localities on the Antarctic Peninsula (King George Island and Seymour Island) were examined for the presence of insect trace fossils. Fossil leaves were preserved as impressions and compressions within siltstones and sandstones and represent leaves that were preserved within a quiet lake environment (King George Island) or shallow marine setting (Seymour Island). The floras were dominated by leaf morphotypes that resemble modern Nothofagaceae (Southern beeches), but leaves similar to other Southern Hemisphere families were also present, including the Cunoniaceae, Proteaceae and Lauraceae.

Over 2,000 fossil leaves were examined for traces of past insect activity. Over 150 fossil leaves (6.9%) contained evidence of feeding traces on the leaves (54 trace types from King George Island and 19 from Seymour Island). The trace fossils were grouped into four functional feeding types: general leaf chewing, skeleton feeding, leaf mines and leaf galls. General leaf chewing was the most common trace type at both localities and leaf mines the least common.

The nearest living analogues of the Antarctic Palaeogene forests are the Valdivian and Magellanic forests of Chile and so insect activity in these forests was studied in order to understand past insect activity in Antarctica. The diversity of insect traces in the Chilean forests was investigated at six sites within National Parks, covering a latitudinal range between 37°S and 55°S. Insects associated with two deciduous species, *Nothofagus pumilio* and *Nothofagus antarctica*, were of particular focus. The factors that affected the level of insect damage and the proportion of leaf mines and galls included height within the tree, orientation of leaf within the tree, altitude, season, leaf age, latitude, plant species and insect species.

Insects which created similar general leaf chewing traces in the modern forests in Chile similar to those on the fossil leaves were larvae of Lepidoptera (Geometridae), Hymenoptera (Symphyta) and the larvae and adults of Coleoptera (Chrysomelidae, Curculionidae, Cerambycidae). Leaf mines were created by species of Coleoptera and Hymenoptera. Species of Diptera (Cecidomyiidae) and Hymenoptera (Cynipidae) created leaf galls. Other invertebrates (Acari (Eriophyidae) and Nematoda (Tylenchida)) also created leaf galls in Chile, similar to fossil leaf galls from Antarctica.

Herbivory types on the fossil flora from King George Island were most similar to modern types in Puyehue (a northern study site, Chile) and those from Seymour Island were most similar to Torres in the south, indicating a possible climatic control on their distribution. Based on this relationship, estimates of palaeoclimate of Antarctica suggest that the climate of King George Island to the west of the Peninsula was warmer and wetter (3.5°C – 10.4°C mean annual temperature, 3.5°C -

24.3°C maximum and minimum mean monthly temperature and 1500 mm annual precipitation) than the cooler and more stable environment at Seymour Island to the east (3.5°C - 10.4°C mean annual temperature, -0.4°C - 16°C maximum and minimum mean monthly temperature, and 570 mm annual precipitation).

The studies of fossil and modern insect traces in Antarctica and Chile have provided a unique opportunity to reconstruct past insect life of Antarctica during the Palaeogene. This is the first documented evidence of insect life during the Palaeogene on Antarctica and highlights the value of modern analogue comparisons to obtain a greater insight into past insect ecology.

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Chapter 1. Introduction

1.1. Background

Today most of continental Antarctica is permanently covered by snow or ice, and only about 0.3 % consisting of ice-free terrestrial habitats (Convey & Stevens, 2007), restricted to the coasts and inland refuges (nunataks). The majority of terrestrial habitats are covered in snow for most of the year and are subject to widely variable, unpredictable weather conditions, both in the short term (daily or weekly) and seasonally (Convey, 1997). Therefore, it is not surprising that there are only two species of insects known to be living in Antarctica, both flightless midges (Diptera: Chironomidae) (Ashworth & Kuschel, 2003). They occur only along the northwest coast of the Antarctic Peninsula (Figure 1.1) under warmer and wet conditions, and currently none have been found in continental Antarctica. The only arthropods with adaptations to survive the low temperatures and aridity of these southerly latitudes are springtails (Collembola) and oribatid mites (Acari) (Convey, 1997). Four species of mites in the genus *Maudheimia* are known to inhabit nunataks on the continental interior. The genus is endemic and is thought to be a relict from the ancient Gondwana fauna (Marshall & Pugh, 1996; Marshall & Coetzee, 2000).

The Antarctic flora today is also sparse. It is dominated by cryptograms that mostly grow in sheltered coastal locations north of 65°S. Only two species of vascular plants are known: a grass, *Deschampia antarctica* (Poaceae) and a herbaceous carophyllid, *Colobanthus quitensis*l (Caryophyllaceae) (Ashworth & Cantrill, 2004). Both species can be found on islands of the Scotia Arc, along the western coast of the Antarctic Peninsula, and southwards to their current southernmost site on Alamode Island, Terra Firma Islands (68°43'S 67°31'W)(Figure 1.1). Both are considered to have colonised the Antarctic Peninsula during the Holocene. Such low floral diversity is however, in marked contrast with the rich fossil record of Antarctica.

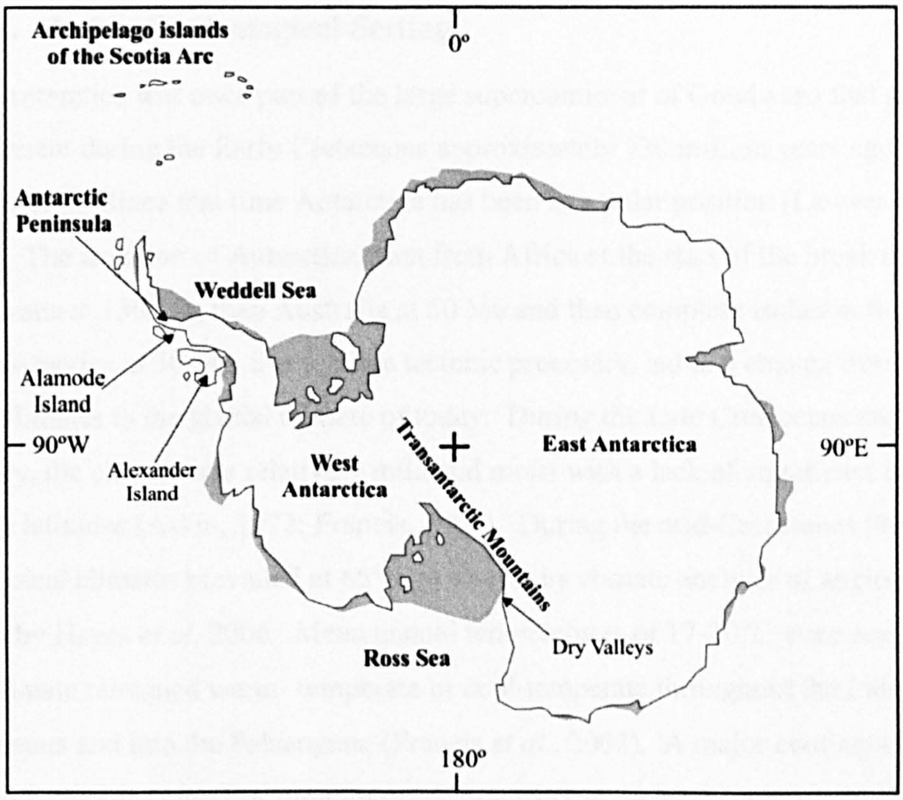


Figure 1.1. Map of Antarctica indicating the localities mentioned in section 1.1.

Plant fossils from Antarctica are common (see section 1.1.2), but insect fossils are extremely rare, especially insect body fossils. A record of past insect life is however, preserved indirectly on fossil leaves in the form of traces that insects created on leaves. The aim of this thesis is thus to study herbivore traces on Palaeogene fossil leaves to determine the diversity and intensity of herbivory created by insects that were present in the ancient forests of Antarctica. The Palaeogene is an interval of geological time that covers the Palaeocene, Eocene and Oligocene in 65 – 24 Ma. It is used in this thesis to describe the age of the fossils in this study, although most of the leaves are of Palaeocene and Eocene age.

1.1.1. Antarctic Geological Setting

Antarctica was once part of the large supercontinent of Gondwana that started to fragment during the Early Cretaceous approximately 130 million years ago (Figure 1.2). Since that time Antarctica has been in a polar position (Lawver *et al.*, 1992). The isolation of Antarctica, first from Africa at the start of the break up of Gondwana at 130 Ma, then Australia at 50 Ma and then complete isolation from South America at 30 Ma, due to plate tectonic processes, led to a change from warm polar climates to the glacial climate of today. During the Late Cretaceous and early Tertiary, the climate was relatively mild and moist with a lack of significant ice even at high latitudes (Askin, 1992; Francis, 1996). During the mid-Cretaceous (80 Ma), subtropical climates prevailed at 65°S, as shown by climate analysis of angiosperm leaves by Hayes *et al.* 2006. Mean annual temperatures of 17-20°C were proposed. The climate remained warm- temperate or cool-temperate throughout the Late Cretaceous and into the Palaeogene (Francis *et al.*, 2008). A major cooling was seen in global records at the Eocene-Oligocene boundary at 34 Ma (Zachos *et al.*, 2001), when geological evidence from Antarctica and proxy climate records from marine oxygen isotopes indicate the first appearance of extensive ice on Antarctica (Francis *et al.*, 2008).

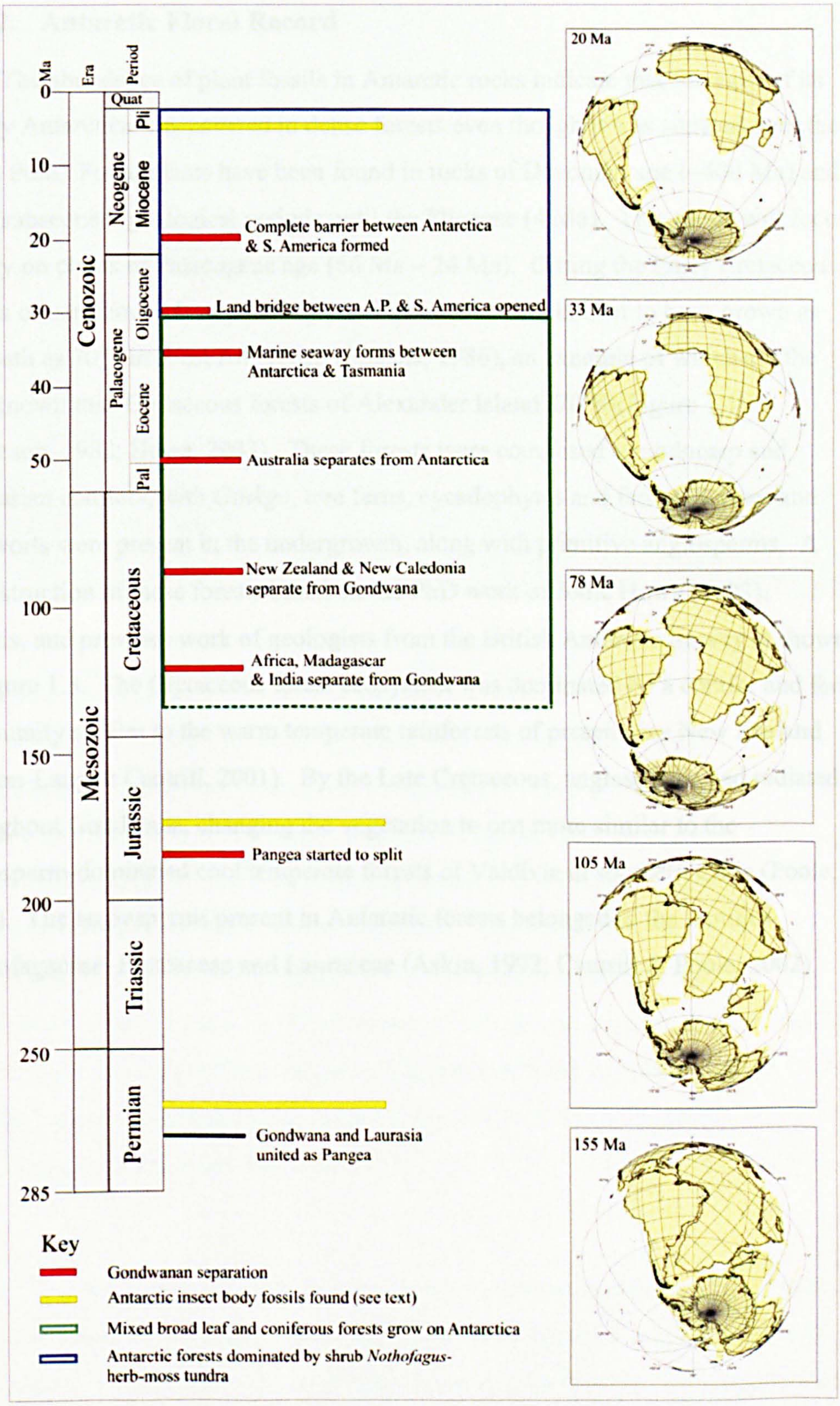


Figure 1.2. The separation of the supercontinent Gondwana through geological time and the main times when Antarctic insect body fossils were found. The major change in forest type on Antarctica is also shown. Abbreviations: Pal = Palaeocene, Pli = Pliocene and Quat = Quaternary. Palaeogeography maps provided by P. Markwick. See text for source references about geological history.

1.1.2. Antarctic Floral Record

The abundance of plant fossils in Antarctic rocks indicate that for most of its history Antarctica was covered in dense forests even though it was situated over the South Pole. Fossil plants have been found in rocks of Devonian age (~400 Ma) and in all subsequent geological periods until the Pliocene (4 Ma). This thesis will focus mainly on plants of Palaeogene age (66 Ma – 24 Ma). During the Early Cretaceous, forests of conifers such as podocarps and araucarians are known to have grown as far south as 70°S in West Antarctica (Francis, 1986), an example of which are the well known mid-Cretaceous forests of Alexander Island (70°S)(Figure 1.1) (Jefferson, 1982; Howe, 2003). These forests were composed of podocarp and araucarian conifers, with *Ginkgo*, tree ferns, cycadophytes and ferns. Mosses and liverworts were present in the undergrowth, along with primitive angiosperms. A reconstruction of these forests based on the PhD work of Jodie Howe (2003), Francis, and previous work of geologists from the British Antarctic Survey is shown in Figure 1.3. The Cretaceous forest ecosystem was dominated by a conifer and fern community similar to the warm temperate rainforests of present day New Zealand (Falcon-Lang & Cantrill, 2001). By the Late Cretaceous, angiosperms had radiated throughout Gondwana, changing the vegetation to one more similar to the angiosperm-dominated cool temperate forests of Valdivia in southern Chile (Poole, 2003). The angiosperms present in Antarctic forests belonged to the families Nothofagaceae, Proteaceae and Lauraceae (Askin, 1992; Cantrill & Poole, 2002).

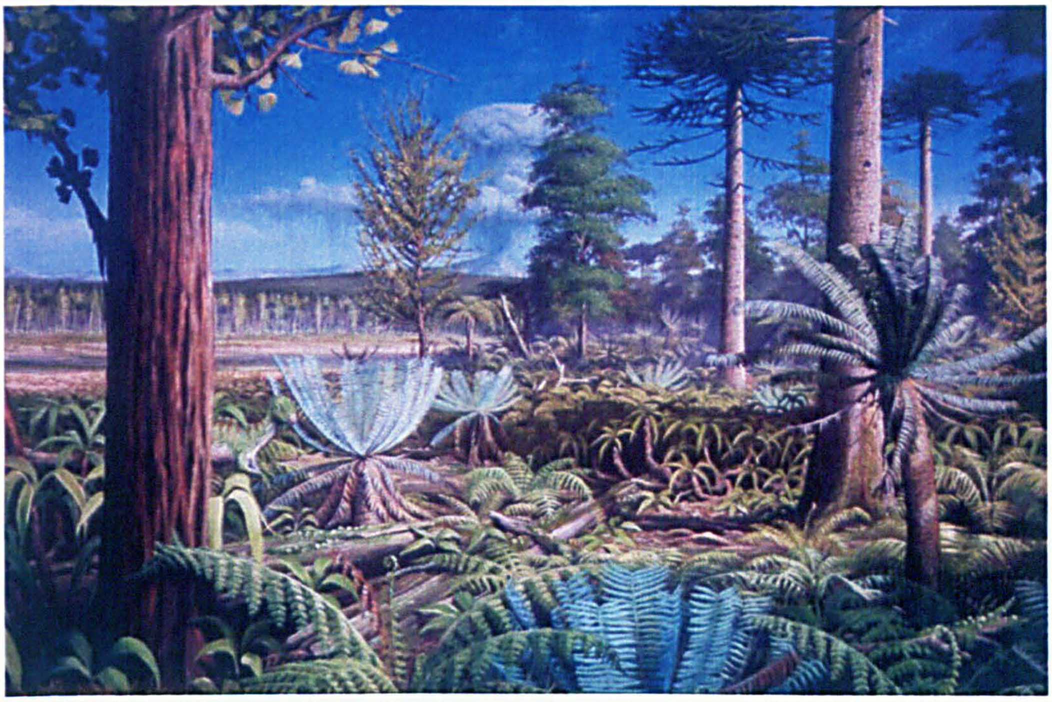


Figure 1.3. Reconstruction of the mid-Cretaceous forests of Alexander Island. Painted by artist Rob Nicholls and based on the PhD work of Jodie Howe (2003), J. Francis, and previous work of geologists from the British Antarctic Survey.

A major taxonomic turnover occurred with the appearance of the Nothofagaceae in the Late Cretaceous, an angiosperm family that became a characteristic component of modern Southern Hemisphere floras (Cantrill & Poole, 2005). Along with the Nothofagaceae, the Laurales and Cunoniaceae were important components of the angiosperm floras. The Nothofagaceae rapidly increased in abundance so that nearly half of the fossil angiosperm wood in Campanian strata (84 Ma – 74 Ma) belongs to *Nothofagus,* southern beech (Cantrill & Poole, 2005). This indicates major changes to the forest structure, with the canopy becoming increasingly dominated by a few taxa and with species of Nothofagaceae being most important.

The onset of glaciations in Antarctica at 34 Ma and the cooling of the climate had significant consequences for the vegetation. The diverse floras of the Eocene were lost and cool to cold-temperate *Nothofagus* species dominated the vegetation, along with mosses and ferns. Prior to the Oligocene, the floras were relatively diverse, but after the major cooling at the Eocene/Oligocene boundary, the record of fossil vegetation is sparse with only a few leaves of *Nothofagus* documented.

Isolated leaves of *Nothofagus* were found in rock cores of Oligocene and Miocene age (Cantrill, 2001). Recently, *Nothofagus* leaves were found in Miocene sediments in the Dry Valleys region of Antarctica (Ashworth *et al.*, 2007). Fossil wood and leaves of *Nothofagus* show that tundra vegetation was able to survive as far south as 80°S, only 300 miles from the South Pole in the Pliocene (Francis & Hill, 1996; Ashworth & Cantrill, 2004).

1.1.3. Fossil Insects of Antarctica

Insects first evolved around 390 million years ago during the Devonian and by the Late Carboniferous many winged insect forms were present that represent most of the major extant orders (Grimaldi & Engel, 2005). They reached a peak in familial diversity during the Permian. An increase in the diversity of insects occurred in the Triassic, a time of change from the wet humid climates of the Permo-Carboniferous in the Northern Hemisphere (cool post-glacial climates in the Southern Hemisphere). Triassic climates were much drier and warmer than the preceding Permian, and the floras changed from those dominated by humid-loving plants to waxy conifers and ferns that were adapted to arid climates. The first modern insect families such as Tipulidae (Diptera), Staphylinidae (Coleoptera), Belostomatidae and Naucoridae (Heteroptera) and Xyelidae (Hymenoptera), to name a few, appeared in the Triassic (Grimaldi & Engel, 2005).

During the Jurassic (~150 Ma), insects evolved that belonged to extinct families or to stem groups of basal recent families (Grimaldi & Engel, 2005). The Cretaceous (141-65 Ma) is one of the best geological periods for fossil evidence of insects with at least twenty-five major deposits worldwide (Grimaldi & Engel, 2005). Most recent families of insects first appeared at this time and groups such as the schizopharan Diptera radiated. During the Cretaceous, the angiosperms, the predominant plant group on land, originated and diversified. It is thought that as insects are closely associated with flowering plants in many ways, such as pollinators and phytophages of angiosperms, this radiation may have facilitated the radiation of the insects (Mitter *et al.*, 1988). When the rates of diversification of insect families are examined, however, it seems that they do not match the rates of angiosperm radiation. The post-Palaeozoic radiation of the insects appears to have started more than 100 million years before the angiosperms appeared in the fossil record (Labandeira & Sepkoski Jr., 1993).

In Antarctica the oldest insect fossils are Permian in age (Figure 1.4). Three documented Permian insect sites in Antarctica have yielded mayfly nymphs (Ephemeroptera*)* and several homopteran insect wings have been found (Carpenter, 1969; Tasch & Riek, 1969; Tasch, 1971). Jurassic deposits have preserved a single odonata wing, mayfly nymphs and a coleopteran elytron in Southern Victoria Land (1973). Elytra of this type are common in Mesozoic deposits globally (Tasch, 1973). The only published record of an insect body fossil from the Antarctic Peninsula (Figure 1.4) is by Zeuner (1959), of coleopteran fragments identified as aquatic beetles from the Jurassic Mount Flora Formation, Hope Bay.

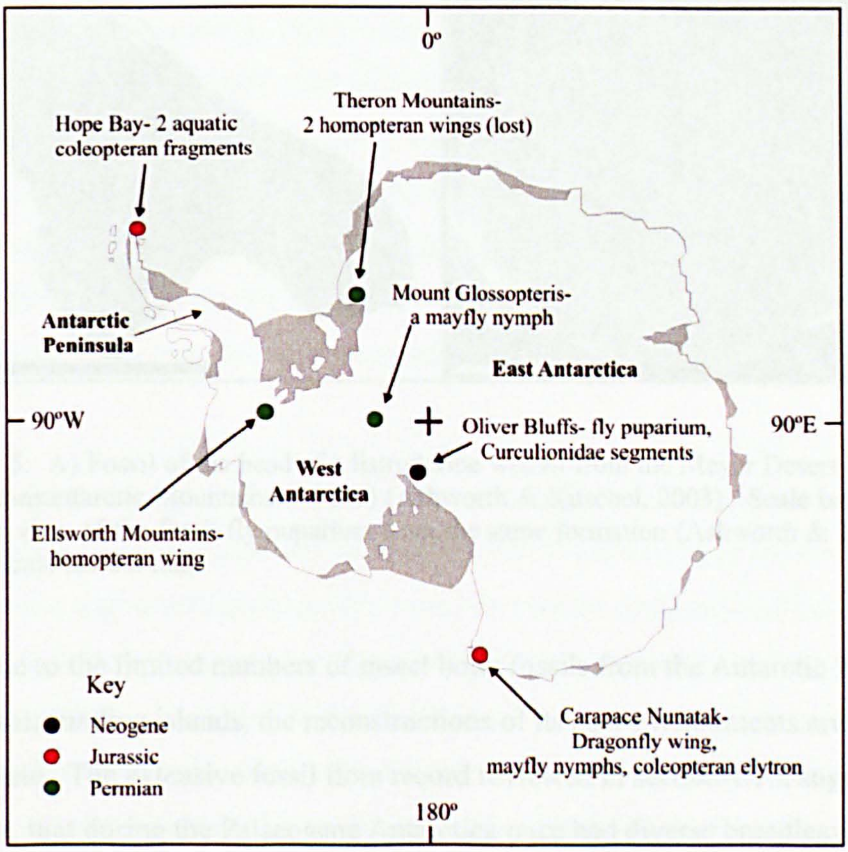


Figure 1.4. Map of Antarctica showing the areas in which insect body fossils have previously been found and from which time period. The homopteran wings from the Theron Mountains were lost prior to formal identification, although they were documented to have been homopterous (Carpenter, 1969).

In younger deposits, insect body fossils have been found on East Antarctica (Figure 1.4). For example, a single leg segment of a weevil was found in Pliocene deposits of the Sirius Group sediments (Ashworth *et al.*, 1997), and two other species of fossil listroderine weevils (Coleoptera: Curculionidae: Rhytirhinini: Listroderina) are known from the Meyer Desert Formation in the Transantarctic Mountains (~5 Ma)(Ashworth & Kuschel, 2003) (Figure 1.5A). It was suggested that the weevils were the descendants of Gondwanan species that lived in Antarctica continuously from the Late Cretaceous or Early Palaeogene until they became extinct in the Neogene (Ashworth & Kuschel, 2003). This is also supported by the discovery of a fossil fly puparium (Diptera: Cyclorrhapha) from the same formation (Ashworth & Thompson, 2003)(Figure 1.5B). The extinction of such insects from Antarctica coincided with the expansion of the ice sheets and the onset of extreme glacial climates.

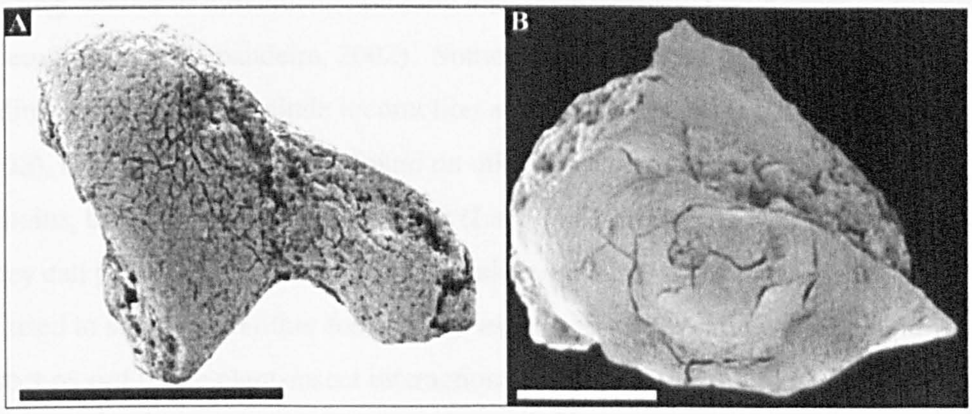


Figure 1.5. A) Fossil of the head of a listroderine weevil from the Meyer Desert Formation in the Transantarctic Mountains (~5 Ma) (Ashworth & Kuschel, 2003). Scale bar 1 cm. B) Posterior view of the fossil fly puparium from the same formation (Ashworth & Thompson, 2003). Scale bar 0.5 mm.

Due to the limited numbers of insect body fossils from the Antarctic Peninsula and its surrounding islands, the reconstructions of its past environments are incomplete. The extensive fossil flora record reviewed in section 1.1.2 suggests, however, that during the Palaeogene Antarctica once had diverse broadleaved and coniferous forests growing there. In modern systems such environments support a rich insect fauna, with abundant evidence of plant-insect interactions found on the leaves. Therefore, it is more than likely that insects thrived in Antarctica in such forests and that additional evidence for insect life can now be obtained by studying the fossil plants. Globally, the first evidence of feeding on living plants occurred during the Carboniferous. By the Late Carboniferous there is evidence of diversification of plant-insect interactions (Scott, 1991; Scott *et al.*, 1992). By the Cretaceous the majority of different feeding types on leaves had evolved (Scott *et al.*, 1992; Labandeira, 1998, 2002). Therefore, it is likely that examination of the Palaeogene flora should yield new evidence of such plant-insect interactions in Antarctica.

1.2. Evidence of plant-insect interactions (herbivory) of the past

The study of past plant-insect interactions is possible through examination of trace fossils. Insect trace fossils represent the preservation of an insect's behaviour ranging from its reproduction technique (Genise *et al.*, 2007); how it survived by feeding, shelter or movement; and how the insect interacted with other organisms in its environment (Labandeira, 2002). Some trace fossils can be preserved within the sediment itself; these include locomotion and resting traces (Smith & Hasiotis, 2008), or are secondary traces found on other preserved materials such as plant remains, bones or vertebrate coprolites (Laza & Reguero, 1990; Britt *et al.*, 2008). They can provide the sole evidence for palaeoecological interpretation or they can be used to supplement other fossil material. Trace fossils can provide a unique and direct record of the plant-insect interactions in the past (Grimaldi & Engel, 2005).

Although the trace fossils may have been created by both vertebrates and invertebrates, insects are however the major group of herbivores in modern forests, and, because they create characteristic marks that can be distinguished from other organisms, it can be assumed that the trace fossils on fossil leaves were mainly created by insects. Trace fossils can sometimes indicate the type of insect that made the trace, to order if not to family level, by the shape, size and position of the damage. When considering insect damage on the plant, there are a range of traces that can be preserved, such as mines on the leaves, chewing marks on the leaves and stems, galls and wood borings. Examples of trace types on leaves are described in the following sections and a summary of the fossil record of those insect trace types that are the focus of this study is shown in Figure 1.6.

1.2.1. Types of herbivory trace

1.2.1.1. General Leaf Chewing

General leaf chewing refers to feeding on the leaf, which can be marginal, in a continuous or discontinuous manner, as well as non-marginal bullet-like holes (shot-hole) within the leaf laminae (Figure 1.6). Trace fossils displaying general leaf chewing provide the most common palaeontological evidence of plant-insect interactions (Scott & Paterson, 1984). The earliest known examples are on the Late Carboniferous seed fern *Neuropteris* and also on *Glossopteris*, small to large woody trees that were widespread throughout Gondwana during the Permian (Scott *et al.*, 1992). All samples studied showed marginal feeding, mostly of a continuous form (Figure 1.7A). Similar patterns were observed on Triassic and Jurassic fossil leaves, but the information is more limited. Evidence is more abundant on Cretaceous leaves, particularly on mid-Cretaceous leaves, which coincides with the evolution of the angiosperms (Labandeira *et al.*, 2002a; Labandeira *et al.*, 2002b). Various types of leaf feeding have been observed on Tertiary leaves, including examples of continuous marginal, discontinuous marginal and non-marginal feeding (Scott, 1991; Stephenson & Scott, 1992; Wilf & Labandeira, 1999; Currano *et al.*, 2008)(Figure 1.7B-H).

Non-marginal feeding is more difficult for an insect than marginal chewing as it requires specialised mouthparts (Bernays, 1991), therefore, it is seen as an advanced feeding strategy and a derived character. The fossil evidence seems to reiterate this, with an increase in not only the amount of leaf feeding from the Devonian to Tertiary, but also the complexity of the feeding habit. Edwards and Wratten (1980) observed that some taxa have specific modes of feeding, such as the Orthoptera (first appeared in the Late Carboniferous) that bite holes in the edges of the leaves, and the Curculionidae (a Coleopteran family known from the Jurassic), which are known to scoop out the edges of leaves at intervals. It is still however, the most difficult type of feeding damage from which to accurately identify the causal insect. In addition, care has to be taken that an overestimation of herbivory is not made because both aquatic and terrestrial invertebrates damage leaves and the damage can occur post leaf-fall (Scott & Titchener, 1999).

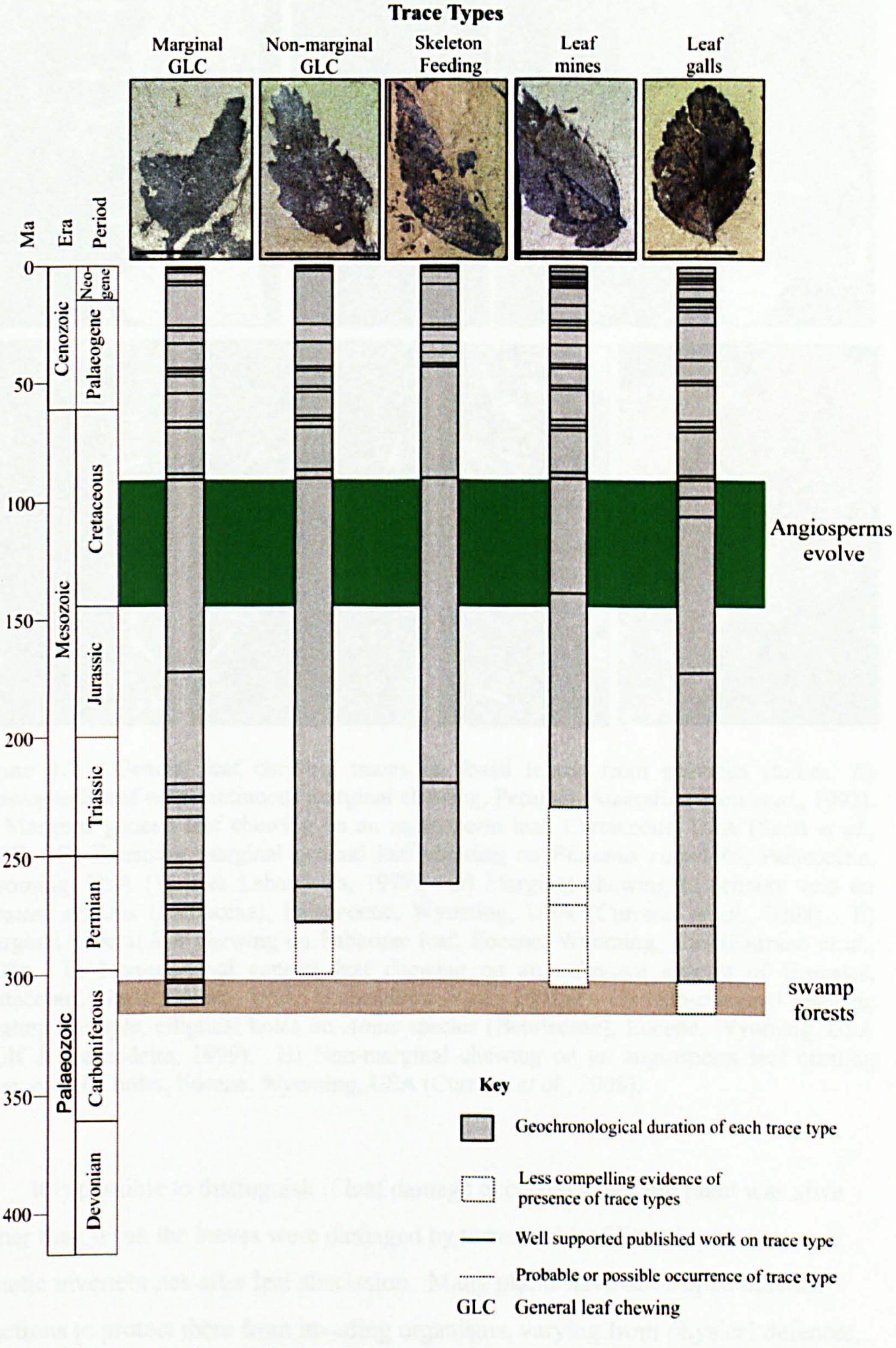


Figure 1.6. The fossil record of different insect traces on fossil leaves summarised from published work. Diagram is adapted from Labandeira (2002). Photograph scale bar 1cm. Photographs of insect traces are of fossil leaves from Antarctica that are examined in this thesis.

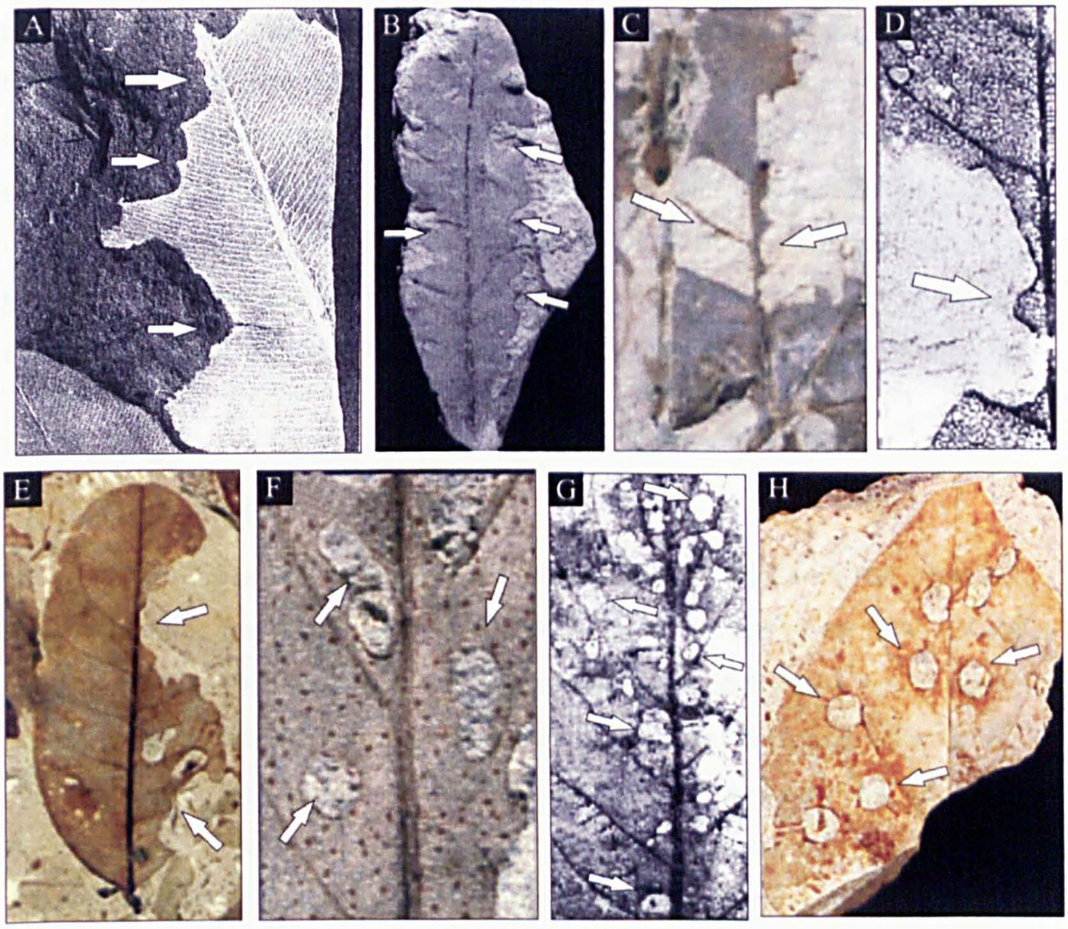


Figure 1.7. General leaf chewing traces on fossil leaves from previous studies. A) *Glossopteris* leaf with continuous marginal chewing, Permian, Australia (Scott *et al.*, 1992). B) Marginal general leaf chewing on an angiosperm leaf, Cretaceous, USA (Scott *et al.*, 1992). C) Extensive marginal general leaf chewing on *Platanus raynoldsi*, Palaeocene, Wyoming, USA (Wilf & Labandeira, 1999). D) Marginal chewing to primary vein on *Persites argutus* (Lauraceae), Palaeocene, Wyoming, USA (Currano *et al.*, 2008). E) Marginal general leaf chewing on Fabaceae leaf, Eocene, Wyoming, USA(Currano *et al.*, 2008). F) Non-marginal general leaf chewing on an unknown species of Urticales, Cretaceous, North Dakota, USA, (Labandeira *et al.*, 2002a). G) Non-marginal chewing creating multiple, elliptical holes on *Alnus* species (Betulaceae), Eocene, Wyoming, USA (Wilf & Labandeira, 1999). H) Non-marginal chewing on an angiosperm leaf creating large, circular holes, Eocene, Wyoming, USA (Currano *et al.*, 2008).

It is possible to distinguish if leaf damage occurred when the plant was alive rather than when the leaves were damaged by terrestrial leaf litter invertebrates or aquatic invertebrates after leaf abscission. Many plants have developed defence reactions to protect them from invading organisms, varying from physical defences, such as leaf spines and thorns on the stem, to production of noxious chemicals at the site of wounding (Strong *et al.*, 1984; Coley & Barone, 1996). A range of defences is involved that varies not only among species, but also differs with leaf age (Forkner *et al.*, 2004; Brunt *et al.*, 2006). Due to chemical defence systems, leaf tissue forms a darkened ridge at the site of damage, which hardens and may deter the organism from feeding further (Labandeira & Sepkoski Jr., 1993). This ridge can be seen in some fossil specimens, making it possible to determine that the damage occurred when the leaf was still alive. Generally, the wound reaction is best preserved on impression fossils, where it can be identified by a distinctive darker colour or indentation around the damaged area (Wilf *et al.*, 2001).

1.2.1.2. Leaf mines

Leaf mining is a highly specialised form of feeding behaviour that gives the insect protection from both predators and dehydration. A leaf miner is the collective name given to an insect whose larvae feed inside the parenchyma or epidermis of the leaves, creating distinctive channels. The adult insects lay their eggs either on the surface or inside the tissue of the leaf. The emerging larvae feed and develop within the leaf tissue where they may remain until metamorphosis or emerge to pupate. Alternatively, older insect larvae may complete their development in another area of the plant such as the petiole or stem, or feed outside of the mine (Hering, 1951). The insect produces a characteristic mine with a distinctive shape and form, allowing them to be identified in the juvenile stage. The larvae may leave behind faecal material within the mines, which can also help with identification. Leaf mining has evolved independently in several insect orders including the Lepidoptera, Diptera, Coleoptera and Hymenoptera.

The characteristics of the channel of the mine are dependent on the way in which the larva eats the plant tissue. It can be categorised into two main forms. If the larva only moves in one direction a linear (serpentine) mine is produced, however, if the larva eats in several directions a blotch mine is made (Hering, 1951). There are a variety of forms for each of the two main mine types. In addition, one species of insect may be able to create both types of mine, but at different stages of larval development. Often, leaf mines are initially linear and then form blotches as the larvae grow. Nevertheless, the identification of the leaf mining species is often possible due to the characteristic mine appearance produced, at least in well studied regions such as the United Kingdom.

Although many insect species have been identified as leaf miners in modern ecosystems, the fossil record of this habit is poor. The evidence for insect groups containing leaf mining species is limited before the Cenozoic, but some records suggest Late Carboniferous origins for both blotch and linear mines (Scott *et al.*, 1992). The leaf mining habit had however, certainly originated at least by the Early Cretaceous (Labandeira *et al.*, 1994). Figure 1.8 provides an example of the range of leaf mines on fossil leaves that have been previously described. Further evidence suggests that leaf mining may be one of the few types of plant damage that originated during the Mesozoic (Labandeira, 2002). The fossil record of mines may also reflect difficulties in the successful preservation of this trace type as both the leaves and the mines can lose key morphological characters during transport, decay and burial which can affect how they are interpreted. For example, shallow blotch mines are vulnerable because the top surface of the mine can easily be removed and the frass (insect faeces) detached, making them resemble surface feeding traces.

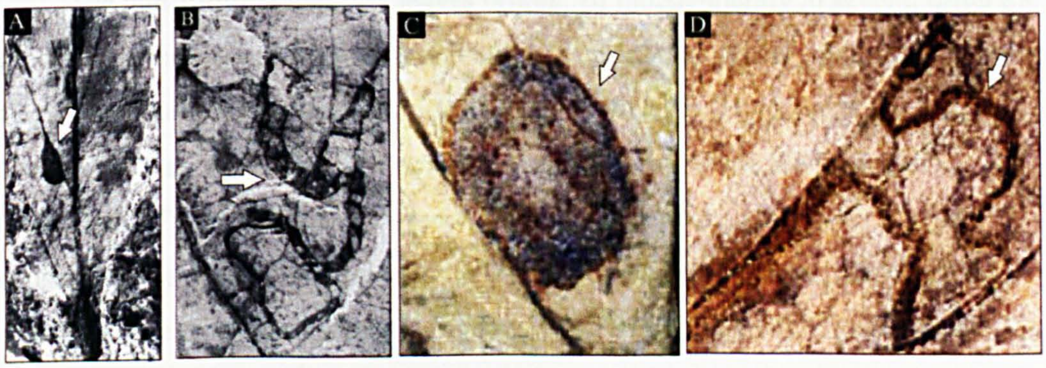


Figure 1.8. Leaf mines on fossil leaves from previous studies. A) Trumpet-type leaf mine on angiosperm leaf, Cretaceous, USA (Scott *et al.*, 1992). B) Serpentine mine on angiosperm leaf, Palaeocene, Wyoming, USA (Wilf & Labandeira, 1999). C) Blotch mine with distinct terminal chamber on an angiosperm leaf, Eocene, Wyoming, USA (Currano *et al.*, 2008). D) Serpentine mine with solid frass trail on an angiosperm leaf, Eocene, Wyoming, USA (Currano *et al.*, 2008).

1.2.1.3. Leaf galls

Galls are defined as any deviation in the normal pattern of plant growth produced by a specific reaction to the presence of a foreign organism (Bloch, 1965). The modified structure supplies the causal organism with both protection and nutrition throughout its development. In contrast to leaf miners, gallers obtain a continuous supply of food and shelter without the need to move to new feeding sites. The galling organism initiates extensive growth of the surrounding plant tissue, either by cellular hypertrophy, cell proliferation or by the appearance of new cellular differentiations (Stephenson & Scott, 1992). The galler controls the plant tissue growth, forming structures foreign to the plant. Galls are induced by a wide variety of organisms, ranging from viruses and bacteria to nematodes and insects (Dreger-Jauffret & Shorthouse, 1992). There are many gall types, depending on the causal organism and the plant species involved. A common type is the covering gall, which as the name suggests, leads to the inducer being enclosed within the gall. Such galls are usually induced externally, provoking strong hypertrophy of the surrounding leaf tissue, leading to the creation of a highly specialised gall structure (Shorthouse & Rohfritsch, 1992).

The gall-forming habit on leaves originated independently in several different groups of arthropods, such as the gall mites (Acari: Eriophyidae), gall flies (Diptera: Cecidomyiidae), Hemiptera (Homoptera) and gall wasps (Hymenoptera: Cynipidae)(Larew, 1992). The non-arthropod gallers include the nematodes (Nematoda: Tylenchida) and bacteria and fungi. There are many possible gall causers, but as insect galls are both highly tissue and host specific, identification is often possible from gall morphology alone (Raman *et al.*, 2005). Comparisons between fossil galls and extant forms can give a broad identification of the gall-causing insect.

The majority of known fossil galls induced by invertebrates are from Cenozoic deposits or younger. The oldest fossil leaf galls were reported, however, on seed ferns from the Permian (Potonié, 1893). The oldest insect galls are on cone stalks of Early Triassic age (Larew, 1992), even though approximately 80% of extant galls occur on leaves (Labandeira, 2002). There is then a distinct gap in the fossil record of about 65 million years. Leaf galls reappear in the Cretaceous, but on angiosperms (Figure 1.9). Highly structured leaf galls were found on Lauraceae from the Late Cretaceous (115 Ma) (Hickey & Doyle, 1977) and sawfly-like galls on *Salix* leaves were also found in Late Cretaceous deposits (Larew, 1992). Fossil leaf galls have been found throughout the Cenozoic (Wilf & Labandeira, 1999; Currano *et al.*, 2008), although less frequently documented from Quaternary deposits (Stone *et al.*, 2008).

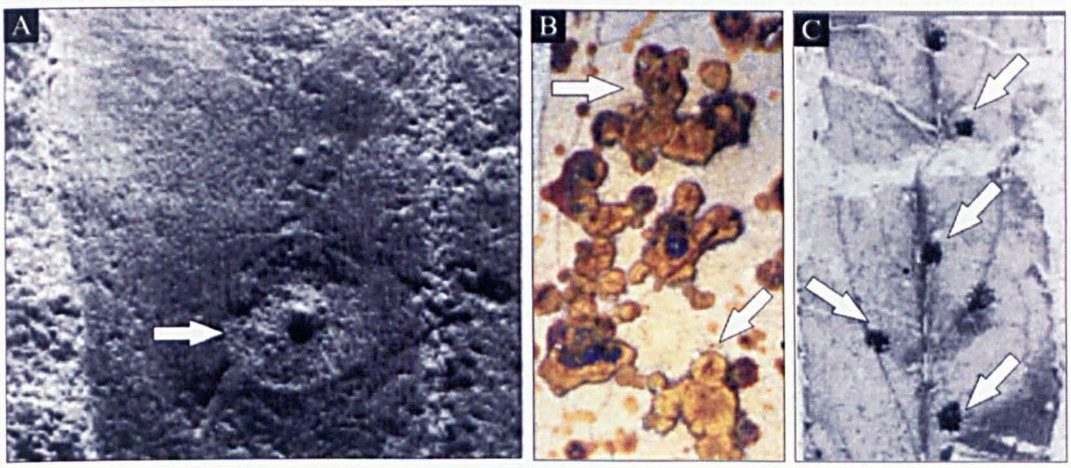


Figure 1.9. Leaf galls on fossil angiosperm leaves from previous studies. A) Angiosperm leaf with spot gall with central exit hole from Dakota Formation, Cretaceous, USA (Scott *et al.*, 1992). B) Clustered polylobate galls on angiosperm leaf (Fabaceae), Eocene, Bighorn basin of Wyoming, USA, (Currano *et al.*, 2008). C) Galls on primary and secondary veins of *Stillingia casca* (Euphorbiaceae), Eocene, Southern west Wyoming, USA, (Wilf & Labandeira, 1999).

1.2.2. Palaeogene insect trace fossils from the Southern Hemisphere

Previous sections of this thesis have documented the different types of plant-insect interactions preserved in the fossil record, with examples given of each. Most studies have however been concentrated in the Northern Hemisphere and there are many fossil leaf collections especially from the Southern Hemisphere, both in museums and the field, that are not utilized to their full potential. One study in Australia reported several lepidopteran leaf mines on angiosperm leaves from Eocene deposits in Victoria (Rozefelds, 1988). There are limited discoveries of insect trace fossils from Antarctica (Hunt, 2001), but no detailed published work. The most relevant trace fossil findings are on fossil leaves from tuffaceous lake beds in Chubut, Argentina (palaeolatitude ~47°S) from the Early Eocene (52 Ma) (Wilf *et al.*, 2005). Four functional feeding groups consisting of general leaf chewing, leaf mines, leaf galls and piercing and sucking traces were collected (Figure 1.10). A greater diversity of both damage types and functional feeding groups was found at that site than in several Early to Middle Eocene North American localities (Wilf *et al.*, 2005). Although of lower latitude than the Antarctic Peninsula during the Eocene, the study indicated that the presence of such insect trace types on Antarctic fossil floras was possible because South America and Antarctica were connected by a land bridge during the Eocene. No Palaeocene or Eocene herbivory traces have however been studied in detail from Antarctica and in general are very sparse from the Southern Hemisphere.

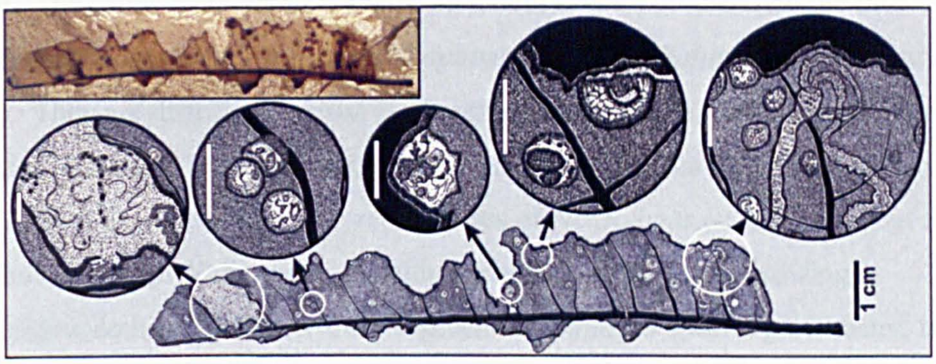


Figure 1.10. The range of insect traces that occurred on a single leaf specimen from the Laguna del Hunco flora, Early Eocene (Chubut, Argentina). The photographed specimen at the top of the figure is depicted as a camera lucida drawing on the bottom, with circular insets. Scale bars, 2 mm. Insets show the following details (from left to right): a blotch mine with frass; a cluster of three galls with exit holes; a gall which has been eaten by an external feeder; two galls, one of which has been eaten by an external feeder; and two linear, probably lepidopteran mines. Figure is adapted from Wilf et al.(2005).

1.3. Modern Analogue of forests and insects for Antarctica

To obtain a greater understanding of the insect trace fossils found on Antarctic plants, comparisons are made in this thesis with traces created on extant tree species in largely similar ecosystems. Palaeoecological reconstructions have suggested that the vegetation growing on Antarctica during the Palaeogene would have been very similar to that of the Valdivian rainforests of southern Chile (Poole, 2003). Further fossil evidence supports this as the tree types that grew on King George Island during the Eocene were also similar in composition to those in extant forests (Poole *et al.*, 2001). Torres *et al.* (1994) described a total of 6 taxa of fossil woods having affinities with extant trees growing in cold temperate rainforests of southern South America, specifically the Valdivian and Magellanic forests. This indicates that the forests of southern South America are suitable modern analogues for the Palaeogene forests of Antarctica. Hence, modern analogues for the fossil insect traces could also be found in southern South America forests such as the forests in Chile.

1.3.1. Forests of Southern Chile

In southern central Chile, *Nothofagus* tree species dominate the forest canopy during the early to middle stages of succession in most forest types, undergoing gradual replacement by more shade tolerant species in older stands (Veblen *et al.*, 1996). The genus *Nothofagus* contains 35 extant species divided into the four subgenera *Brassospora, Fuscospora, Lophozonia* and *Nothofagus* (Hill & Dettmann, 1996). They are distributed across the Southern Hemisphere, being found in South America, Australia, New Zealand, New Caledonia and Papua New Guinea (Poole, 1987). Both deciduous and evergreen species of *Nothofagus* occur throughout the distribution area of *Nothofagus*. All other species that are found growing in *Nothofagus*-dominated forests are evergreen. *Nothofagus* species grow faster, taller and are less shade-tolerant than most other long-lived broad leaved trees in south-central Chile (Veblen *et al.*, 1996). *Nothofagus* usually forms extensive monotypic stands and has a temperate phenology with one synchronous leaf flush and full extension in early spring. Seasonality is marked in both flowering and wood production and leaf age ranges from six months for deciduous species up to three years for evergreen species (Dawson, 1966).

On the central and western parts of the southern Andes in Chile, forests are dominated by evergreen species of *Nothofagus*. At mid elevations (600-950m) *N. dombeyi* is generally present, but at higher altitudes *N. betuloides* is more common. At low altitudes where annual precipitation is at least 3000mm, *N. dombeyi* is found with broad-leaved evergreen trees such as *Laureliopsis philippiana* and *Eucryphia cordifolia* and the conifers *Fitzroya cupressoides* and *Pilgerodendron uviferum*. The understorey is commonly the bamboo *Chusquea culeou*. At higher altitudes, pure stands of *N. pumilio* are found between 1400-1600m (Veblen *et al.*, 1981).

Between, 37°30'S and 40°03'S in the Chilean forests *Araucaria araucana* is associated with both deciduous and evergreen *Nothofagus* species (Wardle *et al.*, 2001).

For Chile, the tree limit is defined as the maximum altitude reached by trees of *Nothofagus*, which rises with increasing distance from the western coasts and decreases with latitude towards the south. In southern forests in Chile the evergreen trees are replaced by the deciduous species *N. pumilio* and, at varying distances below the tree limit, krummholz (a feature of subarctic vegetation at the tree line where exposure to extreme conditions deforms the growth of the tree) of *N. pumilio* and patches of low growing *N. antarctica* are found (Wardle *et al.*, 2001).

1.3.2. Modern insect traces of southern Chile

The *Nothofagus* forests of Chile support an endemic insect fauna that includes relict species of significant conservation importance (Spagarino *et al.*, 2001). *Nothofagus* supports about 30 genera of insects, many of which are specialised on the host plant (McQuillan, 1993). The main groups of insects that form such associations can be highlighted as follows. The leaf chewers include the larvae of several lepidoteran families: Geometridae, Hemileucidae and Lasiocampidae, as well as the larvae of Hymenoptera (sawflies). Chrysomelidae (Coleoptera) and Curculionidae (Coleoptera) are also major elements in the fauna of certain *Nothofagus* species. A shield-backed katydid, *Coniungoptera nothofagi* (Orthoptera: Tettigonidae), occurs in the forests of central Chile and aggregations can defoliate an individual tree in 10-15 days (Rentz & Gurney, 1985). A recent study on the insect fauna in canopies of *Nothofagus* trees and *A. araucana* found a large coleopteran fauna with approximately half of the species yet to be described (Arias *et al.*, 2008). Finally, a species in the genus *Araucanomela* (Coleoptera: Chrysomelidae) is known to feed on *Nothofagus*, including *N. betuloides*, although its biology and life history is still unknown (Reid, 2002).

There are several types of leaf miners associated with species of *Nothofagus*. Heterobathmiidae (Lepidoptera) were found during spring on deciduous *Nothofagus*, with eight species attacking *N. obliqua* and one feeding on *N. antarctica* and one species on *N. pumilio* (Kristensen & Nielsen, 1983; McQuillan, 1993). The evergreen, *N. dombeyi* is mined by *Basileura* species (Lepidoptera: Incurvariidae) (Nielsen, 1985; Parra & Ibarra-Vidal, 1994). The gall-causing insects documented on *Nothofagus* include several species in the genera *Espinosa* and *Aditrochus* (Hymenoptera: Pteromalidae) (De Santis *et al.*, 1993), as well as species of cecidomyiid (Diptera).

Nothofagus has several characteristics that could represent a defence strategy against herbivores. For example, most species have tough leaves, hard seed capsules and the presence of phenolic compounds and tannins. Despite this, *Nothofagus* still has an extensive insect fauna although the extent of feeding damage may vary between species, with *N. antarctica* and *N. obliqua* showing the greatest leaf damage (Russell *et al.*, 2000). A variety of factors can also affect the species composition and diversity of the forests in southern Chile and this is well documented for *Nothofagus* species, as they are the most important native Chilean timber species (Lusk & Ortega, 2003). The insect diversity and abundance in forests of *N. pumilio* were found to change significantly between harvested forests and virgin forests (Spagarino *et al.*, 2001).

The *Nothofagus* dominated forests in central and southern Chile provide an ideal habitat to examine the diversity of insect traces made on both deciduous and evergreen *Nothofagus* species and across a latitudinal range. Insect faunas associated with several species of *Nothofagus* are known and they include a wide range of functional feeding types. Therefore, a range of modern equivalents of insect trace fossils are available for comparison. In addition, the life history and ecology for many insect species remains unknown and a study of the diversity of insect traces can provide evidence of not only new plant-insect interactions, but expand existing knowledge about the distributions of species and their development.

1.4. Climatic Significance

There are several ways to reconstruct the climates of past ecosystems, depending on the fossil samples that are examined, the preservation quality and the type of climate data needed. Fossil plants have been widely used to give palaeoclimate estimates, using wood (Francis, 1986; Francis & Poole, 2002), leaves (Hayes *et al.*, 2006) and pollen (Askin, 1992; Hunt & Poole, 2003). The accuracy of the estimates is dependent on the type of fossil samples, their preservation quality, the type of climate data required and also the relative age of the section under study. The estimates generated are used as climate proxies for climate models to improve their predictive power (Markwick, 1998).

The nearest living relative (NLR) method is a systematic technique used widely by palynologists and palaeobotanists to reconstruct palaeoclimates. The climatic tolerance of a fossil plant is simply extrapolated from that of its nearest living relative. This is valid when using Quaternary material, but it is more limited when examining older fossil specimens as it assumes evolutionary and climatic change has had no influence on the plants. This method also assumes that because a plant lives in a particular habitat it should be regarded as taxonomically similar to plants living in a similar environment even if this is at a different time or place (Herman & Spicer, 1997). Despite these circumstances, NLR is still widely used and has been the basis for further techniques using leaf physiognomics, such as the coexistence approach (CA) (Mosbrugger & Utescher, 1997) and overlapping distribution analysis (ODA) (Yang *et al.*, 2007).

Leaf margin analysis has been used for many years and in many different regions to estimate the mean annual palaeotemperature (Wolfe, 1971, 1979; Wilf, 1997). This analysis is based on the principle that there is a positive linear correlation in living forests between the proportion of woody dicotyledonous species with entire margined leaves in a flora and the mean annual temperature of that site (Wolfe, 1993). Therefore, mesic floras in areas with cool mean annual temperatures have smaller proportions of entire margins amongst their species than those living in areas of warmer mean annual temperatures (Burnham *et al.*, 2001). Although this method has been widely used, the strength of the correlation is not fully tested. It was found to be more robust when a larger number of species were used (Wolfe, 1993), but the frequency of non-entire margined species can vary dramatically between different habitats of the same forest. For example, the mean annual temperature estimates for woody plants growing beside lakes and rivers are underestimated, while those in a closed canopy forest provide very accurate predictions. This has important implications for angiosperm fossil records that contain predominately lake and river deposits (Burnham *et al.*, 2001). Also, the morphology of the leaf is always compromised between conflicting constraints, therefore, a single character analysis provides only a partial climatic signal (Herman & Spicer, 1997).

To overcome these limitations a multi-character leaf physiognomic technique was developed by Wolfe (1993). Leaf characters in modern taxa are analysed at sites for which climatic conditions are known and characters that were strongly correlated with climate variables are identified, such as the mean annual temperature and mean growing season. A database of various sites is generated, part of the Climate Leaf Analysis Multivariate Program (CLAMP) (Wolfe, 1993; Wolfe & Spicer, 1999). The CLAMP technique relies on the assumption that leaf physiognomy is determined by physical laws related to fluid flow, diffusion and irradiance/heat balance that will constrain the plant architecture within certain limits. Therefore, there is an optimum leaf architecture to deal with environmental constraints. In CLAMP there are 31 physiognomic character states included in the analysis and 13 palaeoclimatic variables can be generated. A range of modern systems are used to give calibration points for the analysis and the fossil leaves are scored in the same manner as the modern leaves. The overall score of the fossil system is compared with the modern data to generate climatic estimates.

Leaf area analysis (LAA) can also be used to estimate the mean annual precipitation of an area as the morphology of living leaves, and in particular leaf size, are greatly influenced by the amount of available moisture (Wilf *et al.*, 1998; Wing *et al.*, 2005). Leaves transpire water to the atmosphere and thus smaller leaves are more beneficial in drier environments because they minimise water loss. In such environments a plant cannot afford the high water loss required to maintain large leaves. An example of this technique is provided by precipitation estimates for the Eocene for the Western United States, which indicated that the region was far wetter than the same area today (Wilf *et al.*, 1998).

All the techniques mentioned above generate various climatic estimates that can be used to constrain palaeoclimate models. The model simulations can be compared with the estimates generated using fossils to test their validity. If the models can generate reliable estimates for the past, then their climate estimates for the future may also be more accurate. The fossils of any organisms can be used to generate climate estimates: crocodilians, marine molluscs and coleopterans are examples (Hoganson & Ashworth, 1992; Markwick, 1998; Marra & Leschen, 2004; Ivany *et al.*, 2008). The coleopterans have been used to generate climate estimates for Quaternary ecosystems as beetle morphology has not changed significantly since that time and fossil beetles can be related to modern beetles, often to the species level (Ashworth, 2001). For older deposits and for areas where insect body fossils are rare, other proxies have to be used. Insect trace fossils are known to change in diversity and abundance with climate variability (Wilf & Labandeira, 1999; Wilf *et al.*, 2001). Therefore, they can be used to provide additional support for the estimates generated by the fossil plant proxies. They may also have the additional benefit of narrowing the climate range, as certain species of insect may be less tolerant to a decrease in temperature and be absent from the system, even when the host plant can still tolerate the temperature (Pollard, 1979).

1.5. Summary

- During the Tertiary, mixed deciduous and evergreen angiosperms and conifer forests were present on Antarctica and a warm temperate climate prevailed until 34 Ma.
- Despite the rich forest community, no insect body fossils for the Cenozoic have as yet been found except for insects from Pliocene sediments in the Transantarctic Mountains. There is only one published record of an insect

body fossil from the Antarctic Peninsula and that is from the Jurassic Period. The only other evidence of insect life during the Cenozoic is of dung beetle brood balls from the Antarctic Penninsula.

- Insect trace fossils on leaves have been used for palaeoecological reconstructions and to assess changes in insect diversity in the past. They provide an indirect method to study insect diversity when body fossils are rare.
- Studies have shown that the modern analogue of the Palaeogene forests of Antarctica are the Magellanic and Valdivian forests in southern Chile (based on both the composition of the vegetation and the climate).
- The forests in southern Chile are dominated by species of *Nothofagus*, which have an endemic insect fauna associated with them. Such forests provide an ideal area to investigate the diversity of phytophagous insects and the traces they leave behind on leaves that may be preserved. Comparisons between modern and fossil traces can enable a greater understanding of both the level and diversity of insect damage on leaves within forest systems.
- Insect body fossils have been used to predict past climates for the Quaternary and insect trace fossils can also highlight past changes in climate. Therefore, insect trace fossils may also be used to support past climate estimates from both climate models and fossil floras.

Chapter 2. Aims & Objectives

2.1. Overall Objectives

The aim of this thesis is to determine the type of herbivory that existed in Antarctic Palaeogene forests and the possible climatic significance of such herbivore traces, by comparison with similar modern analogues in southern Chile. In light of the topics discussed in Chapter 1, the following objectives were the focus of the research documented throughout this thesis:

1. To compile a complete database of insect trace fossils from two localities in Antarctica and examine the range of trace types present.
2. Investigate the diversity of insect traces in the modern day analogue of Antarctica during the Palaeogene, which is the forests of southern Chile.
3. Examine the effect of several abiotic factors on the intensity and rate of insect damage within the modern forests. This includes factors that differ between site, such as latitude and elevation, as well as within tree differences, such as height and orientation.
4. Compare the fossil and modern insect traces, both in terms of presence or absence of specific types of trace, as well as an examination of the intensity of insect damage on the leaves studied.
5. Examine the similarity of fossil sites to specific modern sites in southern Chile using both the diversity of insect traces and the intensity of insect damaged leaves.
6. Investigate the distribution of specific species of insect, leaf mines and leaf galls to suggest climatic ranges which can then be related to the fossil traces.
7. To reconstruct the insect faunal diversity of the Palaeogene forests of Antarctica and the possible climatic tolerances of such fauna.

The abovementioned objectives are addressed in specific chapters of the thesis Each chapter has more detailed objectives in order to answer the overall objectives and hence the aim of the thesis. These are considered in the remainder of this chapter.

2.1.1. Objectives of Chapter 3.

In **Chapter 3**, the first aim is addressed by providing a detailed examination of fossil leaf collections from two fossil localities in Antarctica. Two Palaeogene floras from islands adjacent to the Antarctic Peninsula were studied for the presence of invertebrate trace fossils. Therefore, the objectives of **Chapter 3** are to:

- Document the range of herbivory traces found on the fossil leaves from Antarctica and classify them.
- Quantify the level of leaf damage within the fossil systems to enable a valid comparison of the fossil herbivory in Antarctica with that from other regions.
- Investigate whether certain herbivory traces are specific to certain localities or plant types.

2.1.2. Objectives of Chapter 4.

The nearest modern analogues to the Palaeogene forests of Antarctica are the living forests in southern South America, specifically the Valdivian and Magellanic forests of Chile (Poole, 2003). A field collection of modern insect faunas was undertaken in several sites in southern Chile. The methodology applied during the modern fieldwork and the chosen study sites are presented in **Chapter 4,** as well as preliminary analysis and presentation of the field data. The objectives of this chapter are:-

- To obtain an understanding of the diversity of insect species and the traces they make on leaves of two species of deciduous *Nothofagus* in southern Chile, *N. pumilio* and *N. antarctica.*
- To examine the latitudinal range of insects and their traces on the two deciduous species.
- To collect leaf mines, leaf galls, insects and their traces on leaves of an evergreen *Nothofagus* species for comparison with those on the deciduous species.

- To compare the number of insect-damaged leaves on two deciduous *Nothofagus* species.
- To investigate the effects of elevation, season and within-tree variation on the distribution of insects, leaf mines and leaf galls. The effect of changing height and orientation within the tree was of particular interest.
- To examine the validity of the sampling technique using sample effort curves.

2.1.3. Objectives of Chapter 5.

In **Chapter 5**, a detailed description of the diversity of herbivorous insect species and the range of insect traces collected in Chile is documented and the causal insect identified where possible. The objectives of this chapter are:

- To examine the range of modern insect traces in Chilean forests.
- To identify the range of insect species that create unique traces and the range of insect species that create the same type of trace. This will aid the identification of the trace makers in Antarctic forests.
- To examine the diversity of leaf mining and leaf galling species in Chilean forests to aid interpretation of those in the fossil record in Antarctica.
- To investigate the latitudinal distribution of the modern insect herbivory types to determine their environmental significance, in order to estimate the environmental conditions represented by the fossil traces on Antarctic leaves.
- To compare the types of insect herbivory traces from an evergreen and a deciduous species of *Nothofagus*.

2.1.4. Objectives of Chapter 6.

Chapter 6 includes a brief introduction to statistical analysis using the software R. The results of the statistical analysis to investigate the effect of external factors on the presence of insect-damaged leaves and the number of leaf mines in Chile are summarised and the implications for analysis of fossil data discussed. The objectives of **Chapter 6** are:

- To compare the abundance of insect-damaged leaves between two deciduous species of *Nothofagus*.
- To determine the effect of abiotic factors, such as leaf orientation and height within a tree, on the abundance of insect-damaged leaves, leaf mines and leaf galls.
- To test for the effect of latitude on the occurrence of insect-damaged leaves, the intensity of leaf damage, and leaf mine and leaf gall numbers.
- To validate the collection technique with respect to observer bias.
- To test if sampling the branches of the tree from the ground provided a representative sample of the levels of insect damage and the types of insect traces within the tree as a whole.
- To examine the effects of other factors on the levels of insect damage, including elevation, season and age of the leaves.
- To examine which factors may have influenced insect herbivory on leaves in Antarctic Palaeogene forests.

2.1.5. Objectives of Chapter 7.

The fossil and modern insect traces are compared in **Chapter 7** using direct comparisons and quantitative comparisons of the ratios of each general trace category and cluster analysis. Thus, the objectives of **Chapter 7** are:

- To examine the range of insect trace fossils that are comparable with insect feeding traces collected in Chile.
- To determine the abundance of insect species that created individual traces on the modern leaves and thus provide an estimate of the abundance for the fossil traces.
- To determine the degree of similarity between the fossil sites and modern sites by assessing the proportion of different trace types found.
- To perform cluster analysis to explore the similarities amongst the fossil and modern sites in terms of the proportion of insect traces.

2.1.6. Objectives of Chapter 8.

The climatic tolerance of site-specific comparable traces is used to generate climate estimates for both fossil localities in **Chapter 8**. The estimates are compared with climate estimates from an Early Eocene global climate model. Therefore, the objectives of **Chapter 8** are to:

- Investigate climatic variability at the different sites examined in Chile.
- To determine climatic boundaries of the insect, leaf gall and leaf mine distributions described in Chapters 5 and 7.
- Use these to estimate palaeoclimates associated with the fossil herbivory traces.
- Compare computer model outputs with the climate ranges determined from the insect trace fossils to reconstruct the climate of King George Island and Seymour Island during the Palaeogene.

2.1.7. Objectives of Chapter 9. & Chapter 10.

Chapter 9 compares the results of these observations with previous work and the validity of modern and fossil comparison studies is discussed. The main conclusions of the project are discussed, along with the potential for further research in the insect trace fossils of Antarctica in **Chapter 10**.

Chapter 3. The Insect Trace Fossils of Antarctica

3.1. Geological Setting

3.1.1. King George Island

The fossil leaves examined were collected from two islands adjacent to the Antarctic Peninsula. King George Island is situated to the west of the Antarctic Penninsula and was part of a volcanic terrain that forms part of the South Shetland Islands. The island is situated at a latitude of 62°S, similar to its position during the early Tertiary (Lawver *et al.*, 1992), and lies on the western fore-arc margin of the former peninsula magmatic arc (Figure 3.1). Seymour Island is located to the east of the Antarctic Peninsula and is formed of sediments that were originally deposited in the James Ross Basin, a large sedimentary basin that formed on the back-arc of the volcanic arc that now forms the Antarctic Peninsula.

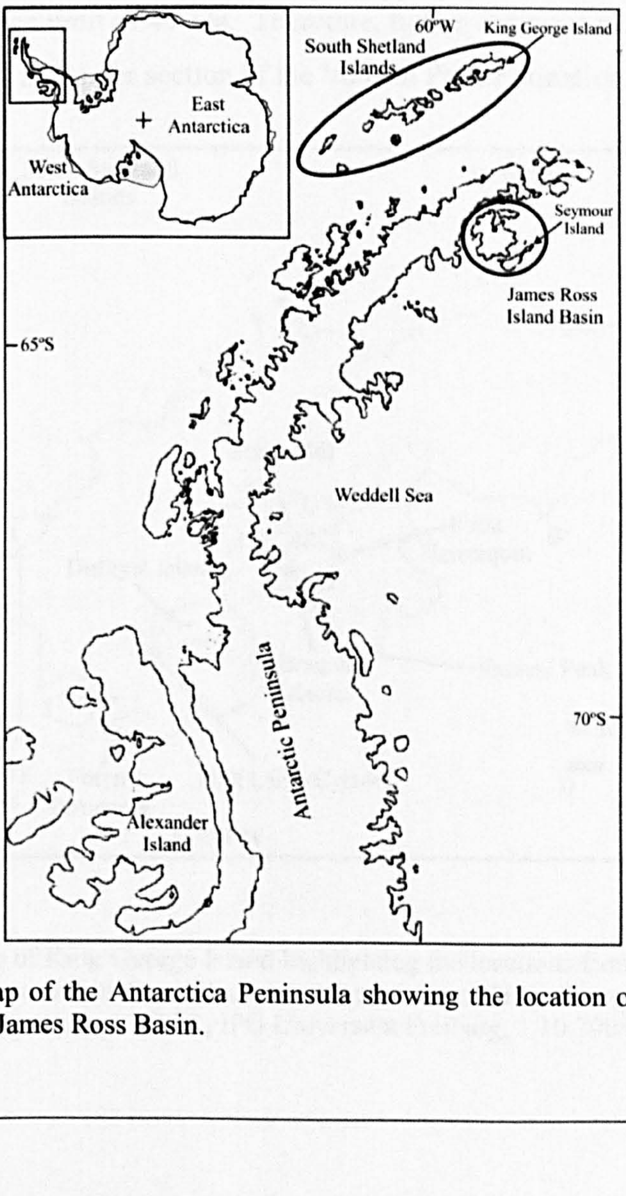


Figure 3.1. Map of the Antarctica Peninsula showing the location of the South Shetland Islands and the James Ross Basin.

The fossil leaves studied in this project are from several sedimentary formations on King George Island, including the Fossil Hill, Point Hennequin and Vaureal Peak formations (Figure 3.2). The Fossil Hill Formation on Fildes Peninsula is dated as Late Palaeocene to Middle Eocene in age, based on K-Ar geochronology of the underlying lavas (Shen, 1999) and also on dating of the sediments (Hunt, 2001). The flora from Collins Glacier and Potter Peninsula, on Fildes Peninsula, are also of Eocene age (Birkenmajer & Zastawniak, 1989). The dating of the Point Hennequin Group sediments is controversial; earlier studies date the upper formations of the sequence as Oligocene-Miocene in age (Zastawniak *et al.*, 1985; Birkenmajer & Zastawniak, 1989), but further K-Ar dating suggests an Eocene age for the entire sequence (Smellie *et al.*, 1984), specifically a Middle Eocene age (44-49 Ma) (Hunt, 2001). The Vaureal Peak Formation was dated using the underlying volcanic strata to provide a minimum age for the sequence. This gave an upper age limit of 49 Ma. Therefore, further dating is required to obtain an age estimate for the upper section of the Vaureal Peak Formation.

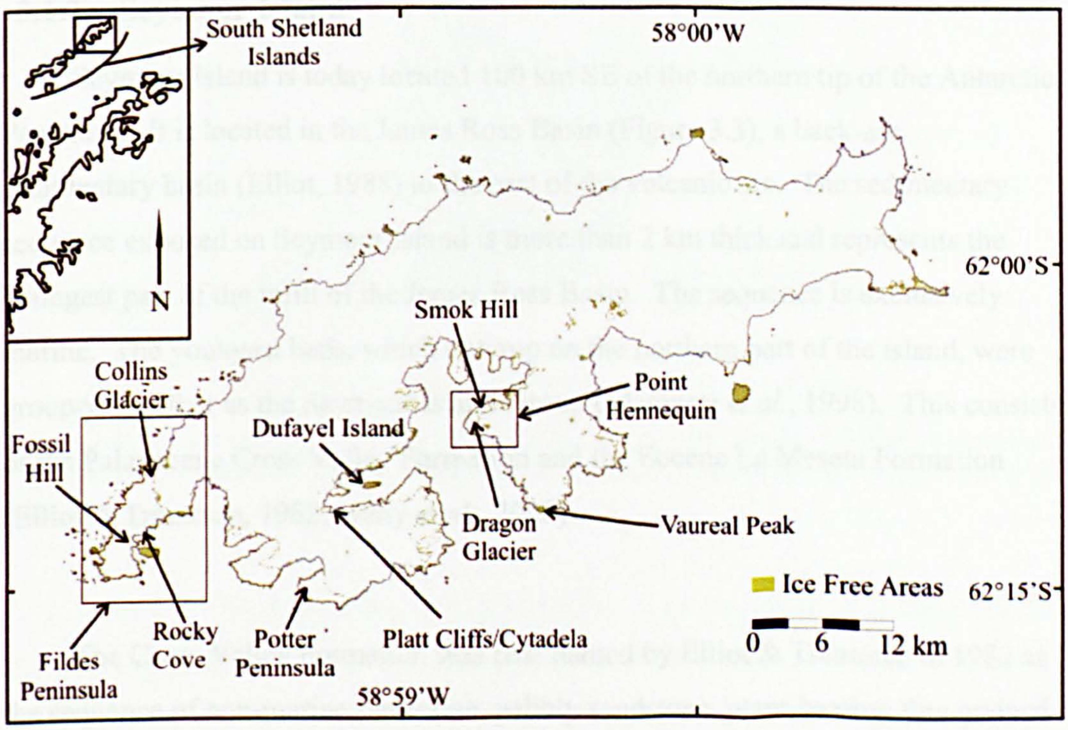


Figure 3.2. Map of King George Island highlighting the locations from which samples used in this study were originally collected. Adapted from King George Island GIS Project (http://www.kgis.scar.org/, SCAR, IPG Universität Freiburg, 1.10.2008).

The fossil floras of King George Island are found within a range of sediment types, including mudstones, siltstones and sandstones. These represent deposition in a range of environments, including shallow and deep water lacustrine settings and low energy fluvial deposits. The interbedding of fossil soils and lake sediments indicates cyclical draining and refilling of a freshwater lake. The most abundant fossil plants are found at the localities called Point Hennequin, Vaureal Peak and Fossil Hill and represent vegetation that grew on land areas in this region, but which was subsequently washed into adjacent lakes. The leaves are preserved as impression and compression fossils, with thin coatings of carbonaceous material. Leaf cuticle is not preserved. Other Cenozoic fossil floras from King George Island have been found in volcanic tuffs on Dufayel Island that have been dated between 51.9-56.8 Ma (Palaeocene age) (Birkenmajer, 1980; Birkenmajer & Zastawniak, 1986). Also, the Platt Cliffs flora is considered to be Late Eocene to Early Oligocene in age (Birkenmajer & Zastawniak, 1989). Further details about the samples are given in section 3.2.1.

3.1.2. Seymour Island

Seymour Island is today located 100 km SE of the northern tip of the Antarctic Peninsula. It is located in the James Ross Basin (Figure 3.3), a back-arc sedimentary basin (Elliot, 1988) to the east of the volcanic arc. The sedimentary sequence exposed on Seymour Island is more than 2 km thick and represents the youngest part of the infill of the James Ross Basin. The sequence is exclusively marine. The youngest beds, which outcrop on the northern part of the island, were grouped together as the Seymour Island Group (Marenssi *et al.*, 1998). This consists of the Palaeocene Cross Valley Formation and the Eocene La Meseta Formation (Elliot & Trautman, 1982; Ivany *et al.*, 2008).

The Cross Valley Formation was first named by Elliot & Trautman in 1982 as the sequence of non-marine sandstone, pebbly sandstone, plant-bearing fine grained sandstone, and unconsolidated sand and clayey sand which represents distributary channels and inter-distributary areas of a deltaic environment. The fossil leaves are from the top section of the formation, Nordenskjöld's bed, and are Late Palaeocene in age. The flora studied from the formation has been interpreted as a para-tropical forest growing in a warm rainy climate (Gandolfo *et al.*, 1998).

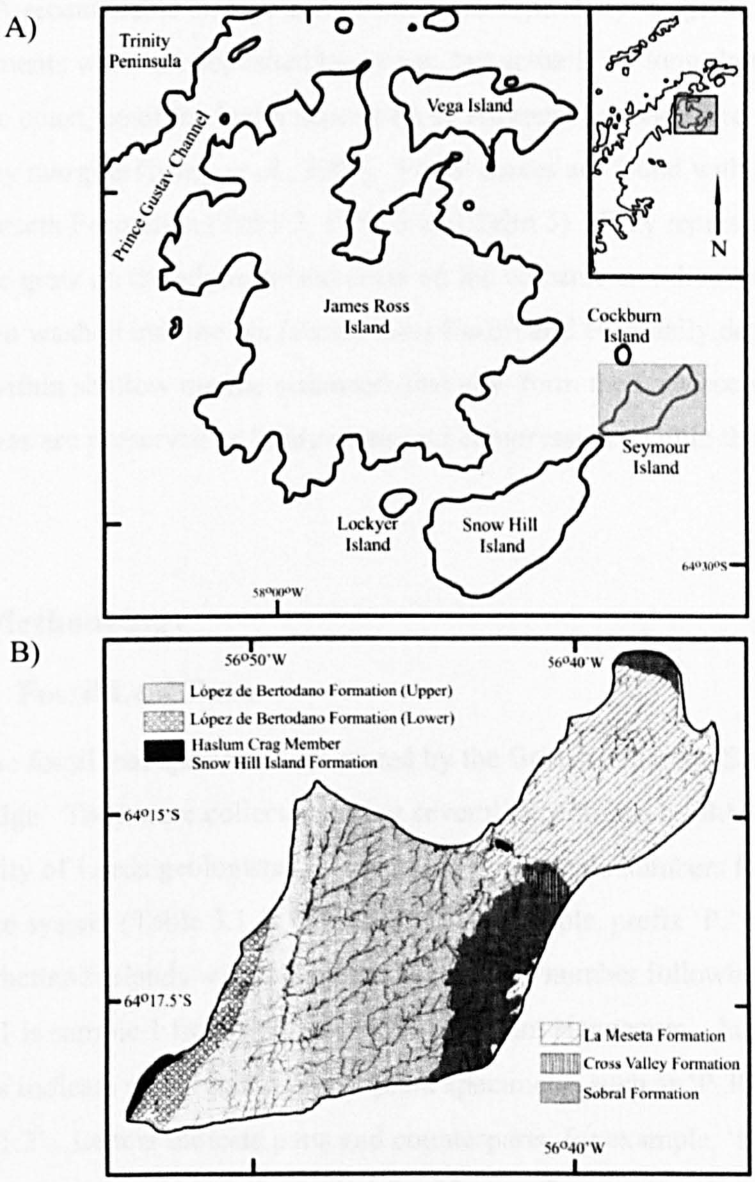


Figure 3.3. A) Location of Seymour Island in the James Ross Basin. B) The geographical extent of the Cretaceous-Tertiary formations on Seymour Island (adapted from Poole *et al.* 2003).

The La Meseta Formation is the name given to the sequence of unconsolidated marine sandstones and mudstones exposed in the north-eastern part of the island. The sandstones and mudstones contain very thin clay beds, pebble horizons, calcareous beds and conglomeratic shell beds. The sediments of the La Meseta Formation originated from the west-northwest, from source rocks that crop out on the Antarctic Peninsula (Marenssi *et al.*, 1999). There have been various interpretations of the depositional environment of the La Meseta Formation from estuarine or deltaic to channel or valley fills (Elliot & Trautman, 1982; Sadler, 1988). A recent stable isotope and strontium isotope study suggests, however, that the sediments were not deposited by a river, but actually by long shore transport along the coast, combined with deposition of sediment and localized runoffs from the valley margins (Ivany *et al.*, 2008). Fossil leaves are found within several units of La Meseta Formation (Telm 2, Telm 3 and Telm 5). They represent vegetation that once grew on the adjacent land areas on the volcanic arc. Leaves and wood were then washed into the sea (James Ross Basin) and eventually deposited and buried within shallow marine sediments that now form the La Meseta Formation. The leaves are preserved as impressions and compressions within thin carbonaceous layers.

3.2. Methodology

3.2.1. Fossil Leaf Taxa

The fossil leaf specimens are stored by the British Antarctic Survey (BAS) in Cambridge. They were collected during several expeditions by BAS and the University of Leeds geologists (Appendix I). The sample numbers follow the BAS reference system (Table 3.1 & Table 3.2). For example, prefix 'P.' indicates the South Shetland Islands with the station and sample number following: for example P.3001.1 is sample 1 from field site P.3001 at Point Hennequin. Any additional numbers indicate rocks with multiple plant specimens, such as 'P.3001.1.1, P.3001.1.2'. Letters indicate parts and counterparts, for example, 'P.3001.1.1a, P.3001.1.1b'. The older collections use station prefixes such as G for localities in Admiralty Bay. For Seymour Island, specimens have the prefix 'DJ' and the same numbering system explained above. The prefix "D" relates to specimens collected in 1944 by the Falkland Island Dependencies Survey, the fore-runner of BAS. Numbers in brackets indicate multiple plant specimens on the same rock, for example, as leaf mats.

Table 3.1. The site codes of the fossil leaves examined from King George Island and the age and name of the locality from which they were collected. The number of insect-damaged leaves from each locality is also given.

Age	Formation	Location	Station Numbers	% of insect-damaged leaves from each location
Palaeocene (~ 57 - 55 Ma)	Dufayel Island Group	Dufayel Island	G.53, G.312	13.8
Middle – Late Eocene (~ 49 - 35 Ma)	Cape Vaureal Formation	Vaureal Peak	P.2799	3.8
Middle Eocene 49 - 44 Ma	Mount Wawel Formation	Dragon Glacier	G.9, P.236, P.2810, P.3001-P.3007, P.3010, P.3011, P.3013	71.5
		Smok Hill	P.1404	3.1
Late-Middle Eocene (~ 45 - 40 Ma)	Fildes Formation	Potter Peninsula	P.232	0
		Rocky Cove	P.3029, P.3035	0
		Collins Glacier	P.3025, P.3028	0
Late-Middle Eocene (~ 40 Ma)	Fossil Hill Formation	Fossil Hill	P.3030 - P.3032, P.3034, P.3036, P.935	6.9
?Early Oligocene (34 - 32 Ma)		Platt Cliffs/Cytadela	G.50, G.309, G.319	0.8

Table 3.2. The site codes of the fossil leaves examined from Seymour Island and the age and name of the locality from which they were collected. The number of insect-damaged leaves from each Telm (Tertiary Eocene La Meseta stratigraphic unit) is also given.

Formation	Telm	Station Number	Age	Source	% of insect-damaged leaves from each Telm
La Meseta	5	DJ.1101, DJ.1112	Mid-Early to mid-Middle Eocene (52-45 Ma)	(Ivany *et al.*, 2008)	3.8
	3	DJ1103	Early Eocene (~ 53 - 52 Ma)	(Ivany *et al.*, 2008)	0
	2	DJ.1158, DJ.1105	Early Eocene (~ 55 - 53 Ma)	(Ivany *et al.*, 2008)	30.8
Cross Valley	N/A	D.512, D.517, D.523, D.572, DJ.913, DJ.905, DJ.1111, DJ.1113,	Late Palaeocene (~55 Ma)	(Elliot & Trautman, 1982)	65.4

The fossil samples are impression and compression fossils which vary in preservation quality. The King George Island flora comprises leaves that are preserved either as carbonised compressions lacking cuticle, as pure impressions or as mineralised impressions. The leaf remains occur as dispersed organs or leaf mats ranging from entire leaves to large fragments. In comparison, the Seymour Island leaves are preserved mostly as fragmentary impressions; some fragments are large, but complete leaves are rare. The quality of the leaf margins and the venation in both fossil floras is variable, which can affect the clarity of species specific herbivory traces, such as the leaf mines. The fossil floras for each locality have been previously described (Hunt, 2001; Tosolini *et al.*, 2009) and consist of several genera of angiosperm plants including representatives of Cunoniaceae, Lauraceae, Myrtaceae, Proteaceae and Nothofagaceae. Coniferous tree remains also present include Araucariaceae, Cupressaceae and Podocarpaceae. For this study of herbivory traces only fossil angiosperm leaves were examined.

3.2.1. Insect Trace Fossils

Fossil leaf specimens were examined using a binocular light microscope to search for the presence of insect trace fossils. Traces were then photographed, described and characterised. Only traces with a distinctive plant wound reaction were considered, so as to exclude examples of mechanical damage to the leaf or damage when the leaf was abscised. Generally, the wound reaction is best preserved on impression fossils where it can be identified by a distinctively darker colouration or indentation around the damaged area (Figure 3.5F).

The insect traces were placed into four main leaf damage categories: general leaf chewing, skeleton feeding, leaf galls and leaf mines. The traces from each locality were classified separately to enable comparison of the diversity of trace types between sites. This can also be justified because when the fossil leaves were alive and attached to the tree they would not have been growing in the same area. Hence, they would have been affected by different environmental factors which in turn would have had an effect on the types of invertebrates that could have made the trace.

The traces were subdivided further, depending not only on the plant type, but also the size, shape and positioning of the trace on the leaf. The size measurement of the general leaf chewing traces was based on the proportion of the leaf area that was damaged. The size classes used in the descriptions are as follows: very small is less than 10% of total leaf area damaged, small is 11 to 25% of the total leaf area damaged, medium damage is 26 to 50% of the total leaf area damaged, large is 51% of the total leaf area and above damaged. The total leaf area was estimated for fragmented leaves using the remaining leaf area as a guide. For the leaf mines and leaf galls, size is not a reliable character to distinguish between the different trace types because the size will change depending on the stage of development of the species involved. For description of the trace, however, an estimate of size is given using the same criteria as mentioned for general leaf chewing traces.

Quantitative analysis of herbivore damage on the fossil leaves was carried out to measure the intensity of herbivory. The amount of leaf area lost due to insect feeding was measured using photographs taken with a JVC digital camera KYF750 with a Nikon 55mm lens. Leica IM50 software was used to measure the width, length and area of each of the fossil leaves, as well as the area of the trace. This enabled the percentage area of leaf loss due to herbivory to be calculated. For fragmented leaves, the original total leaf area had to be estimated using the remaining leaf area as a guide. Only a subset of the fossil leaf specimens with traces could be measured (the least fragmented) and used to give an estimated level of herbivory for the forests of King George Island and Seymour Island during the Palaeogene.

3.3. Description of The Trace Fossils

In total, 1241 individual fossil leaf specimens were examined from King George Island and 1027 fossil leaf specimens from Seymour Island. From both localities specimens had to be discounted from further analysis due to extensive fragmentation (6 specimens from King George Island and 297 specimens from Seymour Island). In the King George Island flora, 130 leaves (9.82% of the total number of fossil leaves) were found to exhibit trace fossils (Appendix II). From Seymour Island, 26 leaves (2.64% of the total number of fossil leaves) exhibited trace fossils (Appendix II). Therefore, 54 different trace types for King George Island and 19 different trace types for Seymour Island were documented (Tables 3.3 - 3.6).

For simplification, the trace fossils are considered to be insect trace fossils for the remainder of the thesis, since insects were most likely to have been the most abundant trace-causing organism. This is particularly true for traces within the leaf mining category. For general leaf chewing and leaf galls, however, there could be other causal organisms. For example, terrestrial snails (Gastropoda) have been observed creating general leaf chewing marks, and mites (Acari) can cause the formation of leaf galls (Shorthouse & Rohfritsch, 1992). In the following sections, a complete description of each trace type will be given, focussing on the most characteristic and abundant trace types.

3.3.1. General leaf chewing

General leaf chewing can be divided into either marginal or non-marginal traces. Marginal general leaf chewing occurs when the leaf tissue is removed along the leaf margin or from the leaf margin to the middle of the leaf. Damage can range in size depending on the plant type on which the trace was made, the age and size of the leaf, and the insect species that made the mark. The production of a highly developed rim of reaction tissue is more pronounced with this type of leaf damage. Marginal general leaf chewing can be further divided into continuous and discontinuous damage. This classification depends on if part of the leaf margin is damaged by an individual without stopping (continuous), or if the same insect feeds on the leaf, stops and then continues further along the leaf margin (discontinuous). For fossil traces it is difficult to distinguish this difference, therefore, the assumption was made that if the leaf area damaged was half way or more to the primary vein or that the single area damaged was greater than 10% of the total leaf area it is continuous. Discontinuous marginal leaf chewing was defined as when the leaf area missing was in very small sections (each damage section was less than 5% of the leaf) around the margin.

Within the range of marginal traces found from both King George Island and Seymour Island (Table 3.3) (Figure 3.4 to Figure 3.6) the most common type of trace was the continuous removal of a medium to large, irregularly-shaped part of the leaf tissue on Nothofagaceae leaves (Figure 3.4A). There were ten individual traces of this type found within the King George Island flora and three individual traces in the Seymour Island flora.

Table 3.3. The different trace categories for marginal general leaf chewing found on the fossils from King George Island (K) and Seymour Island (S). The leaf morphotype numbers used for the description of each leaf, such as Morphotype 1.6 or 1.1, are from Hunt (2001) for King George Island flora and Tosolini *et al* (in prep) for Seymour Island. Continued on p42.

Trace Type	**Leaf Morphotype**	**Description**	**Total Number in Trace Type**
K 1.1	*Nothofagaceae*	Continuous removal of leaf tissue on leaf. Medium to large irregularly-shaped portion of the leaf missing. (Figure 3.4A).	10
K 1.2	*Cunoniaceae*	Continuous removal of leaf tissue. Medium to large areas removed. (Figure 3.4B).	6
K 1.3	Morphotype 1.6	Continuous removal of leaf tissue. (Figure 3.4C)	1
K 1.4	*Knightia excelsa* (2.9)	Continuous removal of leaf tissue. (Figure 3.4D)	2
K 1.5	*Lauriphyllum nordenskjöldii* (1.11)	Continuous removal of leaf tissue. (Figure 3.4E).	2
K 1.6	*Dictylophyllum washburni* (1.1)	Continuous removal of leaf tissue around the basal area. (Figure 3.4F).	3
K 1.7	*Dictylophyllum washburni* (1.1)	Continuous removal of leaf tissue which leads to the primary vein in a circular manner (Figure 3.5A)	1
K 1.8	Unknown 2	Distinct small oval part of the leaf tissue removed on basal area of the leaf. (Figure 3.5B)	1
K 1.9	*Nothofagaceae*	Continuous loss of leaf tissue around the leaf edge, but with small areas missing. (Figure 3.5C)	2
K 1.10	Morphotype 2.32	Discontinuous removal of tissue at the apical area of the leaf. (Figure 3.5D)	1
S 1.1	Morphotype 5 + 32 (*Proteaceae*)	Continuous area of leaf missing, medium to large sized sections of the margin in basal area, half way to primary vein. (Figure 3.5E)	3
S 1.2	Morphotype M	Multiple continuous marks, large area removed from edge into primary vein of the leaf, in basal area. (Figure 3.5F)	1
S 1.3	*Nothofagaceae*	Continuous single area missing from the edge into the primary vein, in the middle area of the leaf. (Figure 3.6A)	2
S 1.4	Unknown 3	Continuous medium size area missing at apical area of the leaf (Figure 3.6B).	2

Trace Type	Leaf Morphotype	Description	Total Number in Trace Type
S 1.5	*Nothofagaceae*	Continuous, double area missing on the apical and middle part of the leaf, small size (Figure 3.6C).	1
S 1.6	Unknown 4	Continuous single area missing from edge into the primary vein, in basal area, medium size. (Figure 3.6D)	1

The second most abundant marginal trace type also involved continuous tissue removal, but occurred mostly on fossil leaves of the Cunoniaceae (Figure 3.4B) (five samples). Traces found on other plant species include a continuous marginal trace on fossil leaves of *Dictylophyllum washburni*, on the left side of the apical area, where two large areas are missing (Figure 3.4E). Another trace type was observed on a *Lauriphyllum nordenskjöldii* leaf, where tissue was removed in a continuous manner from two sides of the leaf (Figure 3.4F). A further type of marginal general leaf chewing is highlighted in Figure 3.6D, where a small area of the leaf tissue is removed from the margin. Also present on this leaf is an example of non-marginal general leaf chewing in the form of small multiple irregularly-shaped holes (Figure 3.6D). Sixteen specimens had multiple types of trace.

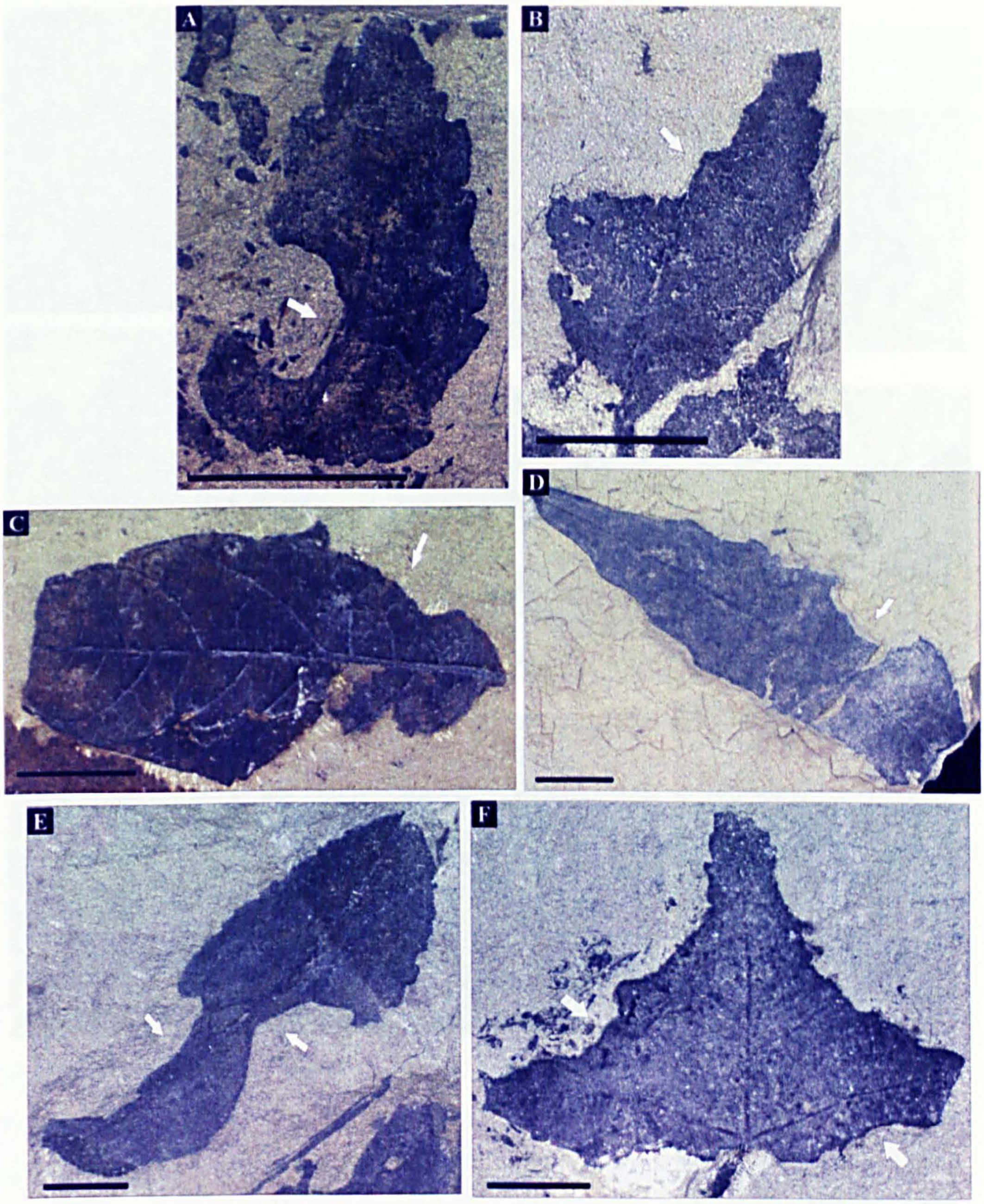


Figure 3.4. Examples of different marginal general leaf chewing trace types on fossil leaves from King George Island (K). A) Type K1.1, P.3001.139, B) Type K1.2, P.3001.116. C) K1.3. P.3001.43. D) K1.4: P.3001.234(2). E) K1.5, P.3001.169. F) Type K1.6, P.3001.5.1(2). Scale bar 1cm. White arrows highlight trace.

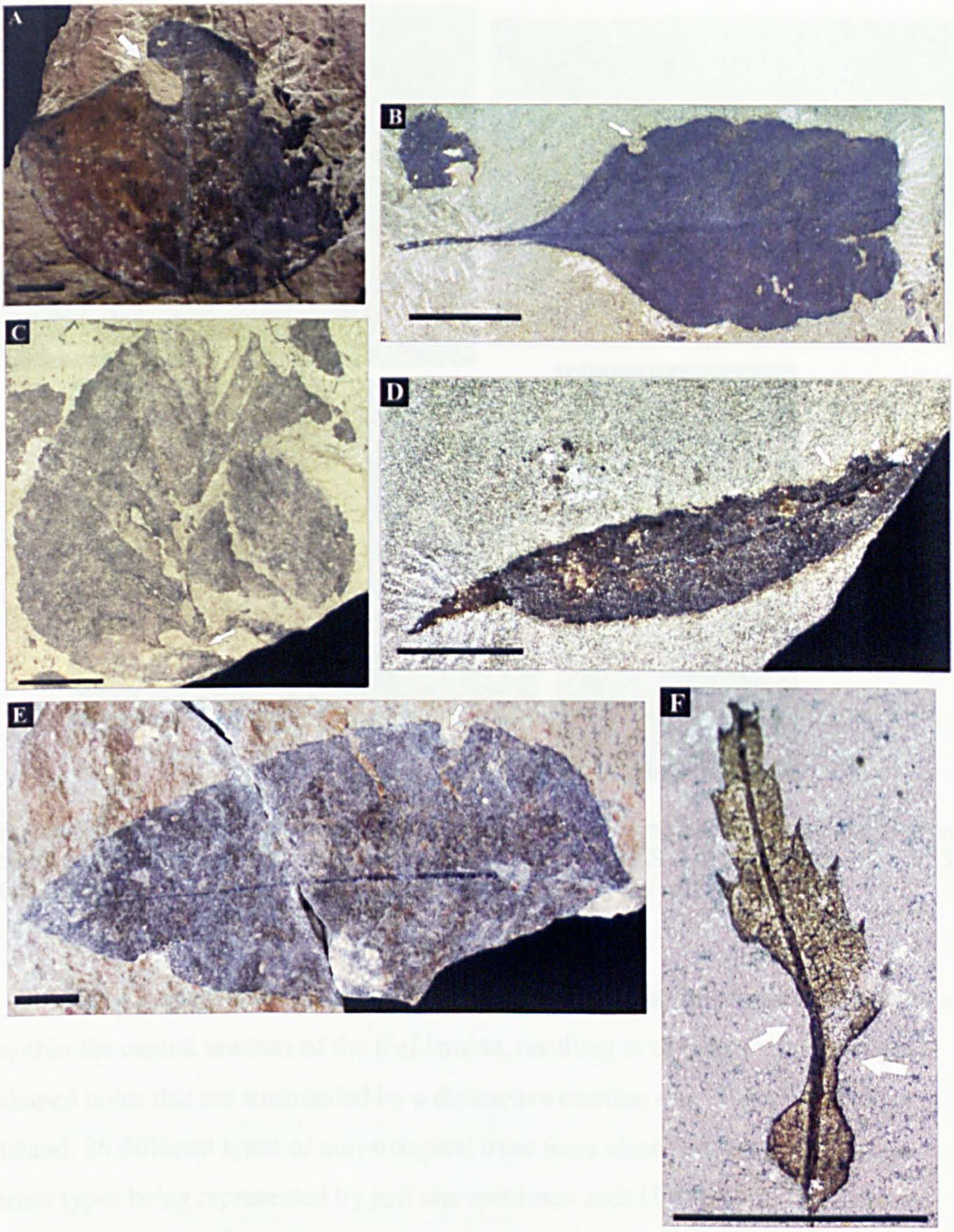


Figure 3.5. Examples of different marginal general leaf chewing trace types on fossil leaves from King George Island (K) and Seymour Island (S). A) K.1.7, P.3001.51(1). B) K1.8, P.3001.58.1(1). K1.9: C) P.3001.42(1), D) K1.10, P.3001.44. E) S1.1, DJ.11105.88. F) S1.2, DJ.1113.117a/b. Scale bar 1cm. White arrows highlight trace.

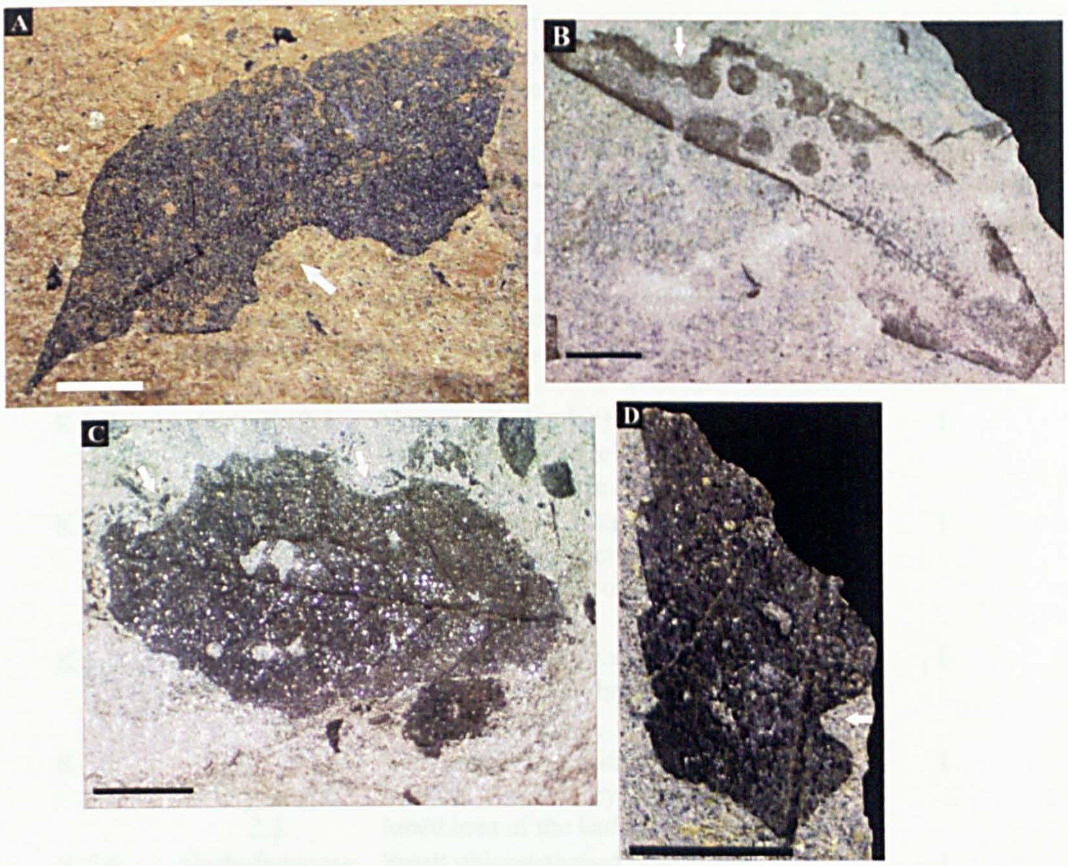


Figure 3.6. Examples of different marginal general leaf chewing trace types on fossil leaves from Seymour Island (S). A) S1.3, DJ.1105.131(1). B) S 1.4, DJ.1113.3. C) S1.5, DJ.1101.39. D) S1.6, DJ.1113.218. Scale bar 1cm. White arrows highlight trace.

Non-marginal general leaf chewing can be defined as the removal of leaf tissue within the central sections of the leaf lamina, resulting in circular to irregularly-shaped holes that are surrounded by a distinctive reaction rim. For King George Island, 26 different types of non-marginal trace were identified, with 50% of the trace types being represented by just one specimen each (Table 3.4). For Seymour Island, 10 different types of trace were identified; 60% of the trace types have only one example (Table 3.4).

Table 3.4. Range of non-marginal chewing trace types identified for King George Island (K) and Seymour Island (S). The leaf morphotype numbers used for the description of each leaf, for example Lomatia 2.4 or Morphotype 2.9, are from Hunt (2001) for King George Island flora and Tosolini *et al* (in prep) for Seymour Island. The family is given when possible with the leaf morphotypes in brackets (Continued on p.47-48).

Trace Type	Leaf morphotype	Description	Total Number in Trace Type
K 2.1	*Dictylophyllum washburni* (1.1)	Elliptical-shaped single mark between two secondary veins, small (Figure 3.7A).	2
K 2.2	*Lomatia* 2.4	Very small circular bullet-hole, single, dark ridge near edge of leaf on the basal region (Figure 3.7B).	1
K 2.3	Morphotype 1.2	Large irregularly-shaped mark near the primary vein and between 2 secondary veins at the middle of the leaf. (Figure 3.8A).	1
K 2.4	*Knightia excelsa* (2.9)	Small, single elliptical mark across the secondary vein in the middle of the leaf (Figure 3.8B).	1
K 2.5	Morphotype 2.5	Very small mark with distinct ridge between 2 secondary veins on the right lobed area of the leaf (Figure 3.8C).	1
K 2.6	Nothofagaceae	Small oblong-shaped hole at the primary vein (Figure 3.8D).	1
K 2.7	Morphotype 2.24	Small elliptical shape between two secondary veins at edge of leaf, single hole (Figure 3.8E).	1
K 2.8	Morphotype 1.4	Very small circle adjacent to the primary vein (Figure 3.8F).	1
K 2.9	Myricaceae (2.18)	Leaf tissue between two secondary veins removed from the primary vein to half way to the margin. Distinct ridge present (Figure 3.8G).	1
K 2.10	Morphotype 2.6	Irregularly-shaped hole next to the secondary and primary vein (Figure 3.8H).	1
K 2.11	Magnoliidae (2.22)	Multiple irregular marks, next to secondary veins on both apical and basal leaf areas. In middle of an inter-vein area (Figure 3.7C).	1
K 2.12	Unknown 5	Multiple, large, oblong-shaped marks in between two secondary veins, throughout the leaf (Figure 3.7D).	3
K 2.13	Cunoniaceae	Single large circular mark covering several secondary veins, near the leaf margin (Figure 3.7F).	2
K 2.14	Cunoniaceae	Multiple, small, bean-shaped elliptical areas on primary veins at the basal area. One per inter-secondary vein area (Figure 3.7E).	1

Trace Type	Leaf morphotype	Description	Total Number in Trace Type
K 2.15	Cunoniaceae	Multiple, very small, circular and elliptical marks over the whole leaf, darkened ridge distinct. (Figure 3.9A).	6
K 2.16	Nothofagaceae	Small, single circle adjacent to the primary vein or the secondary vein (Figure 3.9B).	7
K 2.17	Nothofagaceae	Single elliptical-shaped mark between two secondary veins, small to medium size with distinct ridge. (Figure 3.9C).	7
K 2.18	Nothofagaceae	Single, medium-sized ellipse next to primary vein or secondary vein with an indistinct ridge. (Figure 3.9D).	4
K 2.19	Nothofagaceae	Large, single, ellipse in basal area next to primary vein and secondary vein. (Figure 3.9E).	4
K 2.20	Nothofagaceae	Multiple, irregularly-shaped marks covering the leaf area, medium to large size, adjacent to secondary vein. (Figure 3.9F).	9
K 2.21	Nothofagaceae	Multiple, long, oblong areas missing on the primary vein and into adjacent secondary veins. (Figure 3.9G).	4
K 2.22	Nothofagaceae	Irregular, large, oblong areas between two secondary veins or adjacent to one secondary vein (Figure 3.10A).	8
K 2.23	Nothofagaceae	Multiple, pin-size marks on one leaf, dark ridge, cover whole area of leaf (Figure 3.10B).	7
K 2.24	Nothofagaceae	Multiple, small, irregular marks with distinct ridge, concentrated on secondary veins next to primary vein (Figure 3.10C).	3
K 2.25	*Dictylophyllum washburni* (1.1)	Large, irregular mark between 2 secondary veins and next to primary vein (Figure 3.10D).	1
K 2.26	*Lauriphyllum nordenskjöldii* (1.11)	Several circular marks with distinct ridge between two secondary veins and covering whole leaf vary in size (Figure 3.10E).	1
S 2.1	*Lauriphyllum densinervosa*	Multiple, small, elliptical traces near first secondary vein (Figure 3.11A).	1
S 2.2	Unknown 6	Single, large, circular hole, between two secondary veins (fragments) (Figure 3.11B).	3
S 2.3	Nothofagaceae	Multiple, medium-sized, irregularly-shaped holes, between 2 secondary veins (Figure 3.11C).	3

Trace Type	Leaf morphotype	Description	Total Number in Trace Type
S 2.4	Proteaceae	Single, large, elliptical mark at primary vein and over different secondary vein areas (Figure 3.11D).	1
S 2.5	Proteaceae	Multiple, very small, circular holes next to the secondary veins and in the middle area of leaf (Figure 3.11E).	4
S 2.6	*Phyllites* sp.	Large, circular hole, distinct ridge between 2 secondary veins or possibly a primary and a secondary vein, at basal area (Figure 3.11F).	1
S 2.8	Unknown 7	Multiple, circular to elliptical marks next to the secondary veins (Figure 3.12A).	1
S 2.9	Cunoniaceae	Single elliptical mark next to the primary vein and a secondary vein (Figure 3.12B).	2
S 2.10	Unknown 8	Multiple, small, circular to elliptical holes, between two secondary veins and all over leaf area (Figure 3.13C).	1
S 2.11	Unknown 4	Multiple, oblong, medium-sized holes next to secondary veins (Figure 3.13D).	1

As with the marginal general leaf chewing traces, Nothofagaceae fossil leaves had the greatest variation of non-marginal traces. Fossil leaves identified as Cunoniaceae contained the next most abundant trace types. The non-marginal trace types on Nothofagaceae leaves ranged from a single large area to multiple small areas missing from the leaf tissue (Figure 3.11B & C). The absence of multiple areas of leaf tissue was, however, also observed, ranging from large elliptical-shaped areas (Figure 3.9F) to small irregularly-shaped areas (Figure 3.10A). The reaction rim is particularly evident on the Cunoniaceae leaves that have lost multiple small circular areas of tissue (Figure 3.12B).

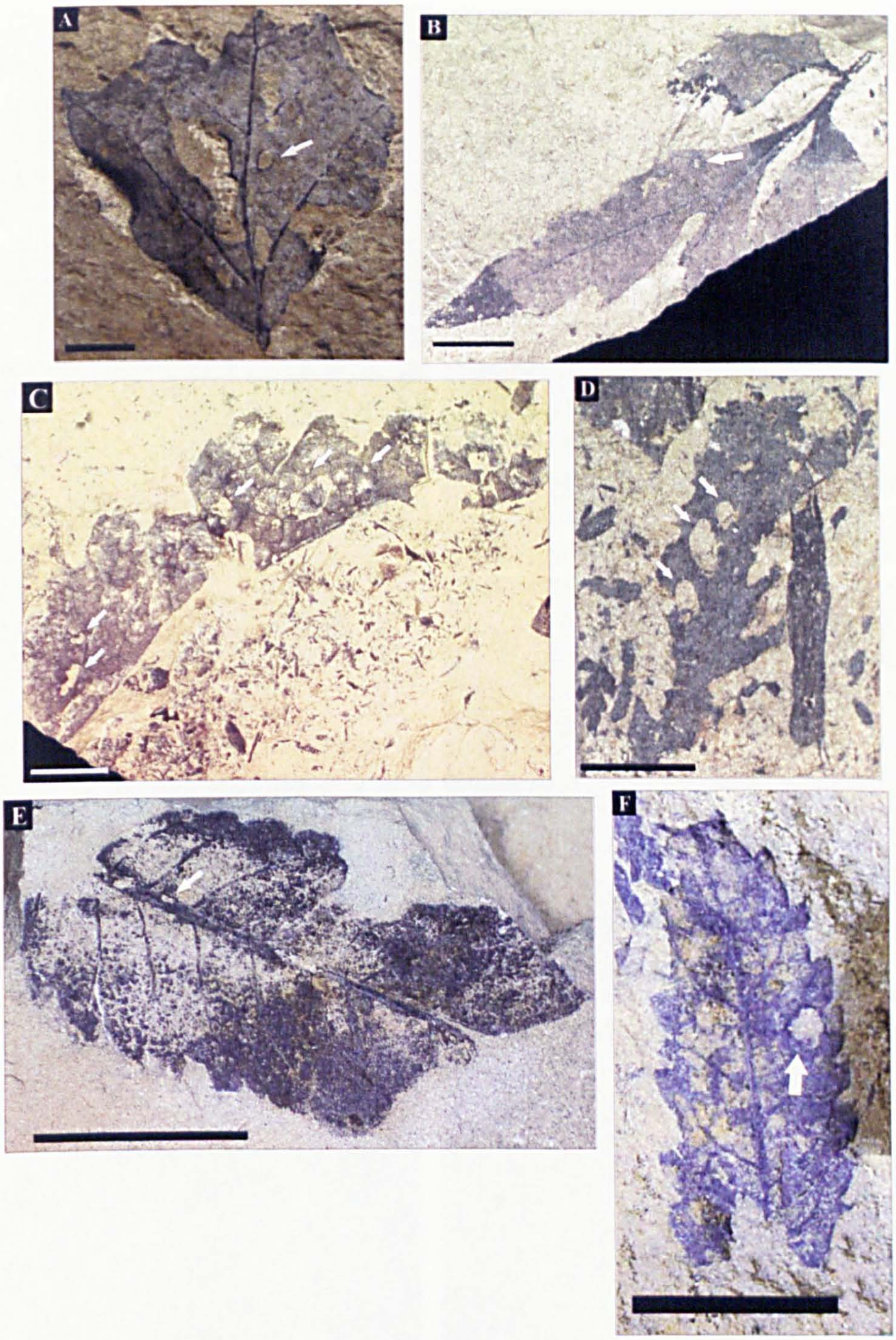


Figure 3.7. Examples of trace types of non-marginal general leaf chewing from King George Island (K). A) K2.1, P.3013.9. B) K2.2, P.3001.59. C) K2.11 P.3001.57(1). D) K2.12, P.236.4(2). E) K2.14, P.2810.14. F) K2.13, P.3001.180A. Scale bar 1cm. White arrows indicate position of traces.

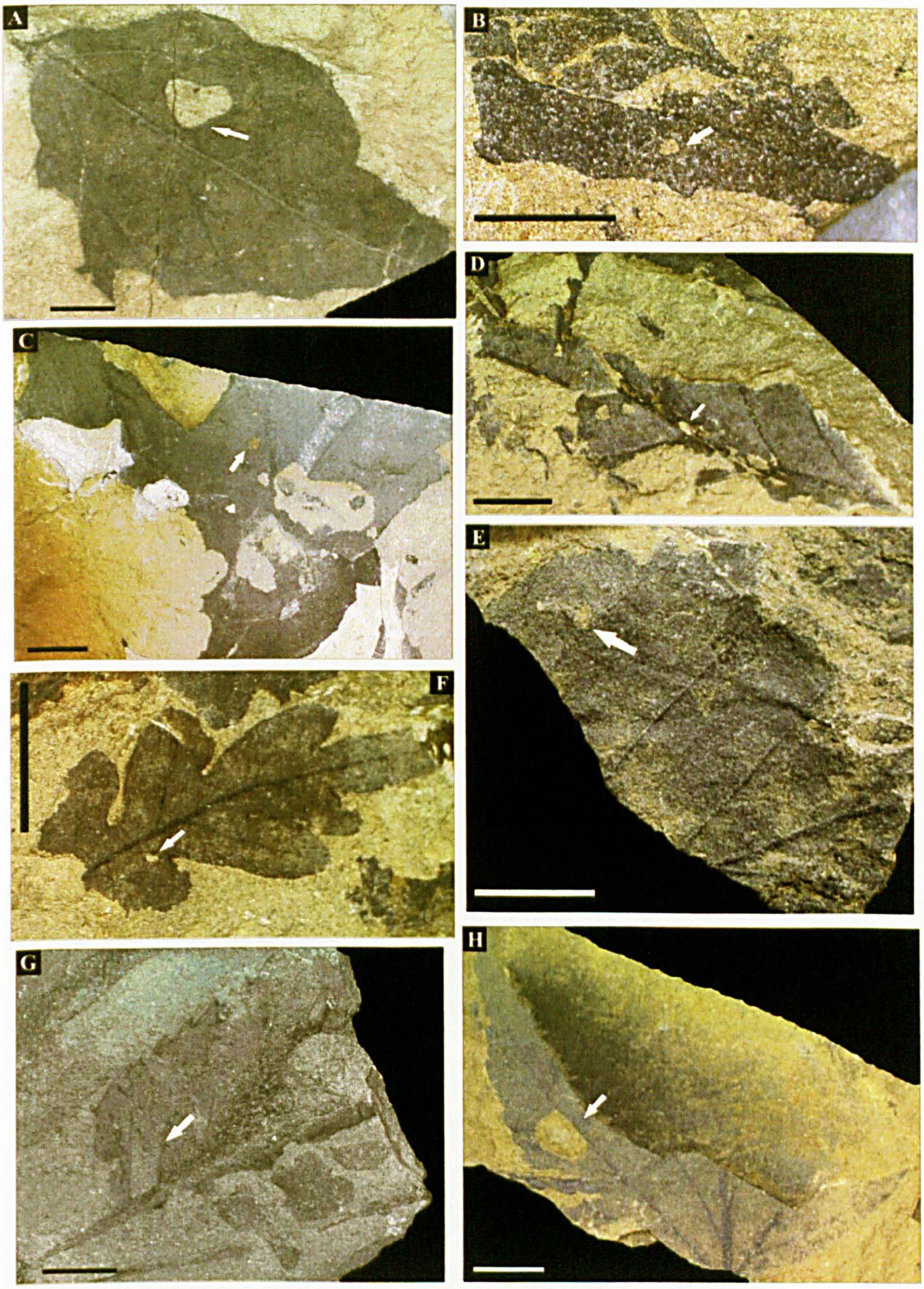


Figure 3.8. Examples of trace types of non-marginal general leaf chewing from King George Island (K). A) K2.3, P.3001.36.1(1). B) K2.4, P.3013.5. C) K2.5, P.3032.59. D) K.2.6, P.3001.89(1). E) K2.7, P. 3032.4. F) K2.8, P.3001.89(3). G) K2.9, G.312.15(8). H) K2.10, Green sticker 2. Scale bar 1cm. White arrows indicate position of traces.

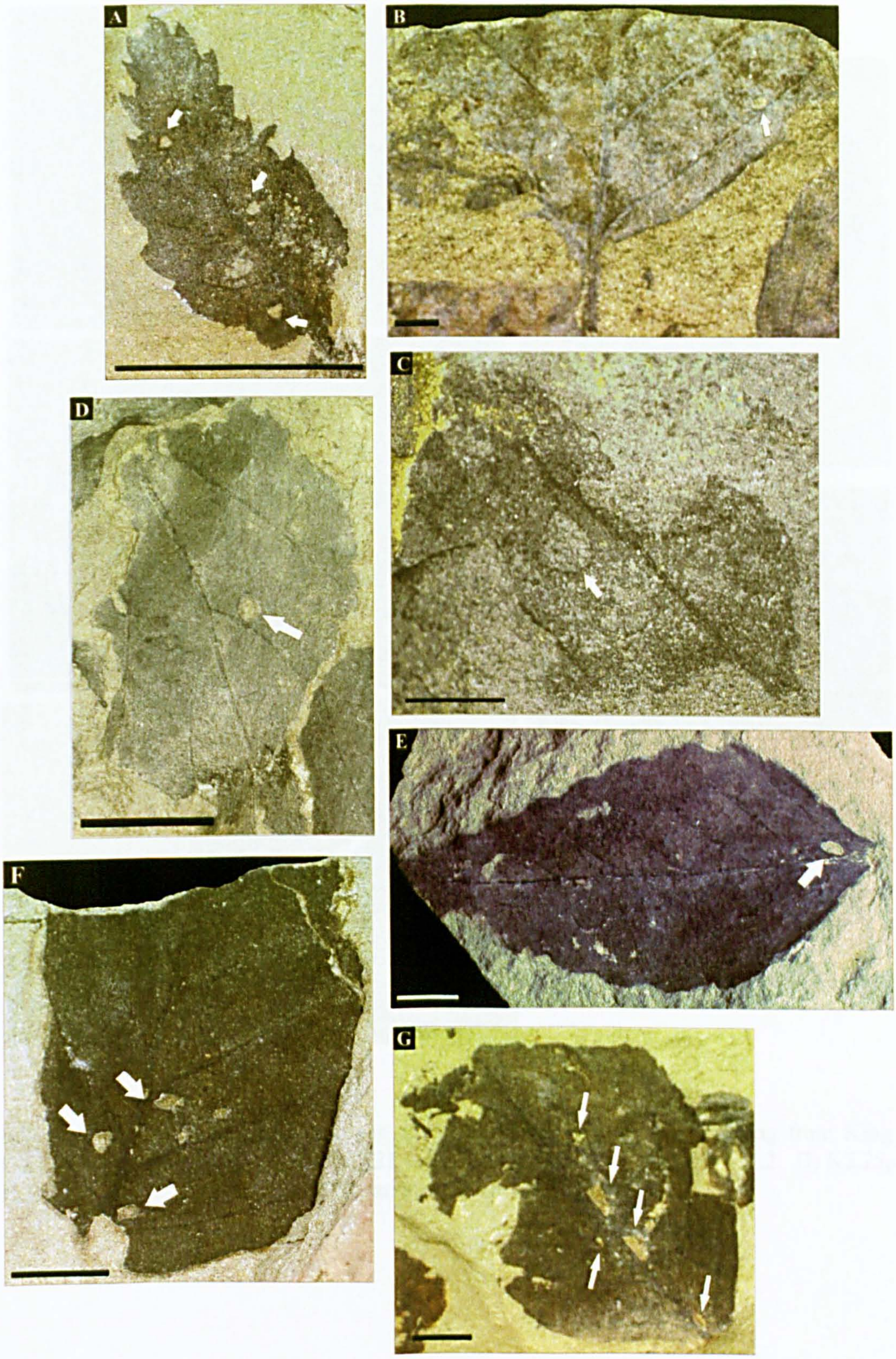


Figure 3.9. Examples of trace types of non-marginal general leaf chewing from King George Island (K). A) K2.15:, P.3001.46(3). B) K2.16, P.3001.186.3(1). C) K2.17, P.3032.23. D) K2.18, P.3001.64(6). E) K2.19, P.3001.108. F) K2.20, P.3001.51(2). G) K2.21, P.3001.7.2(2). White arrows indicate position of traces.

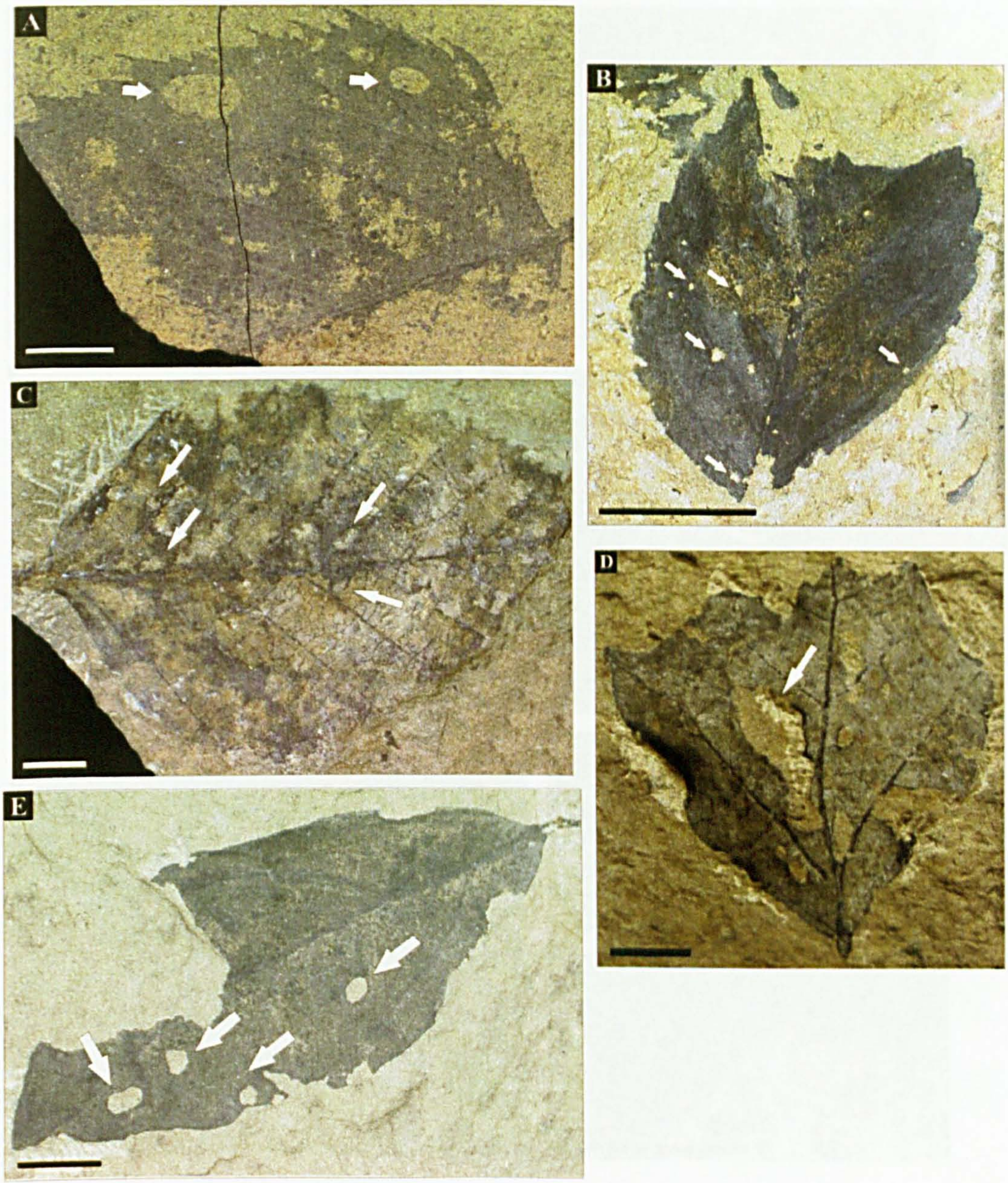


Figure 3.10. Examples of trace types of non-marginal general leaf chewing from King George Island (K). A) K2.22, P.3032.21. B) K2.23, G.9.3(3). C) K2.24, G.9.2. D) K2.25, P.3013.9. E) K2.26, P.3013.7. White arrows indicate position of traces.

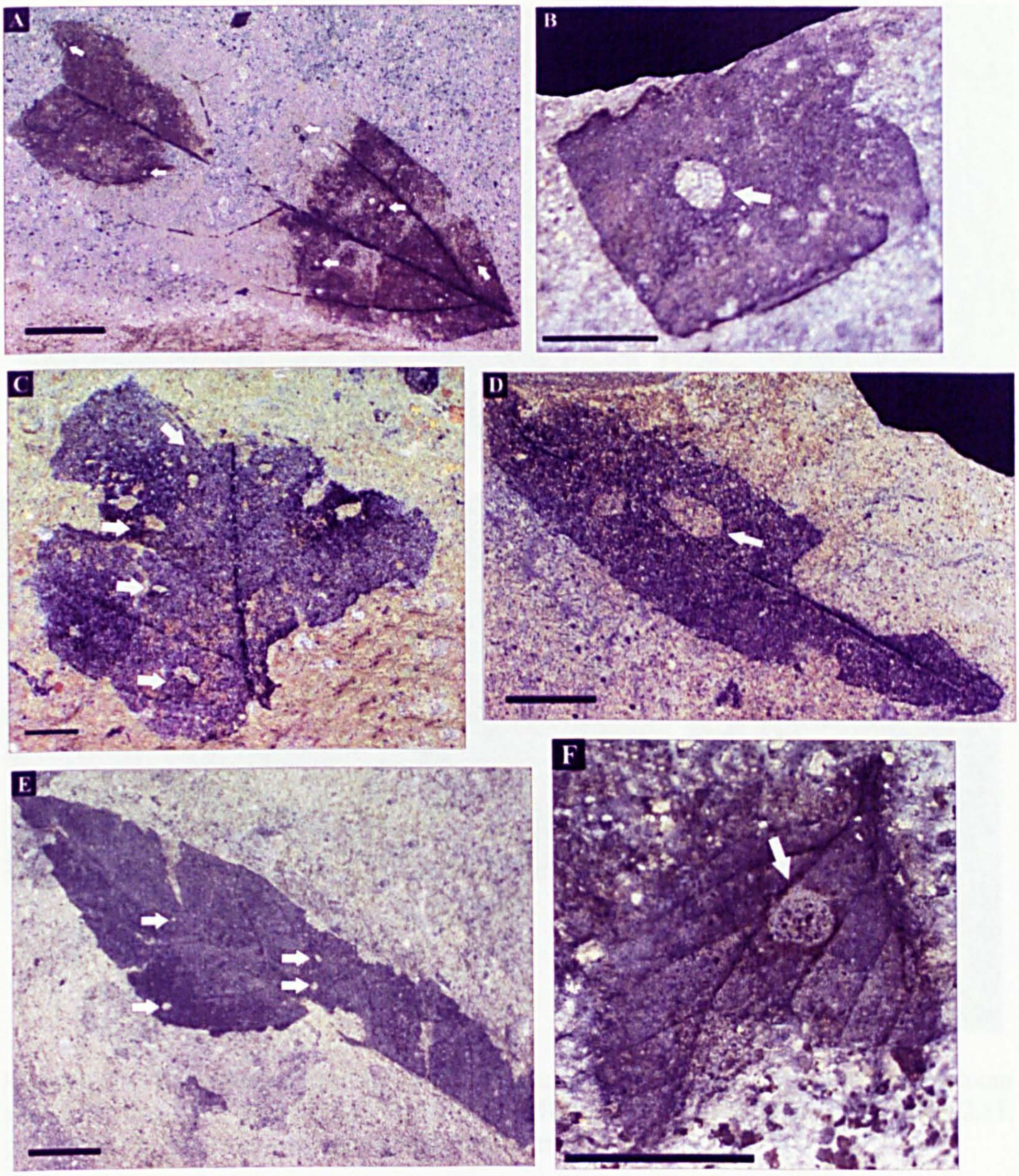


Figure 3.11. Examples of trace types of non-marginal general leaf chewing from Seymour Island (S). A) S2.1, DJ.1113.45b. B) S2.2, DJ.1113.71. C) S2.3, DJ.1105.133. D) S2.4, DJ.1111.11. E) S2.5, DJ.913.86. F) S2.6, DJ.1111.87(1). White arrows indicate position of traces.

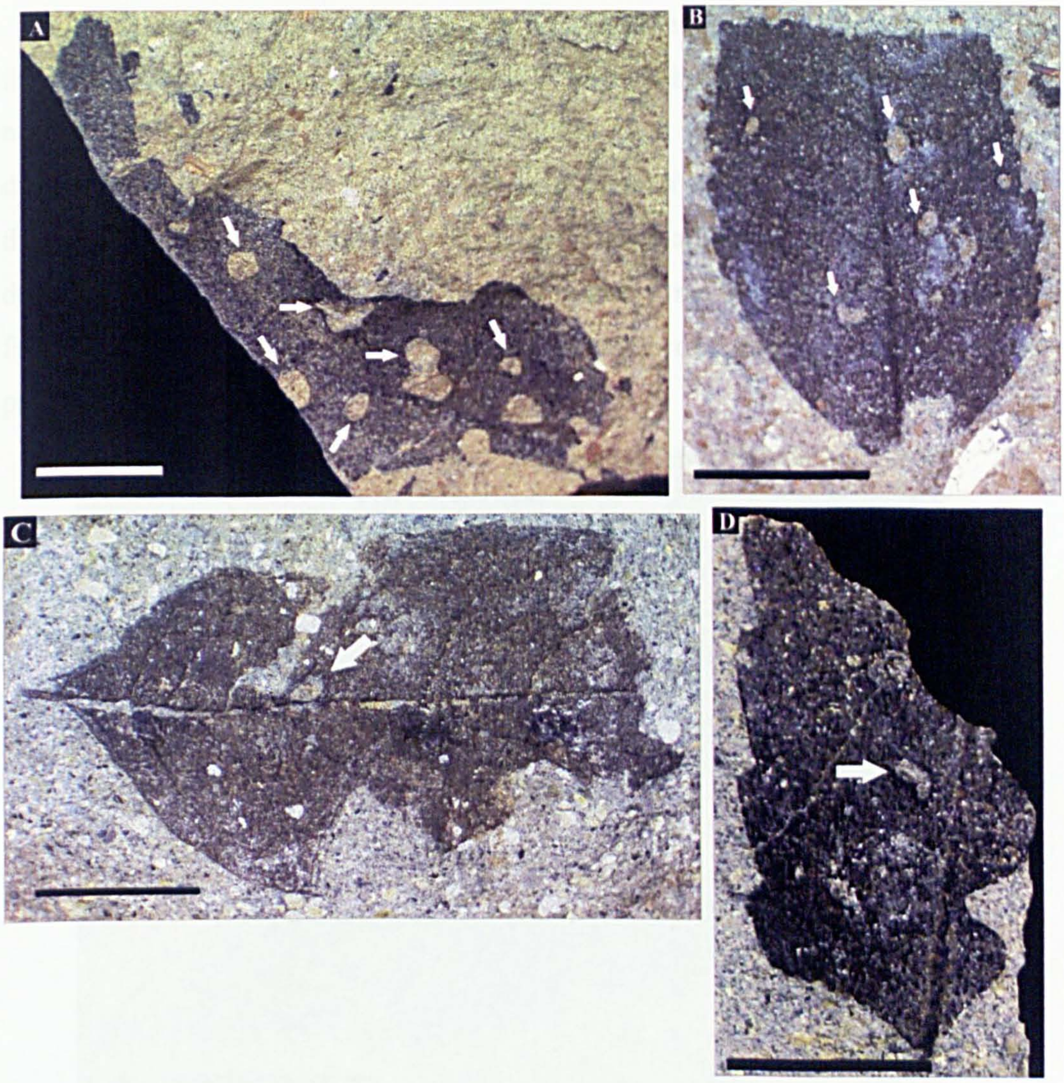


Figure 3.12. Examples of trace types of non-marginal general leaf chewing from Seymour Island (S). A) S2.8, DJ.1105.61. B) S2.9, DJ.1105.141. C) S2.10, DJ.1113.17. D) S2.11, DJ.1105.218. White arrows indicate position of traces.

3.3.2. Skeleton Feeding

In the trace type described as skeleton feeding the tissue (either upper, lower or both epidermal layers) is completely removed, leaving the primary, secondary and lower order venation intact. The area of insect damage is encompassed by a distinctive reaction rim. This differs from extensive non-marginal general leaf chewing because in the latter case the small network of veins are removed and only the primary or secondary veins remain undamaged.

Skeleton feeding was found on only one fossil leaf of Nothofagaceae (K5.1) from King George Island. The vein network is intact but the leaf tissue has been removed (Figure 3.13). The surrounding leaf tissue is still present and there is a distinctive wound reaction around the edge of the area damaged. This indicates that the trace was made when the leaf was still alive and not during or due to decomposition. The rarity of skeleton feeding traces may represent taphonomic filtering because leaves with this trace type would decompose more easily prior to preservation.

Figure 3.13. The only leaf found in all collections with skeleton feeding, King George Island, K5.1, P.3001.140 (2). Scale bar 1cm. White arrow indicates trace.

3.3.3. Leaf Galls

There were 15 recognisably different types of leaf gall observed amongst the fossil leaves, largely reflecting the different plant species on which they were found. For example, at least 11 different leaf types were found with leaf galls on them (Table 3.5). The galls varied in structure, either raised from the surface or preserved flat on top of the leaf. The majority had multiple galls on each leaf specimen. In the King George Island flora 13 gall types were identified. One particularly distinct gall type (K3.2) was found on a single leaf specimen. The leaf had multiple, small, circular galls concentrated around the apical region and adjacent to the primary vein.

The galls have a central exit pore that creates an indentation in the centre of the gall, giving a ring shape (Figure 3.14C & D).

Table 3.5. Description of each type of leaf gall identified on the fossil leaves from King George Island (K) and Seymour Island (S). The leaf morphotype numbers used for the description of each leaf, such as Morphotype 2.32 and Morphotype 1.27, are from Hunt (2001) for King George Island flora and Tosolini *et al* (in prep) for Seymour Island. Continued on p57.

Trace Type	Leaf Morphotype	Description	Total Number in Trace Type
K 3.1	Nothofagaceae	Single small circular gall with raised central hole, smooth wall, next to primary vein and between two secondary veins. (Figure 3.14A)	3
K 3.2	Morphotype 2.32	Multiple, small ring-shaped circular galls, concentrated around the apex adjacent to primary vein, central exit pore (Figure 3.14C&D).	1
K 3.3	Unknown 9	Large gall at the apex covering primary vein and leaf tissue of the apex, circular shaped. (Figure 3.14B).	2
K 3.4	Myricaceae (2.18)	Multiple, medium-sized gall located between two secondary veins, usually 2 or more in the middle area between primary and edge. (Figure 3.14E).	5
K 3.6	Laurales (1.13)	Multiple galls on secondary veins, small-medium size, circular depressions and raised areas. (Figure 3.14F).	7
K 3.7	Nothofagaceae	Multiple, small, circular, raised areas, on secondary veins. (Figure 3.15A).	1
K 3.8	Nothofagaceae (2.55)	Large, single, circular, gall in between two secondary veins, in middle of right side of upper epidermis (Figure 3.15B)	1
K 3.9	Morphotype 1.27	Multiple, small circular galls between 2 secondary veins (Figure 3.15D).	1
K 3.10	Nothofagaceae	Multiple, small medium-sized gall on primary vein, circular to elliptical shape (Figure 3.15C).	1
K 3.11	Unknown 10	Elliptical-shaped gall in apical region near the edge, medium to large size. (Figure 3.15E).	1
K 3.12	Unknown 11	Large indentation raised upwards at base of leaf adjacent to primary vein and basal edge of leaf (Figure 3.15F).	1
K 3.13	Unknown 12	Large, single, circular gall next to primary vein and between 2 secondary veins, basal area, preserved in a raised position. (Figure 3.15G).	1

Trace Type	Leaf Morphotype	Description	Total Number in Trace Type
K 3.14	Morphotype 1.5	Large, single, circular gall, at apex of lobe on the edge, not smooth (Figure 3.15H).	1
S 3.1	*Cunoniaceae* possibly	Multiple, small, circular traces at junction of primary vein and secondary, middle area (Figure 3.16A).	1
S 3.2	Unknown 13	Multiple, small to medium size, circular gall on or adjacent to secondary veins, raised protrusions, apical region (Figure 3.16B).	1

Many fossil leaves from the Dufayel Island locality (Palaeocene) were found to contain leaf gall traces. The fossil leaves with galls were of five different plant types, including the Nothofagaceae, therefore, the locality is not dominated by one particular type of vegetation that was prone to galling. Despite this, 60% of the total number of leaves with galls from King George Island were from the Dufayel Island locality. The specimens had large to medium sized galls of a circular shape and were both found singularly and as multiples per leaf. For example, five different leaf specimens had multiple galls located in the middle section of the leaf and found midway between the primary vein and the leaf margin (Figure 3.14E). There were four trace types that included only specimens from the Dufayel locality (K3.3, K3.4, K3.8 and K3.11) and one trace type (K3.6) that had leaf specimens from both Dufayel Island (6 trace specimens) and Fossil Hill (1 specimen)(Figure 3.14F).

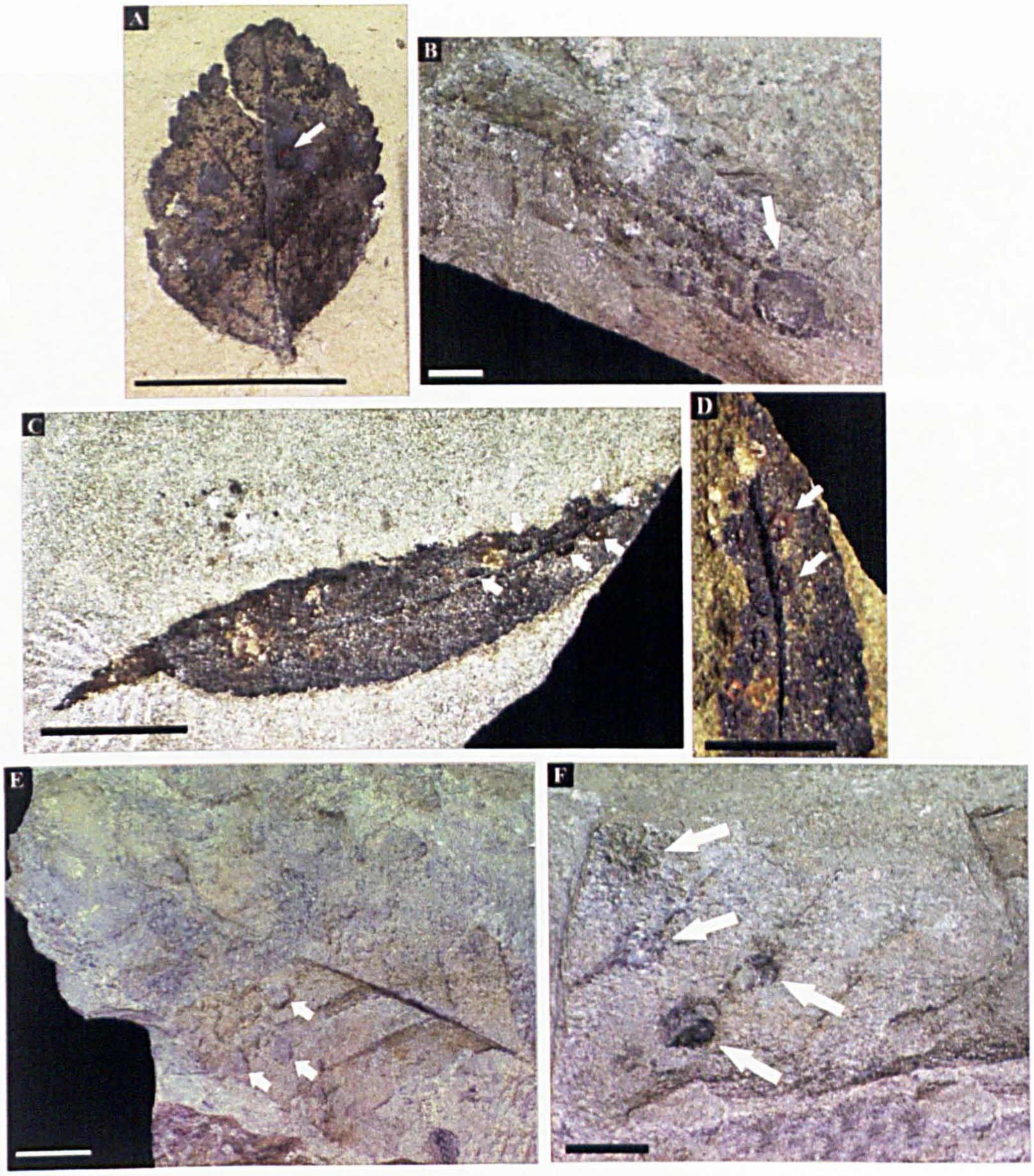


Figure 3.14. Examples of different leaf gall trace types from King George Island (K). A) K3.1, P.3001.136 (1,2). B) K3.2, G.312.13(1). C) K3.2, P.3001.44. D) Close up of K3.2. E) K3.4, G.53.19(1). F) K3.6, G.53.20(2). Scale bar 1cm. White arrows indicate the traces.

Another type (K3.13) is a single large gall at the basal area of the leaf, protruding above the surface of the leaf (Figure 3.15G). For Seymour Island, two specimens had leaf galls present, each with a different trace type (S3.1 and S3.2). For example, one leaf had multiple, small, circular galls only found at the junction of the primary and secondary veins (Figure 3.16A). Seven of the gall types were of singular galls on leaves. An example is K3.1, where a small circular gall is present. The gall is adjacent to the primary vein, in the middle of two secondary veins and is raised from the surface on a single Nothofagaceae leaf (Figure 3.14A).

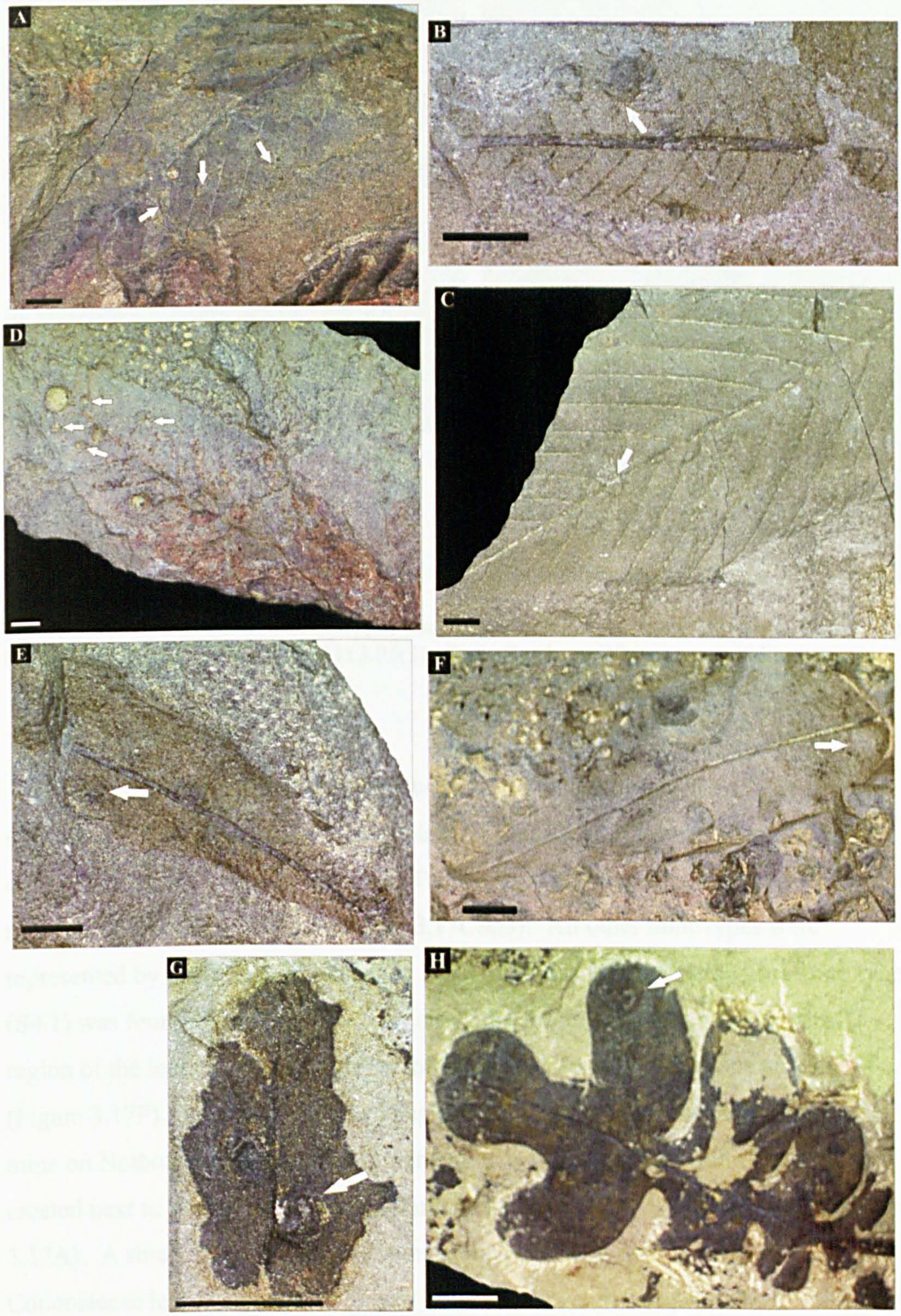


Figure 3.15. Examples of different leaf gall trace types from King George Island (K). A) K3.7, P.2799.1.2(1). B) K3.8, G.53.6(3). C) K3.10, P.2799.8.2-5(1). D) K3.9, P.2799.1.2(3). E) K3.11, G.53.19(2). F) K3.12, P.3031.81(1). G) K3.13, P.3001.58.1(2). H) K3.14, P.3001.187(2). Scale Bar 1cm. White arrows indicate traces.

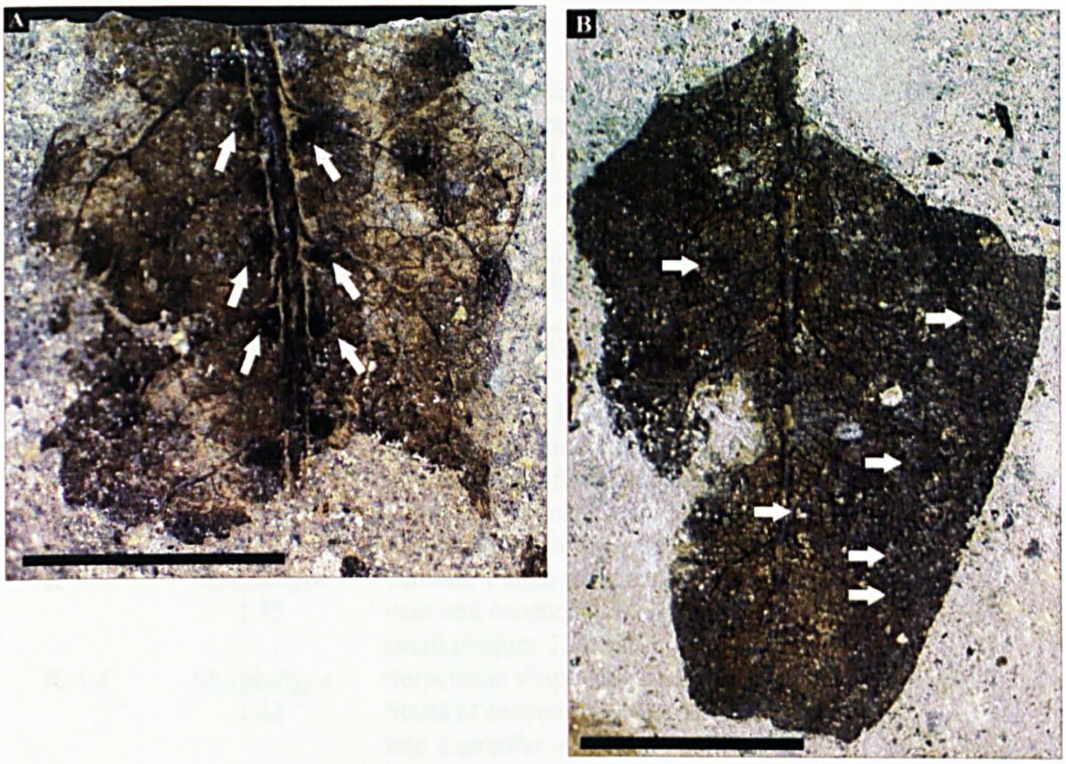


Figure 3.16. Two leaf gall trace types found on fossil leaves from Seymour Island. A) S3.1, DJ.1113.161. B) S3.2, DJ.1113.95(3). Scale Bar 1cm. White arrows indicate traces.

3.3.4. Leaf Mines

Five different types of leaf mine were found on the fossil leaves. They varied in character depending on the type of plant species on which the mine was created and in the size and shape of the mine (Table 3.6). Only one mine type (4.3) was represented by two specimens (Figure 3.17C&D). All other mine types were represented by only single specimens. From Seymour Island, a serpentine mine type (S4.1) was found to run longitudinally along the fossil leaf surface to the apical region of the leaf. Two mines are on the same leaf, directly beside one another (Figure 3.17F). In contrast, on the King George Island specimens there is a distinct mine on Nothofagaceae leaf (K4.1). The mine was a large to medium sized blotch created next to the primary vein and contained between two secondary veins (Figure 3.17A). A small sinuously-shaped mine at the basal area was found on a Cunoniaceae leaf. The mine continues vertically towards the apex into the middle of the leaf area and it is adjacent to the primary vein (Figure 3.17B). A serpentine to blotch mine was observed in which the channel starts in a linear manner and terminates in a blotch (Figure 3.17E).

Table 3.6. The description of leaf mine traces found at King George Island (K) and Seymour Island (S). The leaf morphotype numbers used for the description of each leaf are from Hunt (2001) for the King George Island flora and Tosolini *et al* (in prep) for the Seymour Island flora.

Trace Number	**Leaf morphotype**	**Description**	**Total Number in Trace Type**
K 4.1	Nothofagaceae	Large-medium blotch mine between two secondary veins and the primary vein (Figure 3.17A).	1
K 4.2	Cunoniaceae	Small linear mine runs vertical along several areas. Starts at primary and finishes at primary vein. Distinct serpentine curve (Figure 3.17B).	1
K 4.3	Morphotype 1.15	Circular blotch mine at the primary vein and secondary vein junction, small.(Figure 3.17C&D)	2
K 4.4	Morphotype 1.13	Serpentine shape leading into a blotch. Starts at secondary vein and widens into a circular blotch on secondary nearer the apical area (Figure 3.17E).	1
S 4.1	Unknown	Irregular serpentine mine, 1 lamina still present with distinct ridge near primary vein and cover several inter-secondary areas (Figure 3.17F)	1

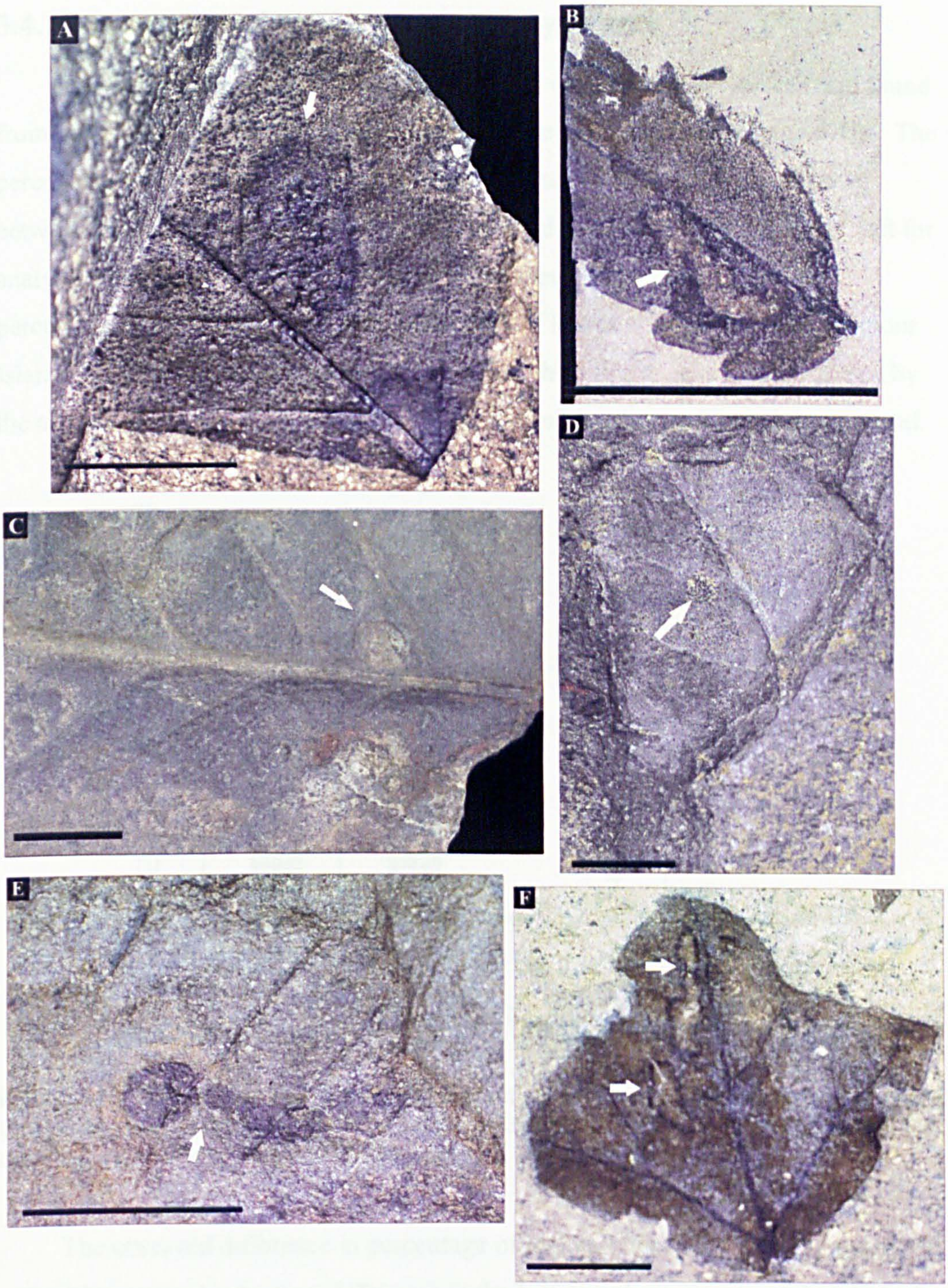


Figure 3.17. Examples of the leaf mine trace types from King George Island(K) and Seymour Island (S). A) K4.1 P1404.20(1). B) K4.2, P.3001.81 A/B. C) K4.3, P.2799.2.2(1). D) K4.3, P.2799.2.2 (2). E) K4.4, G.53 (20)(1). F) S4.1, DJ. 1113.86. Scale bar 1cm. White arrows highlight trace.

3.4. Quantitative Analysis of Herbivory Traces

Non-marginal general leaf chewing was the most abundant type of trace found from both fossil localities; the leaf mines were the least abundant (Figure 3.18). The percentage of leaves in each insect damage category did not differ significantly between the two assemblages (General linear model, poisson error family, χ^2 test for analysis of deviance, d.f. = 4, $P = 0.25$). King George Island had a greater percentage of leaves that contained galls (21% of leaves with traces) than Seymour Island (6.25% of leaves with traces). However, this difference is mainly caused by the abundance of leaf galls from the Dufayel Island locality on King George Island.

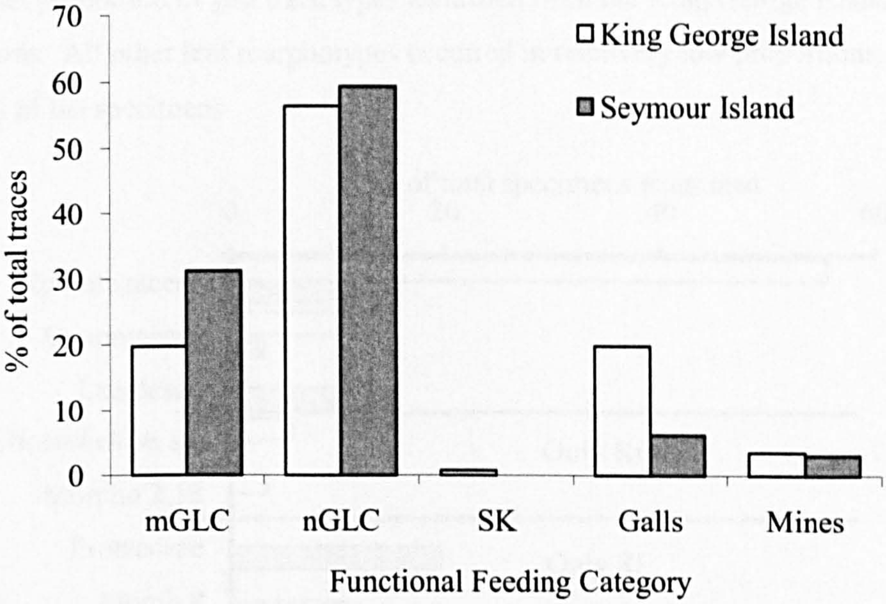


Figure 3.18. Percentage of traces collected within each functional feeding guild (King George Island = 142 traces, Seymour Island = 32 traces). mGLC = marginal general leaf chewing, nGLC = non-marginal leaf chewing, SK = skeleton feeding.

The observed difference in percentage of leaf gall traces found at the two fossil assemblages may be due to a difference in the leaf morphotypes. A total of 40 leaf morphotypes identified from Hunt (2001) and Tosolini *et al.* (in prep), including damaged and undamaged leaf specimens, were examined. The morphotypes with affinities for extant families were grouped into family level to examine possible patterns more easily and the most common leaf morphotypes highlighted. Representatives of the Nothofagaceae, Cunoniaceae and Lauraceae were present in both assemblages. The relative proportions of each leaf morphotype and the leaf morphotype species differed between the two assemblages (General linear model, poisson error family, χ^2 test for analysis of deviance, d.f. = 6, $P < 0.001$) (Figure 3.19). Leaf morphotypes of Nothofagaceae were the most abundant in the King George Island collection (55.45% of specimens), but composed only 11.48% of the specimens examined from Seymour Island. The most abundant leaf type studied from Seymour Island was Proteaceae (19.62% of specimens), with Lauraceae the next abundant (14.81% of specimens). In contrast, the King George Island leaves were dominated by the Nothofagaceae and Cunoniaceae leaf morphotypes, with *Dictophyllum* sp. the next abundant. Morphotype 2.18, the next most abundant leaf type, had several leaf gall traces, all from the Dufayel Island locality. This explains the higher proportion of gall trace types identified from the King George Island collections. All other leaf morphotypes occurred in relatively low proportions, less than 3% of the specimens.

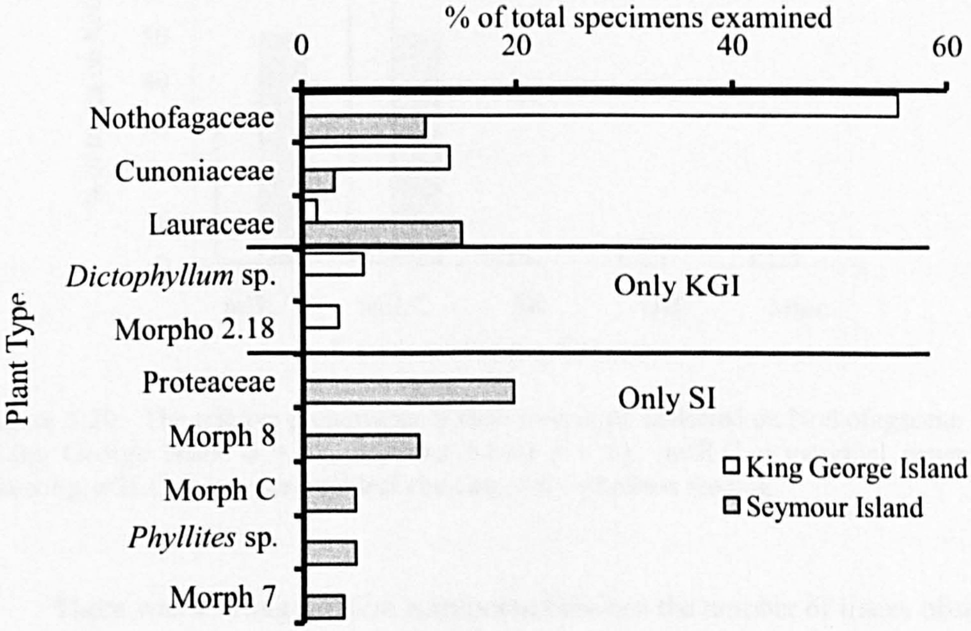


Figure 3.19. The proportion of specimens of the most common leaf types examined at each fossil locality. Leaf types similar to extant families are named, others are given morphotype numbers.

The greatest proportion of traces was found on Nothofagaceae fossil leaves (General linear model, poisson error family, χ^2 test for analysis of deviance, d.f. = 9, $P < 0.001$), therefore, the relative proportion of each of the functional feeding types on Nothofagaceae leaves for each locality was examined (Figure 3.20). There was no significant difference in the relative proportion of each functional feeding type collected from each locality, whether all morphotypes were grouped (Figure 3.19), or the Nothofagaceae morphotypes were examined separately for each locality (Figure 3.20)(General linear model, poisson error family, χ^2 test for analysis of deviance, d.f. = 8, $P = 0.1103$). There were only 6 Nothofagaceae leaves with traces from Seymour Island, all with general leaf chewing trace types. Therefore, despite an abundance of traces observed on Nothofagaceae leaves, the relative proportion of each trace category remained the same. This indicates that the dominance of one leaf morphotype in the fossils does not bias the trace types that will be present.

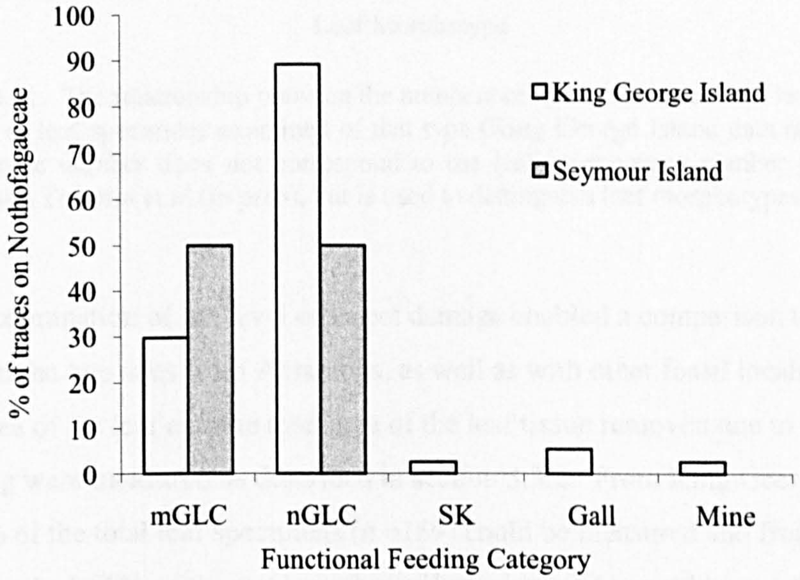


Figure 3.20. The relative proportions of each trace type collected on Nothofagaceae leaves (King George Island n = 37, Seymour Island n = 6). mGLC = marginal general leaf chewing, nGLC = non-marginal leaf chewing, SK = skeleton feeding.

There was a strong positive correlation between the number of traces observed on the leaves of a particular morphotype and the number of leaf specimens of that morphotype (Spearman's rank correlation, r = 0.99, $P < 0.05$). Therefore, the greater the number of leaves preserved of a particular leaf type, the greater the chance of the trace fossils from that plant type being observed (Figure 3.21). Hence, there could be a bias in the proportion of trace types if there is a taphonomic filtering in the plant species preserved. This is especially true for species specific traces such as the leaf mines and leaf galls. Equally, there could be a preservational bias to the more abundant plant species growing during that time period.

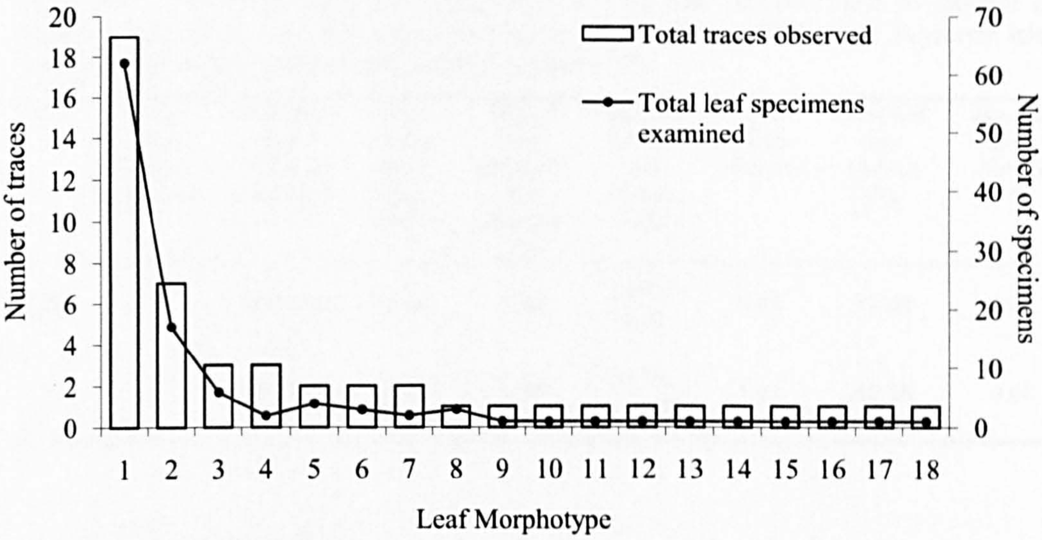
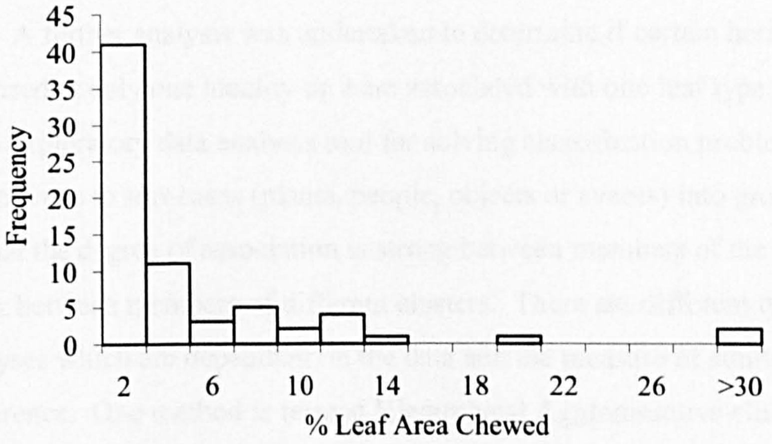


Figure 3.21. The relationship between the numbers of traces observed on a leaf and the total number of leaf specimens examined of that type (King George Island data only). The leaf morphotype number does not correspond to the leaf morphotype number given in Hunt (2001) and Tosolini et al (in prep), but is used to distinguish leaf morphotypes.

Examination of the level of insect damage enabled a comparison to be made between the two sites from Antarctica, as well as with other fossil localities. The total area of the leaf and the total area of the leaf tissue removed due to general leaf chewing were measured as described in section 3.3.2. From King George Island, 11.92% of the total leaf specimens (n =159) could be measured and from Seymour Island, only 1.57% of the total number of leaves (n = 16) could be measured. The area of leaf tissue removed by herbivores was relatively low for both localities, although the proportion for Seymour Island was greater than for King George Island (Table 3.7). The frequency of different levels of leaf damage (Figure 3.22) at each locality was, however, not significantly different (General linear model, poisson error family, χ^2 test for analysis of deviance, d.f. = 3, $P = 0.328$). Both the maximum and the minimum values are similar and the area of leaf tissue chewed per leaf was not greater than 41% of the total leaf area (Table 3.7). The most frequent level of insect damage was, however, less than 5% of the total leaf area. For King George Island, 74.29% of the leaves with general leaf chewing had less than 5% of the leaf tissue damaged and for Seymour Island, 77.8% of the chewed leaves also had less than 5% of the leaf area damaged (Figure 3.22).

Table 3.7. Levels of herbivory (proportion of leaf area removed due to general leaf chewing) for fossil leaf collections from King George Island (KGI) and Seymour Island (SI). Measurements made as described in section 3.3.2.

Site	No. of chewed leaves measured	Estimated total surface area (cm^2)	Total chewed surface area (cm^2)	Surface area removed by herbivory (%)	Mean % of leaf area chewed ± SD	% of leaves chewed	Max leaf area chewed (%)	Min leaf area chewed (%)
KGI	71	12058.90	203.89	1.69	1.69 ± 4.50	9.83	32.09	0.02
SI	9	1230.79	44.12	3.59	4.39 ± 10.42	2.64	40.85	0.08


A. King George Island

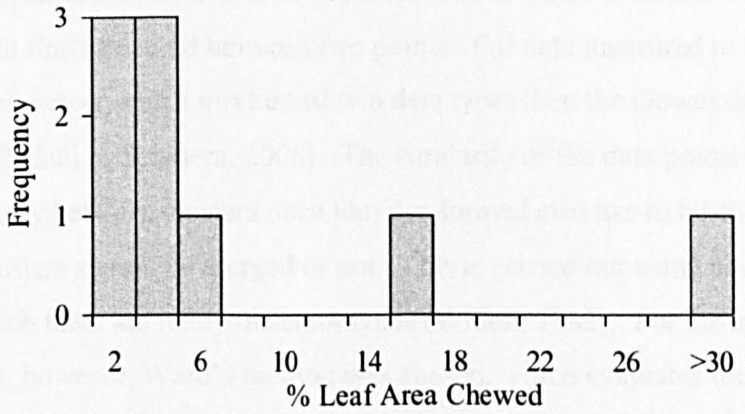

B. Seymour Island

Figure 3.22. The range of leaf area removal by general leaf chewing from the fossil leaves measured from King George Island (A) (n = 71) and Seymour Island (B) (n = 9).

The level of damaged leaves due to general chewing on King George Island and Seymour Island is low when compared to other studies on fossil herbivory levels, particularly in temperate Eocene forest systems (Smith & Nufio, 2004). The estimated percentage of leaves damaged in dry tropical to temperate forests in North America during the Early to Late Eocene ranged from 35% to 19.4% (Wilf & Labandeira, 1999; Wilf *et al.*, 2001; Smith & Nufio, 2004). Where documented, the surface area removed by insects was however similar, 1.4% to 2.5% of total leaf area. This will be explored more fully in the discussion chapter, Chapter 8.

3.4.1. Are there trace characters specific to one locality or one leaf type?

A further analysis was undertaken to determine if certain herbivory traces occurred at only one locality or were associated with one leaf type. Cluster analysis is an exploratory data analysis tool for solving classification problems. The objective is to sort cases (plants, people, objects or events) into groups, or clusters, so that the degree of association is strong between members of the same cluster and weak between members of different clusters. There are different types of cluster analyses which are dependent on the data and the measure of similarity or difference. One method is termed Hierarchical Agglomerative cluster analysis, which starts with all data points as independent units and systematically groups them, depending on their similarity. The similarity of two data points is calculated by a distance measure, such as the Euclidean distance measure, which is defined as a straight line measured between two points. For data measured in a nominal or ordinal way or with a mixture of two data types then the Gower distance method is best (Podani & Schmera, 2006). The similarity of the data points is defined, but the similarity between clusters once they are formed also has to be controlled to see if the clusters should be merged or not. This is carried out using clustering algorithms, of which there are many different types (Willett, 1982). For the analysis on trace fossils, however, Ward's method was chosen, which evaluates the distance between two clusters based on a correlation measurement between every two pairs (Ward Jr, 1963). The clusters that are correlated are merged and the ones that are not are segregated.

Cluster analysis using the Gower distance measure and Ward's algorithm was performed on all trace fossil data using the programme R. The aim was to assess the similarity of traces both between localities and leaf types. All general leaf chewing traces were characterised according to a categorical classification scheme (Table 3.8). The analysis was carried out without the leaf type or locality defined for each trace. The results are displayed graphically using a tree-like diagram called a dendrogram that shows the relationship between the clusters and the final groupings. The trace types mentioned in section 3.3.1 are given as a reference for the trace clusters described from the analyses.

Table 3.8. The different characters used to measure the general leaf chewing fossil traces identified on the leaves from King George Island and Seymour Island. The different categories and codes were used for cluster analysis.

Trace Character	Category	Code
Leaf edge damaged	Yes	1
	No	0
Number of areas damaged	Single	0
	Multiple	1
Size of damage (as defined in section 3.2.2)	Small	1
	Medium	2
	Large	3
Position on the leaf	Primary Vein	1
	Secondary Vein	2
	Primary and secondary vein	3
	Neither vein	4
	Basal area	5
	Apical area	6
	Middle area	7
	Multiple areas	8
Area of the leaf damaged	< 5% of leaf area	1
	6-20% of leaf area	2
	21-50% of leaf area	3
	51-100% of leaf area	4
Shape of damage	Circular	1
	Elliptical	2
	Irregular	3
	Mixture of shapes	4
	Oblong	5
	Continuous damage	6
	Discontinuous damage	7

Different clusters will be generated depending on both the distance and clustering method applied. To ensure the highlighted clusters do not form by chance and that the groupings are reliable, bootstrapping of the results from the cluster analysis was performed. Bootstrapping tests the reliability of the sample by estimating the variation in the cluster analysis. The analysis starts by creating a large number of new samples from the original sample (>1000 samples) of the same sample size, and each sample is drawn with replacement (each data point can be used again in subsequent samples). The required estimate (p-value) is then calculated from each of the new samples yielding a full distribution range. The p-value can then be calculated at the 95% confidence level to confirm that the cluster is not formed only by chance. The higher the p-value the more reliable the cluster.

The results of the cluster and bootstrap analysis indicate that there are no character combinations that can separate one locality from the other, although in conjunction with the leaf type some are distinctive (Figure 3.23). A total of 20 clusters with p-values greater than 95 were defined, indicating the clusters that reliably describe the groupings. There were 8 clusters with traces from only King George Island; the other 12 clusters contained traces from both King George Island and Seymour Island. Of the 8 clusters, 7 included traces on Nothofagaceae leaves, with one cluster containing only traces on Nothofagaceae leaves. The cluster with traces only on Nothofagaceae had a unique character combination which was not seen on any other leaf type or at Seymour Island. The trace was a type of non-marginal general leaf chewing, trace type K2.23 (Table 3.4). The trace had multiple, small, circular shaped areas of damaged leaf, with the damage throughout the leaf surface.

Also evident within the distinct King George Island groups that have both Nothofagaceae and other leaf types grouped, are that the traces on Nothofagaceae show unique character combinations. For example, one cluster contained 4 Nothofagaceae traces and 2 other leaf morphotype groups (M2.6 and M2.18). The Nothofagaceae traces had a unique character combination of non-marginal general leaf chewing, with single, medium sized, elliptically-shaped damaged

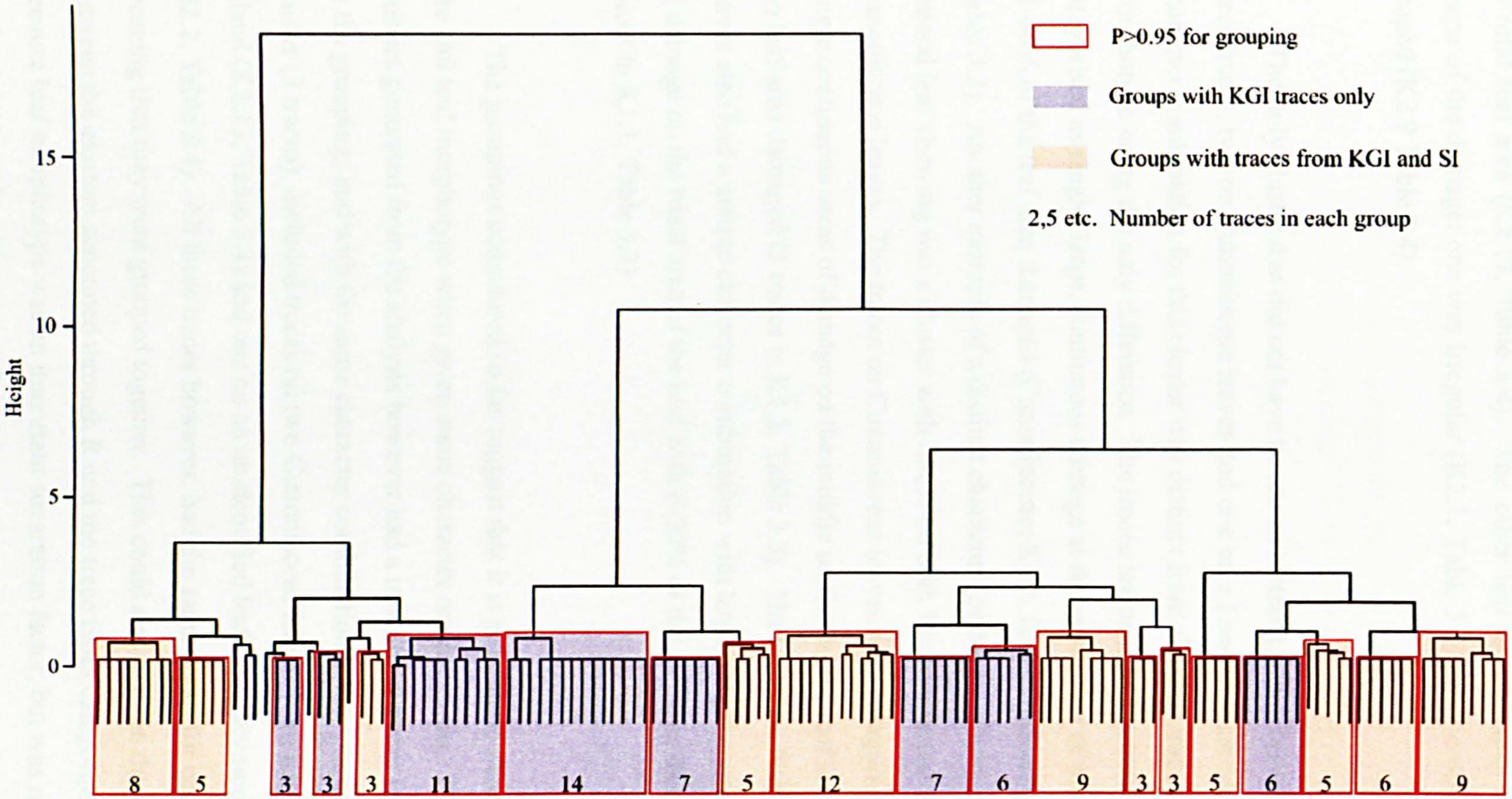


Figure 3.23. Dendrogram highlighting the groups formed after cluster analysis and bootstrapping of all general leaf chewing traces from both King George Island and Seymour Island. Each end point in the tree represents an individual trace. The clusters with red rectangles indicate the reliable clusters with 95% confidence. The y axis is a measure of distance between each separation, which allows the tree to be cut at various points in the analysis.

leaf areas that were found adjacent to the secondary veins and covered less than 5% of total leaf area (K2.17, Table 3.4). The other leaf morphotypes differed in the shape of the damage: one was irregular (K2.1, Table 3.4) and the other was oblong shaped (K2.9 Table 3.4).

The only cluster that did not have traces on Nothofagaceae leaves contained three traces, two on Cunoniaceae leaves and one on a *Lauriphyllum* species. The character combination for this cluster was distinct from all other traces, with the leaf morphotype being the only difference. The traces are marginal general leaf chewing categorised as single, large, continuous damage at the apical part of the leaf, with 21-50% of the leaf area damaged (Cunoniaceae: K1.2, *Lauriphyllum* sp.: K1.5, Table 3.3). Another example of a distinct character combination with marginal general leaf chewing was a cluster with traces on both Nothofagaceae and Cunoniaceae leaves. The traces on Cunoniaceae leaves were categorised as large, single continuous areas of damage on the middle section of the leaf with 6-20% of the leaf area damaged (3 traces in K1.2, Table 3.3). The traces on Nothofagaceae leaves also had a unique character combination with large, single continuous areas of damage on the basal area of the leaf with 6-20% of the leaf area damaged (6 traces in K1.1, Table 3.3)

The groupings considered so far suggest that it is possible to determine both site and leaf morphotype when given some character combinations. The other 12 clusters generated from the analysis however had a mixture of traces from both sites in the groupings and with the same character combinations. For example, one cluster (3 traces), included traces on two Cunoniaceae leaves from King George Island (K2.13, Table 3.4) and one on an unidentified leaf from Seymour Island (S2.2, Table 3.4). All three traces however, had the same codes for each character, meaning that they were grouped together. This could also explain the difference between the clusters generated through R and the trace types categorised manually because leaf morphotype was an important separation factor, but was not included in the R analysis. Also, if a trace differed from another by only one character it is more than likely included in the same cluster. The weighting of certain characters to give exclusion factors, like leaf morphotype, would give more similar groupings.

There were no clusters that grouped together trace types on the same leaf morphotype from King George Island and Seymour Island. Therefore, a trace type on a Cunoniaceae leaf from King George Island (for example K2.14) was not grouped with a trace type on a Cunoniaceae leaf from Seymour Island (for example S2.9). This would be due to the different area of the leaf on which the trace was created as well as the size and shape. This suggests that there are distinct trace types on the same leaf morphotype, but in different localities. The same leaf morphotypes were however not classified at both localities or by the same person. Therefore, difference between localities shown by this cluster analysis may not be due to trace type only.

The cluster analysis has highlighted that there are individual trace character combinations within the least species-specific trace category of general leaf chewing. Specific characters are unique to some leaf morphotypes, but not to locality alone. Hence, a range of character types for a given trace may enable the leaf morphotype to be distinguished, but not reliably the area that the trace originally came from.

3.5. Summary of Insect Traces

- Two fossil leaf collections were examined for the presence of insect trace fossils. For King George Island, 130 leaves were found with traces which could be divided into 54 different trace types. For Seymour Island, 26 damaged leaves were found and 19 trace types.
- The insect damage categories include general leaf chewing, leaf mines and leaf galls. Only one specimen had evidence of skeleton feeding, which could represent a taphonomic filtering due to the leaves being more easily decomposed prior to preservation within the sediment. The taphonomic bias

of trace types is considered further in Chapter 8. General leaf chewing was the most common trace at both localities and leaf mines the least common.

- The frequency of leaf damage due to general leaf chewing was 9.83% for King George Island and 2.64% for Seymour Island, lower than damage levels in previous fossil herbivory studies. The estimates of damage level calculated from the fossils represent a minimum level of leaf damage due to the influence of preservation.
- Cluster analysis has highlighted that for general leaf chewing traces there are specific trace characters that together are unique to certain leaf morphotypes. Some trace characters are specific to site, but only in combination with the leaf morphotype.
- The most common fossil traces were found on Nothofagaceae morphotypes, therefore, species of *Nothofagus* were the focus of studies on modern insect herbivory traces (Chapters 4 - 6).

Chapter 4. The Modern Insect Faunas of Chile

4.1. Data Analysis in Chapter 4

All statistical analyses were carried out using the programme R 2.6.1 (R: A Language and Environment for Statistical Computing, The R Development Core Team, 2008). Generalized linear models were used to test the difference between the numbers of leaves collected at each site for the two deciduous *Nothofagus* tree species (Section 4.4.3.1). A detailed description of all statistical models is given in Appendix VI.

4.2. Study Sites

The fieldwork in Chile was carried out from November 2006 to April 2007. Field sites were chosen after discussion with entomologists and botanists at the Museo Nacional de Historia Natural in Santiago, Chile about the distribution of the two chosen southern beech species, *Nothofagus pumilio* and *Nothofagus antarctica.* Permission was gained for access to the sites managed by the Corporación Nacional Forestal (CONAF), which included both National Parks and Nature Reserves.

4.2.1. Location

The sites visited were distributed across a range of latitudes in southern Chile (Figure 4.1) to allow for sampling of a greater diversity of microclimates (and potentially insects and the traces they leave behind). The study areas ranged from the most northerly at Parque Nacional Nahuelbuta (37°44'S) to the most southerly site on Isla Navarino (55°00'S). The complete list of sample sites and their locations is presented in Table 4.1. The main study area, Parque Nacional Puyehue, was sampled three times (7th-14th December 2006, 21st January 2007-1st February 2007 and 19th-30th March 2007) to enable data collection at different times during the warmest season (section 6.5.1). In the following sections and chapters the sites visited will be described using the codes given in Table 4.1.

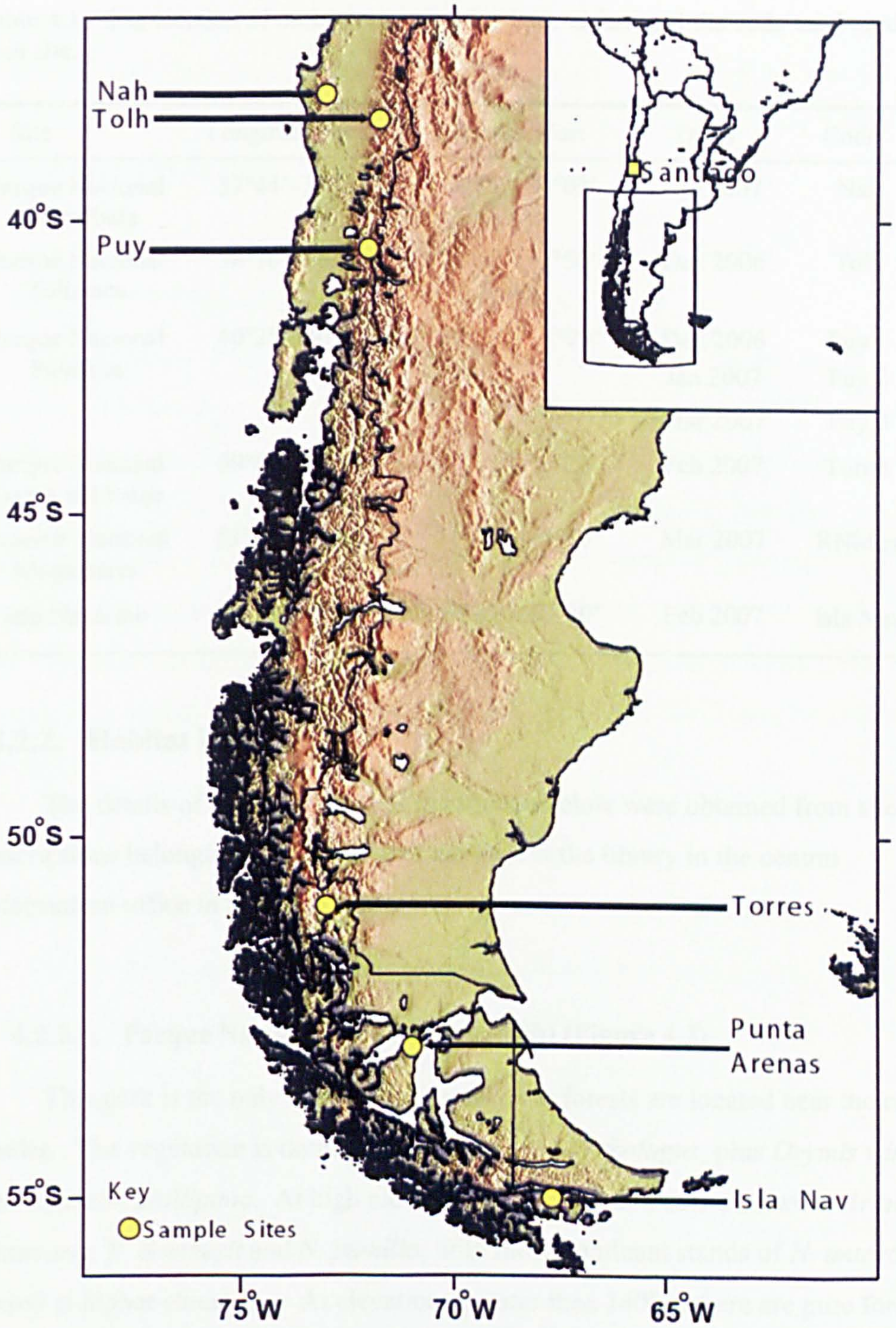


Figure 4.1. Map showing locations of sites surveyed in Chile. The park codes are explained in Table 4.1.

Table 4.1. The location of each survey site, the dates visited and the code used to identify each site.

Site	Longitude South	Latitude West	Dates	Code
Parque Nacional Nahuelbuta	37°44'- 37°51'	72°57'- 73°03'	Jan 2007	Nah
Parque Nacional Tolhuaca	38°10'- 38°15'	71°42 - 71°50'	Dec 2006	Tolh
Parque Nacional Puyehue	40°25'- 40°57'	71°50' - 72°20'	Dec 2006	Puy 1
			Jan 2007	Puy 2
			Mar 2007	Puy 3
Parque Nacional Torres del Paine	50°45'- 51°20'	72°31'- 73°22'	Feb 2007	Torres
Reserva Nacional Magallanes	53°05' - 53°11'	71°01' - 71°15'	Mar 2007	RNMag
Isla Navarino	54°55'- 55°00'	67°45' - 67°30'	Feb 2007	Isla Nav

4.2.2. Habitat Description

The details of all sampling sites mentioned below were obtained from site descriptions belonging to CONAF that are kept at the library in the central information office in Santiago, Chile.

4.2.2.1. Parque Nacional Nahuelbuta (Nah) (Figure 4.2)

This park is the only study area in which the forests are located near the coastal Andes. The vegetation is dominated by species of *Nothofagus*, plus *Drymis winteri* and *Laurelia phillipana*. At high elevations an association exists between *Araucaria araucana*, *N. dombeyii* and *N. pumilio*, with more dominant stands of *N. antarctica* found at higher elevations. At elevations greater than 1400m there are pure forests of *A. araucana*.

4.2.2.2. Parque Nacional Tolhuaca (Tolh) (Figure 4.2)

Many species in this park have been affected by a forest fire that occurred in the summer season of 2002, therefore sampling was limited to areas away from the affected sections. Again, species of *Nothofagus* were present, with *N. allesandri* and

N. betuloides at lower elevations and mixed stands of *N. pumilio* and *N. antarctica* at higher elevations. *Araucaria araucana* trees are the dominant species at the maximum tree limit (1300m) and there is a dense undergrowth of native bamboo, *Chusquea culeou*, in many parts.

4.2.2.3. Parque Nacional Puyehue (Puy) (Figure 4.2)

Six species of *Nothofagus* grow in the park, including *N. antarctica*, *N. pumilio*, *N. betuloides*, *N. dombeyi*, *N. nitida* and *N. obliqua*. Below 900m elevation the forests are dominated by evergreen species such as *N. dombeyi* and *L. philippiana*, with *N. betuloides* and *N. nitida* appearing at higher altitudes. Above 950m, the forest is dominated by *N. pumilio* with *N. antarctica* growing at higher altitudes. The undergrowth is dominated by dense stands of *C. culeou* and *C. macrostachya*. At around 1200m, *N. pumilio* trees are reduced to a maximum height of only four metres and trees of *N. antarctica* dominate until the tree line limit at 1300m.

4.2.2.4. Parque Nacional Torres del Paine (Torres) (Figure 4.3)

The vegetation in this large park can be split into four zones related to their specific climates. To the west where it is predominately wet and cold there is a mix of *Nothofagus* species such as *N. betuloides*, *N. antarctica* and *N. pumilio*. Also present in the undergrowth are *Berberis buxifolia* and *Berberis ilicifolia*, both species that grow in wet areas. From the Andes to the steppe region, *N. pumilio* is the dominant species and at lower altitudes *N. betuloides* and *D. winteri* are also found. As altitude increases and the temperature declines, *N. antarctica* becomes the dominant species. At the start of the steppe zone there is a change into a semi-arid cold climate where the dominant plants are *Festuca* sp. and *B. buxifolia*, and *Chiliotrichium diffusum* appears. There are also areas of tundra in which the predominant species is *Sphagnum magellanicum*, which requires high precipitation and low temperatures (Kleinebecker *et al.*, 2007).

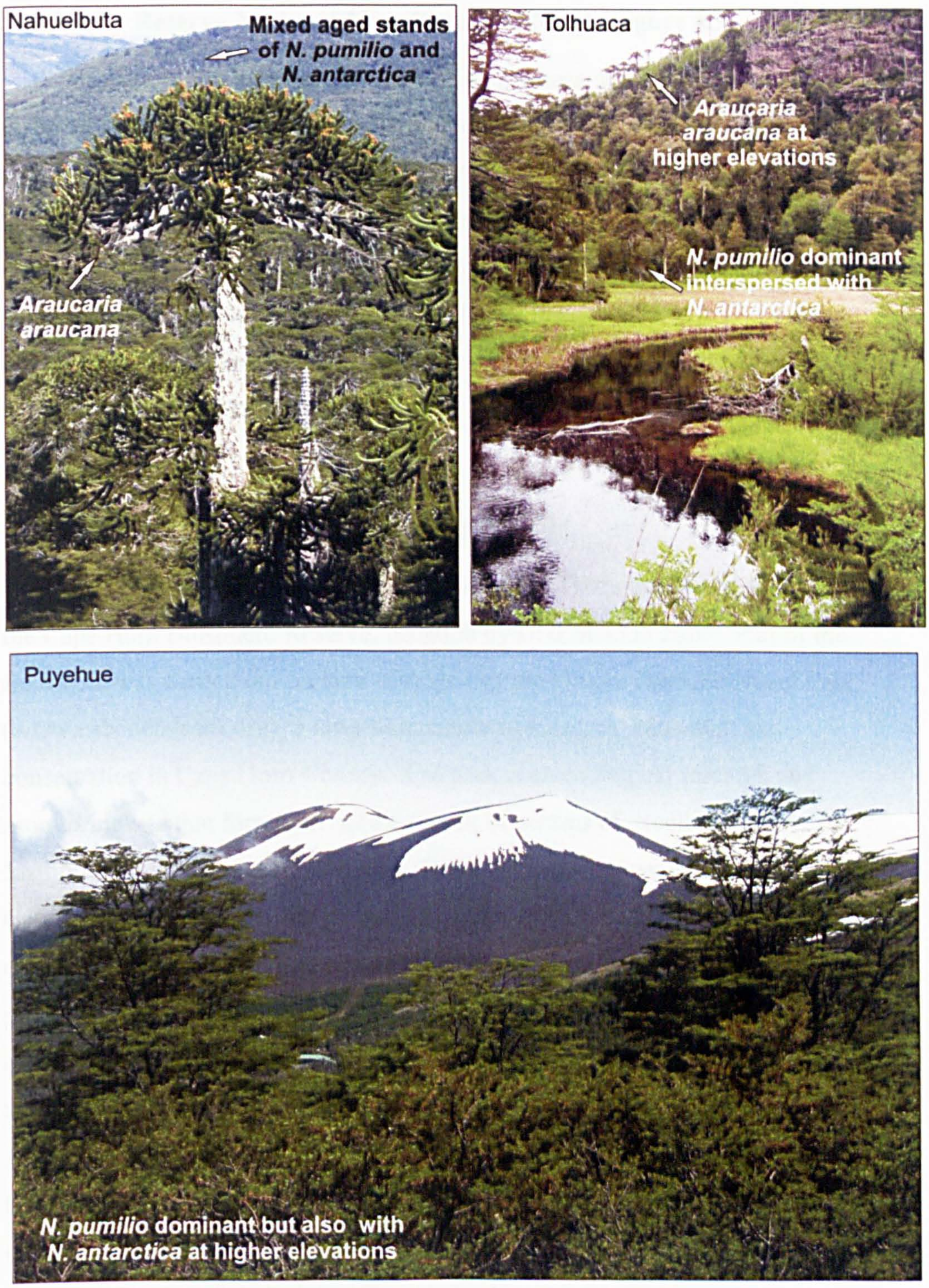


Figure 4.2. Photographs and brief descriptions of the three most northerly sampling sites in Chile: Nahuelbuta, Tolhuaca and Puyehue.

4.2.2.5. Reserva Nacional Magallanes (RNMag) (Figure 4.3)

This site is five kilometres west of Punta Arenas and contains three areas with different vegetation types. Up to 350m above sea level the forest is dominated by *N. pumilio* mixed with *N. antarctica*, with pure stands of *N. antarctica* at higher latitudes. Part of the park near Rio Grande (53°05'S, 71°14'W) in the west is dominated by *N. pumilio* dispersed with *Embothrium coccinem* in shallow permeable soils, but dominated by *N. betuloides* interspersed with *D. winteri* in deeper more impermeable soils. There are also areas of bog in which *Sphagnum magellanicum* dominates, accompanied by *Tetrocium magellanicum.*

4.2.2.6. Isla Navarino (Isla Nav) (Figure 4.3)

Isla Navarino (Isla Nav) is an island south of Tierra del Fuego and is part of the Cape Horn Biosphere Reserve, declared by UNESCO in 2005. Part of the fieldwork was carried out on land managed by the Omora Ethnobotanical Park (www.cabodehornos.org), a long-term centre of research, education and conservation in Cape Horn County. The park is an ecological research and monitoring site that forms part of a network of centres of excellence under the Institute of Ecology and Biodiversity (IEB), formerly known as the Millennium Centre for Advanced Studies in Ecology and Research on Biodiversity (www.ieb-chile.cl). The park is mainly covered by a mixed deciduous and evergreen forest of predominantly *N. pumilio*, *N. antarctica* and *N. betuloides* with interspersed patches of open grassland and sphagnum bog. The fieldwork was carried out further inland towards Los Dientes mountain chain and high altitude lakes, where sparse stands of *N. pumilio* and *N. antarctica* were found on the edges of expanse areas of sphagnum bog. Many of the forests on Isla Navarino have been heavily disturbed due to the introduction of the North American beaver, *Castor canadensis*, and many areas of dead standing forests exist adjacent to sampling areas.

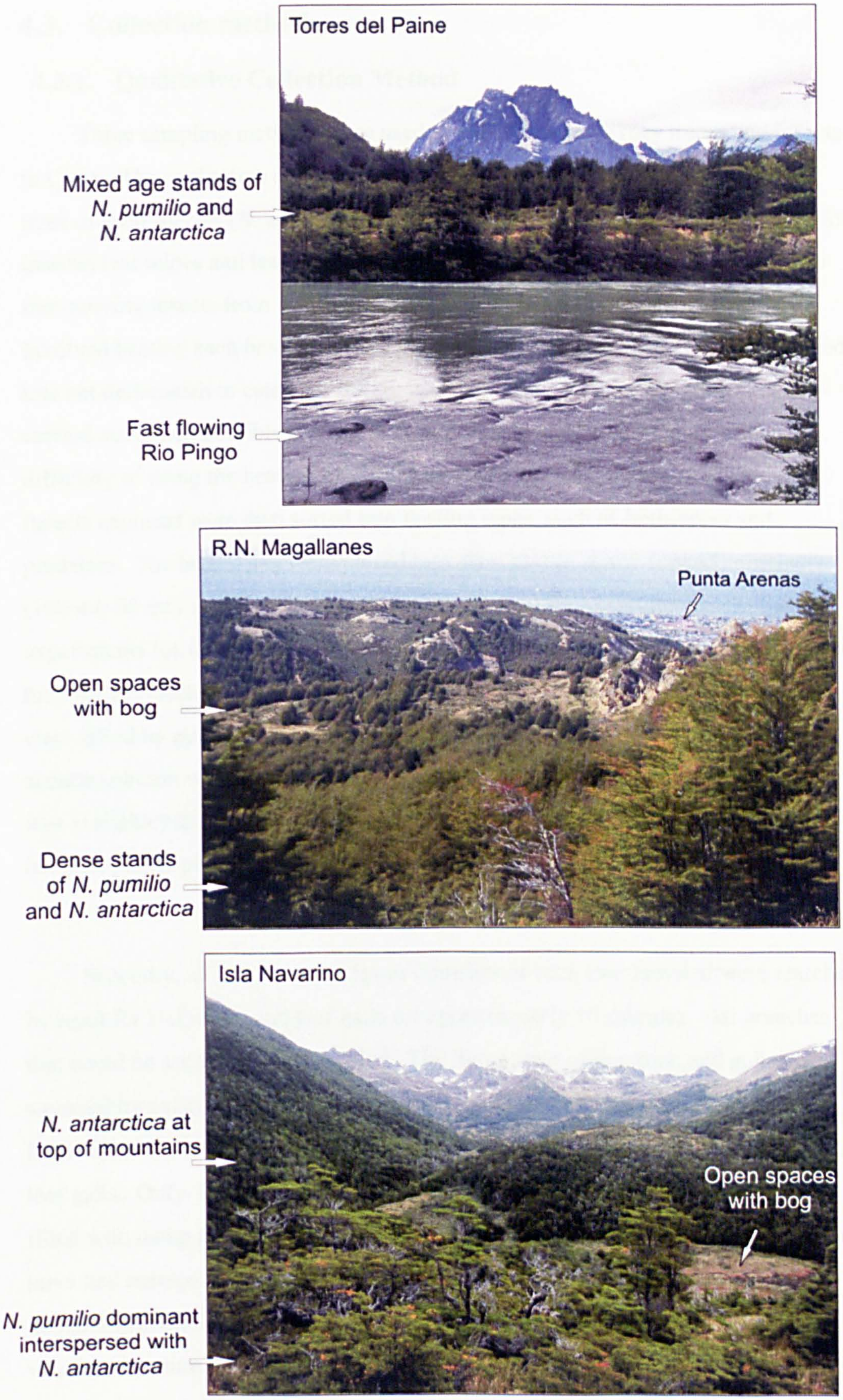


Figure 4.3. Photographs and brief descriptions of the three sampling sites in southern Chile.

4.3. Collection methods

4.3.1. Qualitative Collection Method

Three sampling methods were used to collect the herbivory traces and insects in Chile. The qualitative collection method involved sampling twelve individual trees of each species (*N. antarctica* and *N. pumilio*), that were randomly chosen, for insects, leaf mines and leaf galls. Firstly, a beating technique was used to sample free-moving insects from four lower branches of each tree (Ozanne, 2005). This involved beating each branch with a stick three times, while holding a wide-necked kite net underneath to catch the falling insects (Figure 4.4A). Sampling was only carried out (when possible) on dry days or during dry periods because of the difficulty of using the beating technique and the inactivity of insects in the rain. Insects captured were then sorted into feeding types, such as herbivores and predators. The herbivores were placed into clear plastic screw-topped containers (100 ml; 60 mm x 70 mm and 5ml; 15mm x 57 mm) for observational feeding experiments for which the insects were given fresh leaves from the same tree species they were collected from, and observed until they died. Non-herbivorous insects were killed by putting them in a glass jar in which a tissue with a drop of ethyl acetate solution was placed. Ideally, the dead insects were stored in a freezer (if one was available) and in a cool box during transit. Larvae were stored in 70% ethanol in small plastic pots for preservation.

Secondly, all leaves on the lower branches of each tree sampled were searched by hand for leaf mines and leaf galls for approximately 10 minutes. All branches that could be accessed were sampled. The living state of the mine and gall was assessed by examining for the presence of a larva in the mine when held up to the light and whether there were signs of damage or exit holes in both the leaf mines and leaf galls. Only 'live' mines and galls were placed into standard 9cm Petri dishes filled with damp filter paper. They were then checked periodically until the adult or larva had emerged or a cocoon was formed. The time until emergence varied depending upon the species, the stage at which the mine or gall was collected, and whether the mine or gall had been attacked by a parasitoid. The time until emergence ranged from 20 days to 50 days, after which the mine or gall was dissected if no emergence had occurred. A random selection of leaves with each type of leaf gall were preserved in small plastic pots containing 70% alcohol solution for further investigation at a later stage.

At Parque Nacional Puyehue, an evergreen *Nothofagus* species, *N. betuloides* was also sampled. It was important to sample from both evergreen and deciduous species for comparison with the Antarctic insect trace fossils because it is not known if the fossil leaves were from evergreen or deciduous trees. During the last visit (March 2007), 12 *N. betuloides* trees were searched by hand for insect traces, which were then described and categorised.

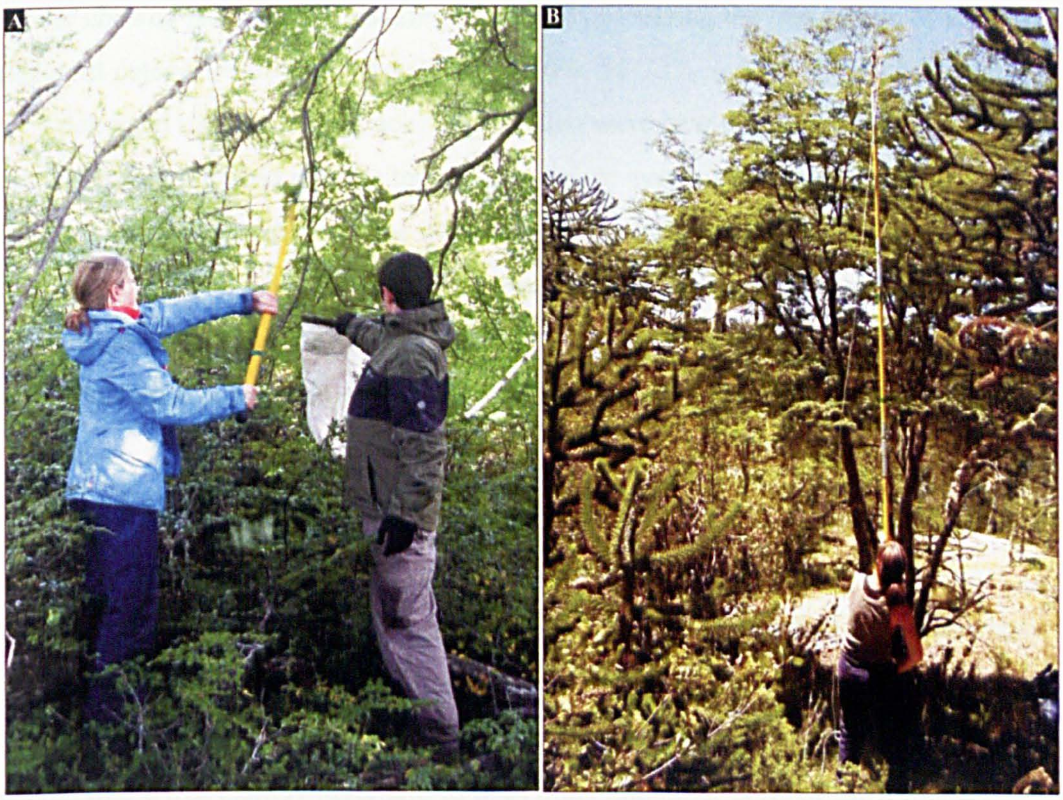


Figure 4.4. A) Qualitative collection of insects from the lower branches of each tree using the beating technique. B) Quantitative collection of leaves from within the tree canopy using extendable pruning poles.

4.3.2. Quantitative Collection Method

After the qualitative collection method had been applied, six trees of each species were sampled further to assess both the presence and intensity of leaf damage. The factors within individual trees that may have affected the presence and intensity of leaf damage were also investigated, such as height and orientation of the tree, and latitudinal differences. This involved collecting three small branches of approximately 30 leaves from different heights on both north and south aspects of the tree. The three samples were collected by measuring 1m intervals through the tree canopy, starting at the lower branches. The leaves were sampled using extendable pruning poles, extended to a maximum height of 5m (Figure 4.4B).

Each branch collected was then processed by counting the age ratio of the leaves (number of young and old leaves was assessed using the method described in Chapter 6), the number of chewed leaves, the number of leaf mines and the number of leaf galls, as well as noting any further traces of interest. The intensity of damage on the chewed leaves was measured by visually assessing the leaf as one of several different categories of damage: <25%, 26-50%, 51-75% and 76-100% of the leaf area damaged due to insect attack. Leaves that were near the limits of two categories were placed into the higher category to avoid underestimating the intensity of damage. As with the qualitative collection method, the insects from all leaf mines and leaf galls collected were reared in Petri dishes lined with slightly damp filter paper until the adults emerged. They were killed and stored until identification was possible using the same method as described in section 4.3.1.

4.3.3. Canopy Collection

To examine the validity of collecting from the ground and to investigate whether there were differences in diversity of insect traces between the canopy and lower levels within a tree, a double rope climbing technique was used to gain access to higher branches within individual trees (Appendix III). This technique enabled access to the tree canopy layer and to both inner and outer parts of the branches (Figure 3.5). All trees surveyed this way were in Parque Nacional Puyehue. Only *N. pumilio* trees were chosen because *N. antarctica* trees were too small to climb. All trees were sampled on dry days only. Trees to be climbed were chosen at random where possible, but safe access had to be considered as a priority. All trees were approximately 30m high but, due to practicalities, the maximum height of sampling was 20m. Quantitative collection of three small branches of approximately 30 leaves was performed within the top, middle and bottom sections of each tree at both north and south orientations. Leaves on the small branches collected were processed in the same way as described in section 3.3.1.

Figure 4.5. A) Collection of leaves in the lower canopy of a *N. pumilio* tree in Puyehue. B) Demonstration of the tree climbing technique used to sample at different height levels in the canopy.

4.3.4. Species Accumulation Curves

The number of leaves collected was constrained by many factors, such as time and access and practicalities, such as space and storage. It was important, however, to know that an adequate number of samples had been collected for analysis and that the level and diversity of herbivory was not misinterpreted due to inadequate data collection. Therefore, for each species, a species accumulation curve, also known as a sample effort curve, was compiled to determine if the maximum number of herbivore types had been collected. The cumulative number of samples (in this case only each tree studied counts as one sample) was plotted on the x-axis against the diversity of herbivore types on the y-axis. Classification of the diversity of all herbivore types was based on the same criteria as the fossil trace categories described in Section 2.3.2. Species accumulation curves were produced using the software package called Species Diversity & Richness IV, Pisces Conservation Ltd, 2007.

The species accumulation curves were produced using the raw data (Appendix IV) from both qualitative and quantitative methods, for all sites and for each species. The average number of herbivory types for each sample was plotted and curves were smoothed by randomising 1000 times the order in which the samples were included. For *N. pumilio* (n = 96), the curve initially has a steep gradient, but gradually levels off as the total sample number is reached (Figure 4.6). For *N. antarctica* (n = 90), the curve also starts with a steep gradient but gets shallower and then gradually levels off, although with a steeper gradient than that for *N. pumilio* (Figure 4.7). The flattening of the curve at the 90 sample point indicates that an appropriate number of samples were collected, providing confidence that the majority of herbivore types had been sampled. Further samples would nonetheless have generated further herbivore types. This largely validates the sampling method in that any further increase in sample effort would not have yielded any major increase in the number of herbivore types.

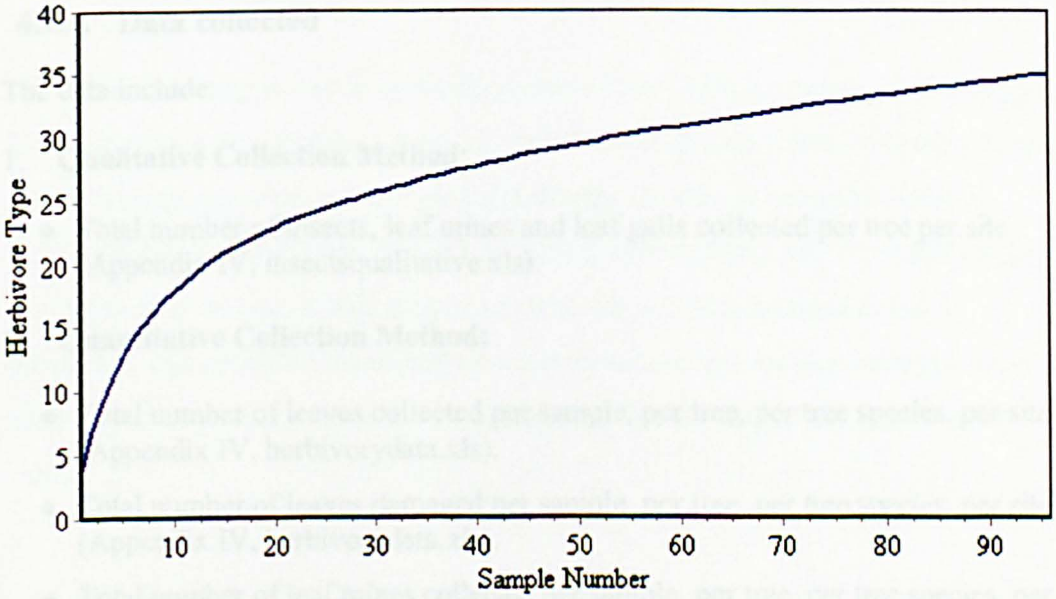


Figure 4.6. Sample effort curves for the diversity of herbivore types, including bite marks, leaf mines and leaf galls, on *N. pumilio* for all sites. The average number of herbivore types for each sample from a randomized sampling order generated 1000 times is plotted against sample number.

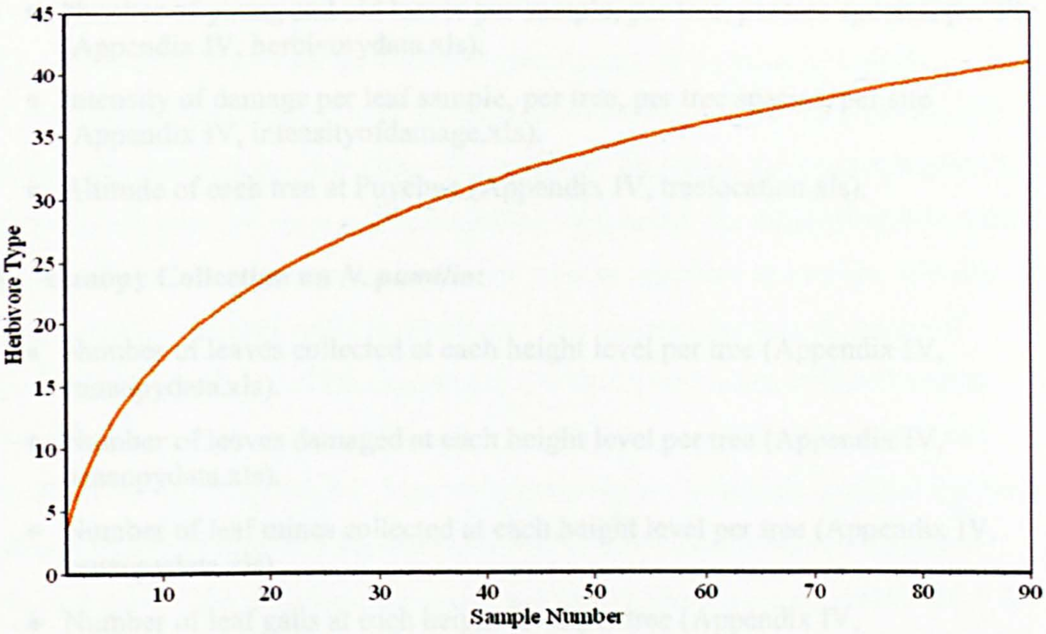


Figure 4.7. Sample effort curves for the diversity of herbivore types, including bite marks, leaf mines and leaf galls, on *N. antarctica* for all sites. The average number of herbivore types for each sample from a randomized sampling order generated 1000 times is plotted against sample number.

4.4. Results

4.4.1. Data collected

The data include:

1. **Qualitative Collection Method**:
    - Total number of insects, leaf mines and leaf galls collected per tree per site (Appendix IV, insectsqualitative.xls).
2. **Quantitative Collection Method:**
    - Total number of leaves collected per sample, per tree, per tree species, per site (Appendix IV, herbivorydata.xls).
    - Total number of leaves damaged per sample, per tree, per tree species, per site (Appendix IV, herbivorydata.xls).
    - Total number of leaf mines collected per sample, per tree, per tree species, per site (Appendix IV, herbivorydata.xls).
    - Total number of leaf galls collected per sample, per tree, per tree species, per site (Appendix IV, herbivorydata.xls).
    - Total number of leaves with skeleton feeding per sample, per tree, per tree species, per site (Appendix IV, skeletonfeeding.xls).

- Number of young and old leaves per sample, per tree, per tree species, per site (Appendix IV, herbivorydata.xls).
- Intensity of damage per leaf sample, per tree, per tree species, per site (Appendix IV, intensityofdamage.xls).
- Altitude of each tree at Puyehue (Appendix IV, treelocation.xls).

3. **Canopy Collection on *N. pumilio*:**

- Number of leaves collected at each height level per tree (Appendix IV, canaopydata.xls).
- Number of leaves damaged at each height level per tree (Appendix IV, canaopydata.xls).
- Number of leaf mines collected at each height level per tree (Appendix IV, canaopydata.xls).
- Number of leaf galls at each height level per tree (Appendix IV, canaopydata.xls).

4. **Insect Data**:

- Description of feeding damage created for identified species (Appendix V, appendix5insectfeeding.xls).
- Identification of parasitoid species reared from leaf mines and galls (Appendix V, parasitoids.xls).

The herbivory traces on *Nothofagus* trees from Chile are described in Chapter 4 and causal insects identified as far as possible. In Chapter 5 data on occurrence of insect damage on leaves are analysed statistically in order to determine which factors (for example altitude, latitude, tree microclimate) influenced insect damage. The following sections in this chapter present the raw data obtained in the qualitative and quantitative methods in order to ensure that the data have not been biased by the collection techniques and thus later interpretations are justified and valid.

4.4.2. Results of the Qualitative Study

In total, 47 individual trees of *N. pumilio* and 42 individual trees of *N. antarctica* were sampled across the six sites using this method. The data briefly summarized in this section will be analysed in Chapter 4 to highlight the presence of insect species and traces of specific interest. The total number of insects collected using the beating technique for both tree species was 1676, but only a small proportion of these insects (13.37% of insects collected) were represented by their feeding traces during the collecting observations. Therefore, the feeding marks of many insects were not recorded because they were either non-phytophagous on the tree species; were of another feeding guild, such as predators or tourists; or death occurred before feeding could be observed. The variation in the percentage of insects that were observed feeding at each site (and which were collected and on which further analysis was carried out) was relatively small, between 0.66% and 2.58% of the insects collected. Figure 4.8 and Figure 4.9 indicate that both the mean number of insects collected and the mean number of insects observed feeding at each site show little variation (General linear model, binomial error family d.f. = 87, $P = 0.07$). This relationship was observed on both species of *Nothofagus* (Figures 4.8. and 4.9). This indicates that comparing insect trace diversity between sites using qualitative data is valid as the number of insect species found only at certain sites is not biased by the variation in the number of insects collected.

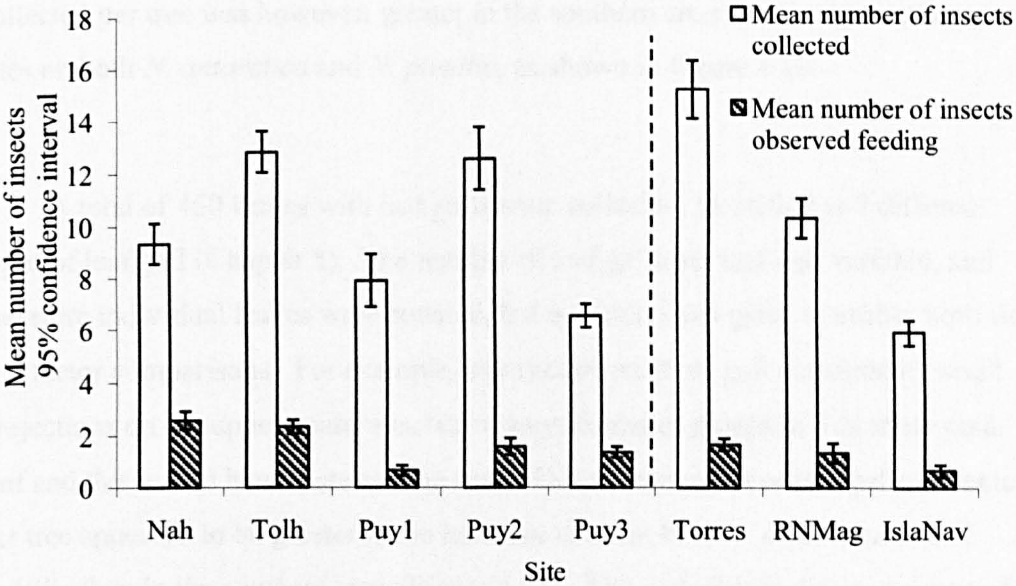


Figure 4.8. The mean number of insects collected by beating and the number of insects observed feeding on *N. antarctica* at each site. The parks are in latitudinal order from North to South and the dashed line represents the major divide between the more northerly sites to the left and the more southerly sites to the right of the line. Bars show the 95% confidence interval.

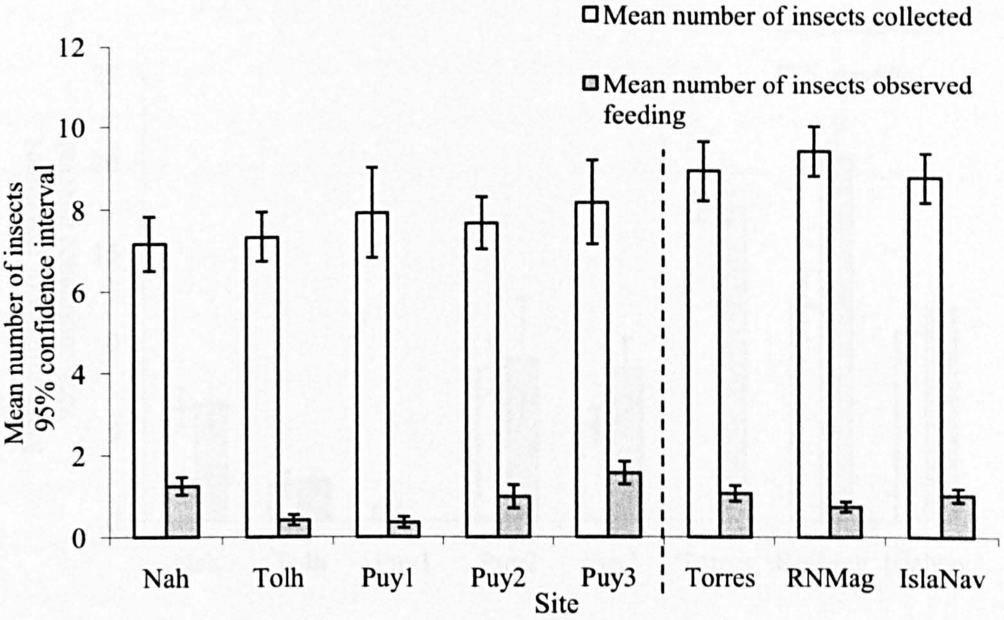


Figure 4.9. The mean number of insects collected and the number of insects observed feeding on *N. pumilio* at each site.

A total of 827 individual mines were collected, assigned to 10 different types of leaf mine (Chapter 5). The types of mines ranged from small serpentine mines to blotch mines that covered the entire leaf area. The mean number of leaf mines collected per tree was however, greater in the southern sites than in the northern sites on both *N. antarctica* and *N. pumilio*, as shown in Figure 4.10.

A total of 460 leaves with leaf galls were collected, identified as 7 different types of leaf gall (Chapter 5). The number of leaf galls per leaf was variable, and therefore individual leaves were counted, but not individual galls, to enable both site and factor comparisons. For example, one type of multiple gall consisted of small projections on the upper epidermis, was always found in groups of 4 or more on a leaf and this would have distorted the data. The mean number of leaf galls collected per tree appeared to be greater in the northern sites for both *N. antarctica* and *N. pumilio* than in the southern sites (Figure 4.11). The variation in the occurrence of leaf mines and leaf galls will be explored fully in Chapter 5.

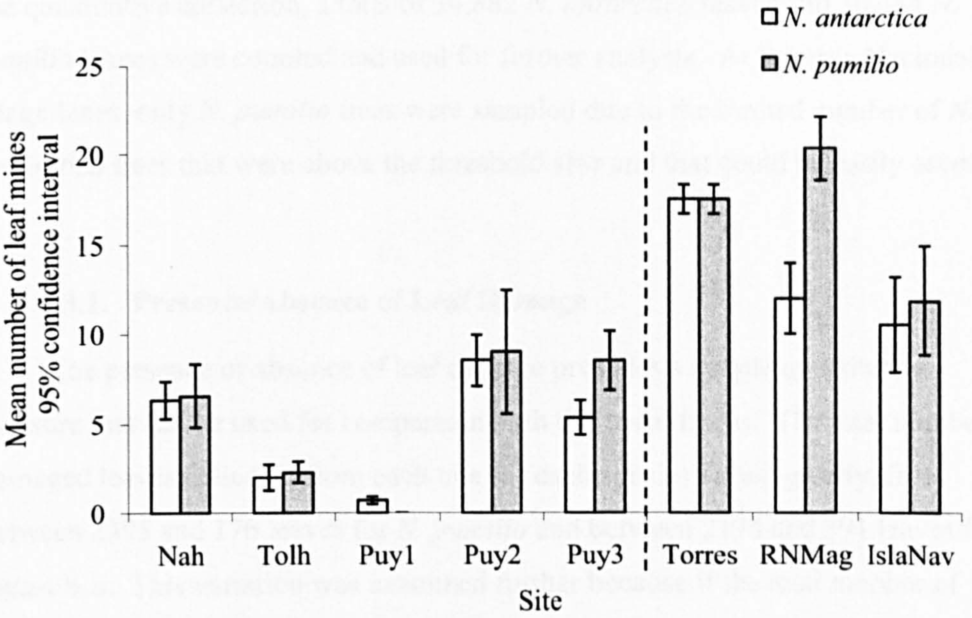


Figure 4.10. The mean number of leaf mines collected per species, per tree, per site on *N. antarctica* (white) and *N. pumilio* (grey), using the qualitative technique.

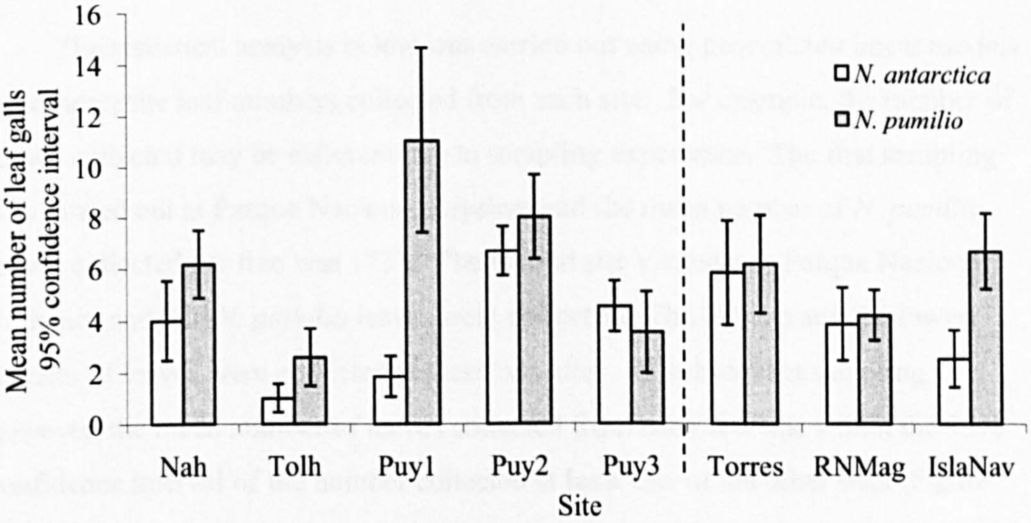


Figure 4.11. The mean number of leaf galls collected per species, per tree, per site on *N. antarctica* (white) and *N. pumilio* (grey), using the qualitative technique.

4.4.3. Results of the Quantitative Study

The data presented in this section will be used in the statistical analyses performed in Chapter 5 to investigate the influence on leaf herbivory of factors such as latitude, altitude, season, plus height and orientation within the tree. Such factors will be examined in order to understand modern systems, as well as to compare the modern insect traces with fossil insect traces and to aid in their interpretation. From the quantitative collection, a total of 34,882 *N. antarctica* leaves and 36,484 *N. pumilio* leaves were counted and used for further analysis. At Reserva Nacional Magallanes, only *N. pumilio* trees were sampled due to the limited number of *N. antarctica* trees that were above the threshold size and that could be easily accessed.

4.4.3.1. Presence/Absence of Leaf Damage

The presence or absence of leaf damage provides a simple quantitative measure that can be used for comparison with the fossil traces. The total number of damaged leaves collected from each tree for each species varied greatly, from between 2395 and 176 leaves for *N. pumilio* and between 2194 and 391 leaves for *N. antarctica*. This variation was examined further because if the total number of leaves was statistically different between each site then comparing the number of damaged leaves at each site is not valid.

The statistical analysis below was carried out using generalized linear models to compare the leaf numbers collected from each site. For example, the number of leaves collected may be different due to sampling experience. The first sampling was carried out at Parque Nacional Puyehue and the mean number of *N. pumilio* leaves collected per tree was 1733. The second site visited was Parque Nacional Tolhuaca and 726 *N. pumilio* leaves were collected. The highest and the lowest number of leaves were collected at these two sites. In subsequent sampling, however, the mean number of leaves collected from each site was within the 95% confidence interval of the number collected at least one of the other sites (Figure 4.12). Therefore, the total number of leaves collected became more consistent with more sampling experience. Statistically, there was only one significant difference in the number of leaves collected between the first visit to P.N. Puyehue and each of the other sites (Table 4.2).

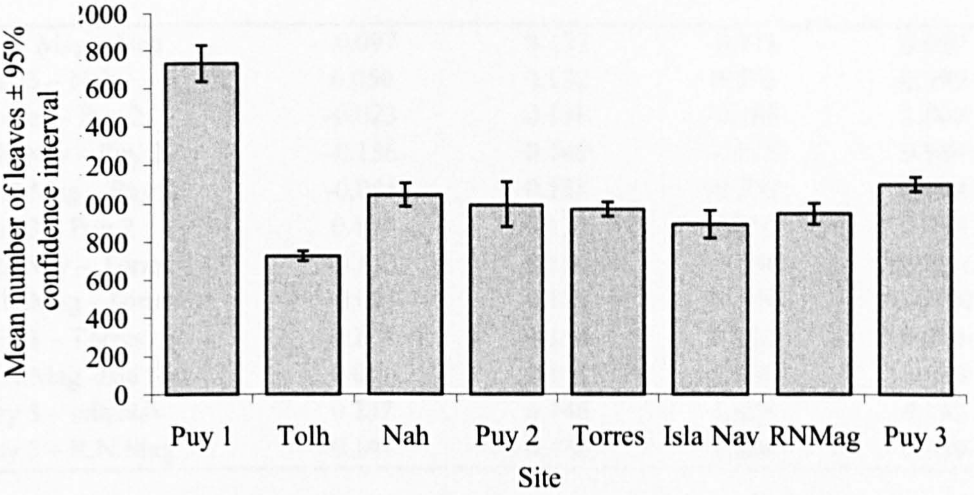


Figure 4.12. Mean number of all leaves collected at each site from *N. pumilio*. The sites are presented in the order they were visited from December 2006 to March 2007.

Table 4.2. A generalized linear model (GLMs) was used to assess the differences in the number of leaves collected per site from *N. pumilio*. The minimal adequate model was fitted using the quasi family for poisson errors. The site comparisons were performed using a simultaneous tests for general linear hypotheses by multiple comparisons of the means using Tukey Contrasts, *z*-values and adjusted *p*-values are given below. Significant differences at the 95% confidence level are marked with *. Continued on p94.

Response Variable	Explanatory Variable	Test	F	*P*
Number of leaves	Site	F-test, d.f = 7	3.88	<0.0001*

Comparisons	Estimate	SE	z	*P*
Puy 1 - Tolh	-0.870	0.135	-6.463	< 0.001*
Puy 1 - Nah	-0.507	0.119	-4.250	< 0.001*
Puy 1 - Puy 2	-0.559	0.121	-4.605	< 0.001*
Puy 1 - Torres	-0.582	0.122	-4.760	< 0.001*
Puy 1 - Isla Nav	-0.695	0.135	-5.148	< 0.001*
Puy 1 - R.N. Mag	-0.604	0.123	-4.911	< 0.001*
Puy 1 - Puy 3	-0.458	0.118	-3.894	0.0027*
Nah - Tolh	0.363	0.147	2.467	0.207
Puy 2 - Tolh	0.312	0.149	2.096	0.414
Torres - Tolh	0.289	0.150	1.931	0.526
Isla Nav - Tolh	0.175	0.160	1.095	0.957
R.N. Mag - Tolh	0.266	0.150	1.770	0.637
Puy 3 – Tolh	0.413	0.146	2.832	0.086
Puy 2 – Nah	-0.05130	0.135	-0.380	0.999
Torres – Nah	-0.074	0.136	-0.548	0.999
Isla Nav – Nah	-0.188	0.148	-1.273	0.908

Comparisons	Estimate	SE	z	*P*
R.N. Mag – Nah	-0.097	0.137	-0.711	0.997
Puy 3 – Nah	0.050	0.132	0.376	0.999
Torres – Puy 2	-0.023	0.138	-0.168	1.000
Isla Nav – Puy 2	-0.136	0.149	-0.915	0.984
R.N.Mag – Puy 2	-0.046	0.138	-0.332	0.999
Puy 3 – Puy 2	0.101	0.133	0.756	0.995
Isla Nav – Torres	-0.113	0.150	-0.756	0.9951
R.N.Mag - Torres	-0.023	0.139	-0.164	1.0000
Puy 3 – Torres	0.124	0.134	0.923	0.984
R.N.Mag -Isla Nav	0.091	0.151	0.601	0.999
Puy 3 – Isla Nav	0.237	0.146	1.625	0.732
Puy 3 – R.N.Mag	0.147	0.135	1.086	0.959

In contrast, the number of leaves sampled per site from *N. antarctica* did not show such a simple relationship (Figure 4.13). The variation in the number of leaves collected could not be explained by the different number of height levels sampled, leading to a different mean number of leaves collected. There was no significant correlation between tree height and the number of leaves collected (Pearson's Correlation, d.f. = 41, r = 0.183, $p = 0.247$), despite there being a significant difference in the mean tree height sampled between sites (Table 4.3). The difference in leaf number collected per site was found to be significantly different (Table 4.4). Therefore, due to this difference in the number of leaves collected, the proportion of leaves damaged by insects per sample for each tree species (Figure 4.14) had to be used in further statistical analyses to enable a valid comparison (Chapter 6).

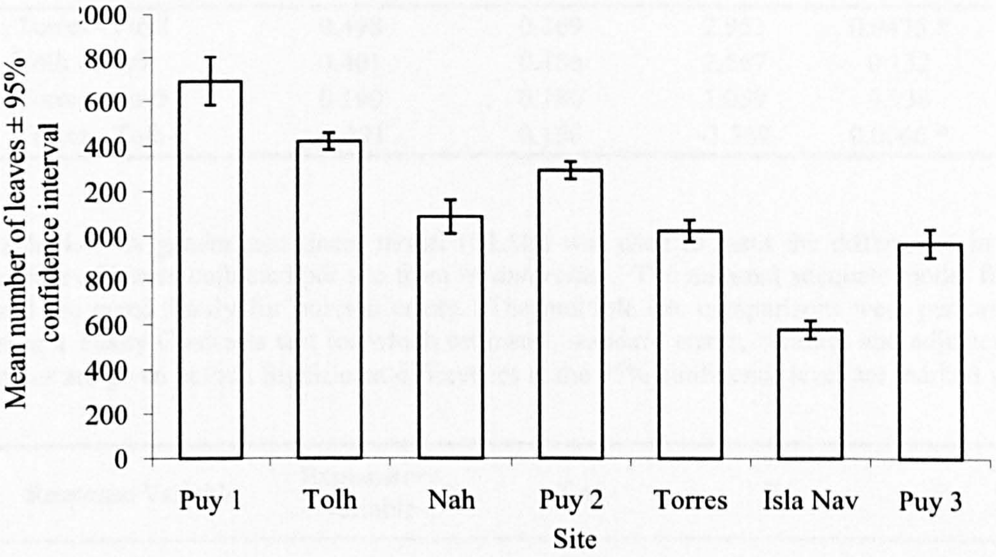


Figure 4.13. Mean number of leaves collected at each site from *N. antarctica*. The sites are presented in the order they were visited from December 2006 to March 2007.

Table 4.3. A generalized linear model (GLMs) was used to assess the differences in the height of each tree collected per site from *N. antarctica*. The minimal adequate model fitted used the quasi family for poisson errors. The site comparisons were performed using simultaneous tests for general linear hypotheses by multiple comparisons of the means using Tukey Contrasts, *z*-values and adjusted *p*-values are given below. Significant differences at the 95% confidence level are marked with *. Continued on p96.

Response variable	Explanatory variable	Test	F	*P*
Height	Site	F-test, d.f =35	8.854	< 0.001*
Comparisons	**Estimate**	**SE**	**z**	***P***
Nah - Isla Nav	0.817	0.204	4.005	0.0012*
Puy1 - Isla Nav	0.039	0.238	0.163	1.000
Puy2 - Isla Nav	0.987	0.199	4.963	< 0.001*
Puy3 - Isla Nav	0.680	0.208	3.262	0.0184 *
Tolh - Isla Nav	1.081	0.197	5.500	< 0.001*
Torres - Isla Nav	0.490	0.216	2.270	0.252
Puy1 - Nah	-0.778	0.201	-3.867	0.002
Puy2 - Nah	0.171	0.153	1.113	0.922
Puy3 - Nah	0.137	0.137	0.827	0.982
Tolh - Nah	0.264	0.150	1.760	0.568
Torres - Nah	0.327	0.174	1.876	0.488
Puy2 - Puy1	0.949	0.196	4.836	< 0.001 *
Puy3 - Puy1	0.641	0.206	3.115	0.0290 *
Tolh - Puy1	1.042	0.194	5.380	< 0.001 *
Torres - Puy1	0.451	0.213	2.115	0.336
Puy3 - Puy2	0.307	0.159	1.931	0.452
Tolh - Puy2	0.094	0.143	0.653	0.995

Comparisons	Estimate	SE	z	*P*
Torres - Puy2	0.498	0.169	2.953	0.0475 *
Tolh - Puy3	0.401	0.156	2.567	0.132
Torres - Puy3	0.190	0.180	1.059	0.938
Torres - Tolh	-0.591	0.166	-3.569	0.0066 *

Table 4.4. A generalized linear model (GLMs) was used to asses the differences in the number of leaves collected per site from *N. antarctica*. The minimal adequate model fitted used the quasi family for poisson errors. The multiple site comparisons were performed using a Tukey Contrasts test for which estimates, standard errors, *z*-values and adjusted *p*-values are given below. Significant differences at the 95% confidence level are marked with *.

Response Variable	Explanatory variable	Test	F	*P*
Number of Leaves	Site	F-test, d.f.=35	15.59	<0.001
Comparisons	Estimate	SE	z	*P*
Nah – Isla Nav	0.632	0.141	4.483	< 0.001*
Puy 1 – Isla Nav	1.077	0.132	8.164	< 0.001*
Puy 2 - Isla Nav	0.807	0.137	5.886	< 0.001*
Puy 3 – Isla Nav	0.515	0.144	3.579	0.0063*
Tolh – Isla Nav	0.902	0.135	6.677	< 0.001*
Torres – Isla Nav	0.573	0.142	4.023	0.00102*
Puy 1 – Nah	0.445	0.106	4.181	< 0.001*
Puy 2 – Nah	0.175	0.113	1.548	0.711
Puy 3 – Nah	-0.117	0.121	-0.965	0.961
Tolh – Nah	0.270	0.110	2.446	0.176
Torres- Nah	-0.059	0.119	-0.494	0.999
Puy2- Puy1	-0.270	0.101	-2.675	0.103
Puy3-Puy1	-0.562	0.110	-5.090	< 0.001*
Tolh – Puy1	-0.175	0.098	-1.778	0.558
Torres – Puy 1	-0.504	0.108	-4.649	< 0.001*
Puy 3 – Puy 2	-0.291	0.116	-2.502	0.155
Tolh – Puy 2	0.095	0.105	0.907	0.971
Torres – Puy 2	-0.233	0.115	-2.038	0.386
Tolh-Puy 3	-0.233	0.115	-2.038	0.0121*
Torres- Puy 3	0.058	0.123	0.472	0.999
Torres – Tolh	-0.329	0.112	-2.930	0.051

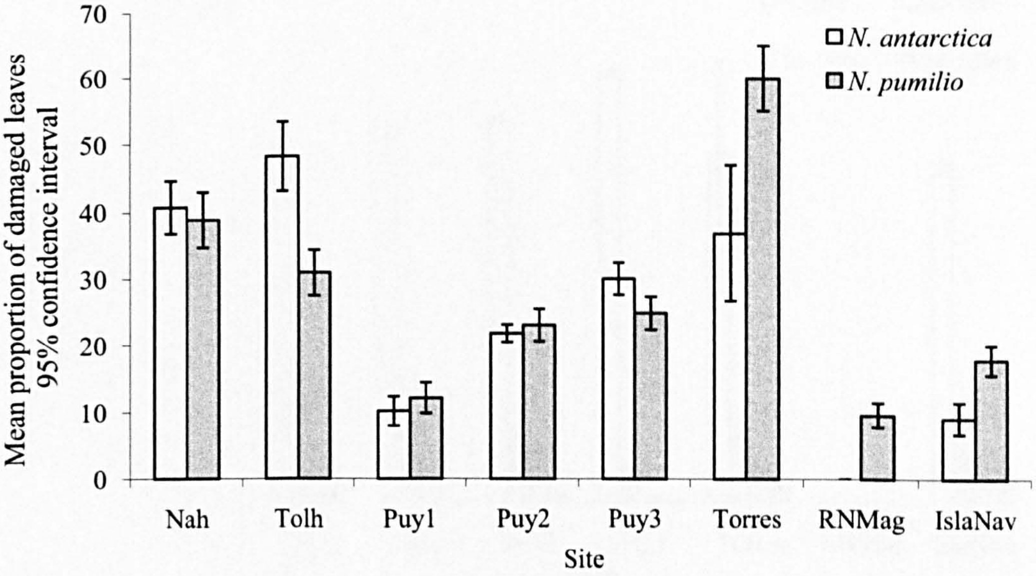


Figure 4.14. The mean proportion of leaves with insect damage collected from each site from both *N. antarctica* (white) and *N. pumilio* (grey). Bars show the 95% confidence interval.

4.4.3.2. Intensity of Leaf Damage

The proportion of damaged leaves collected within the different categories of herbivory traces can be compared across sites to examine the effects of latitude on intensity of insect damage on leaves. The frequency of damage did not vary greatly with site, as both more northerly and southerly areas show the same general trend (Figure 4.15). There was an inverse relationship between the amount of damage per leaf (the damage level category (Section 4.3.2)) and the number of leaves found in a specific category. The majority of leaves had only a small percentage of the leaf area damaged by insects and only a small proportion had an extensive amount of area damaged. This difference will be examined statistically in Chapter 6 and compared with the fossil data in Chapter 7.

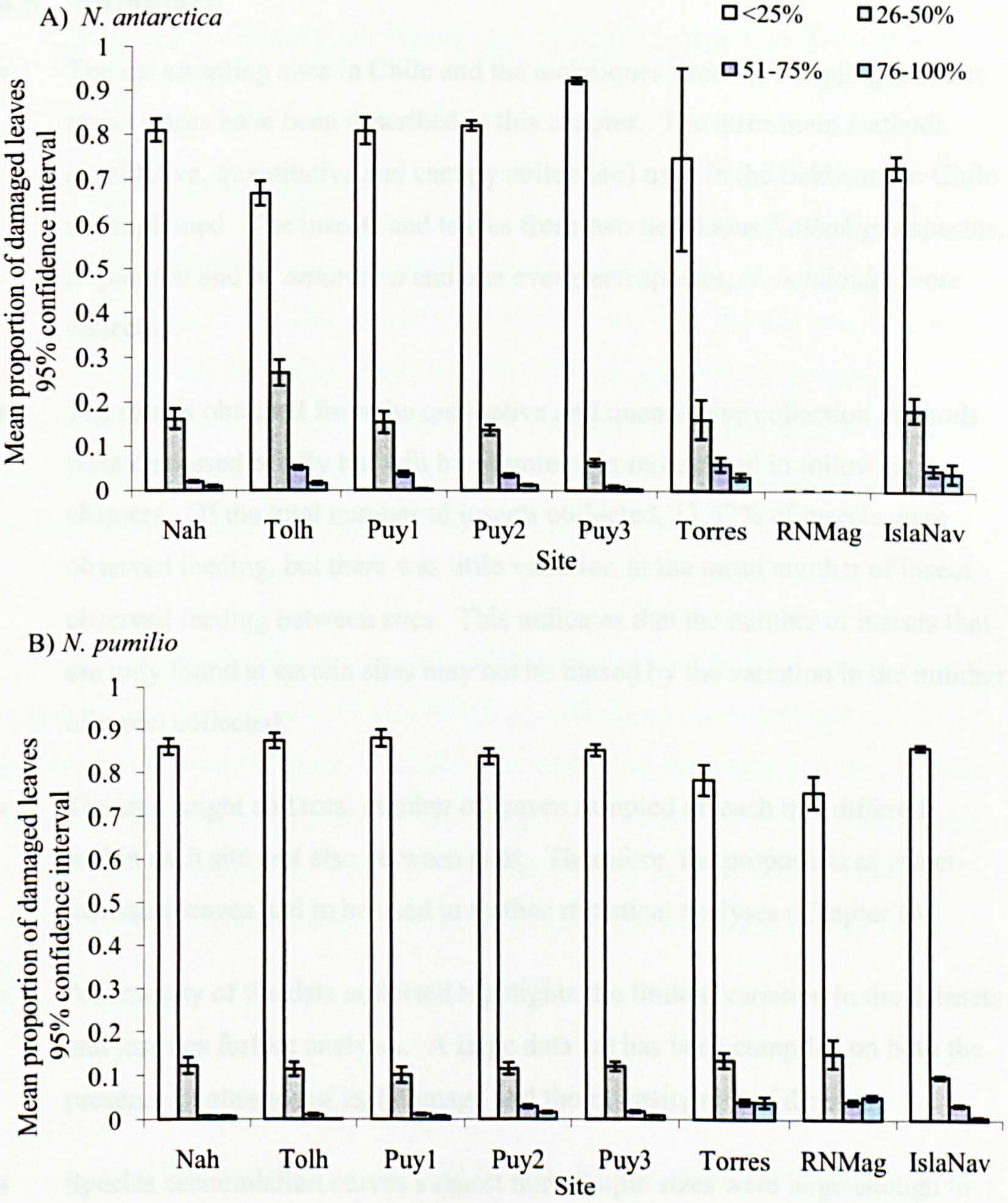


Figure 4.15. The proportion of leaves found within each damage category, <25% (white), 62-50% (grey), 51-75% (purple) and 76-100% (blue) for each site visited and for A) *N. antarctica* and B) *N. pumilio*.

4.4.3.3. Leaf Mines & Leaf Galls

Using the quantitative collection method, a total of 1141 individual mines were collected, representing 10 different types of leaf mine. In total, 1662 leaves with leaf galls were collected, representing 6 different leaf gall types. The types of leaf mines and leaf galls were the same as those collected using the qualitative collection method. The data are analysed further in Chapters 5 and 7.

4.5. Summary

- The six sampling sites in Chile and the techniques used for sampling modern insect traces have been described in this chapter. The three main methods (qualitative, quantitative and canopy collection) used in the fieldwork in Chile are explained. The insects and leaves from two deciduous *Nothofagus* species, *N. pumilio* and *N. antarctica* and one evergreen species, *N. betuloides* were collected.

- The results obtained from the qualitative and quantitative collection methods were discussed briefly but will be examined in more detail in following chapters. Of the total number of insects collected, 13.37% of insects were observed feeding, but there was little variation in the mean number of insect observed feeding between sites. This indicates that the number of insects that are only found at certain sites may not be biased by the variation in the number of insect collected.

- The tree height and total number of leaves sampled on each tree differed within each site and also between sites. Therefore, the proportion of insect-damaged leaves had to be used in further statistical analyses (Chapter 6).

- A summary of the data collected highlights the limited variation in the datasets and justifies further analyses. A large data set has been compiled on both the presence or absence of leaf damage and the intensity of leaf damage.

- Species accumulation curves suggest that sample sizes were large enough to obtain a high diversity of herbivory types, justifying further analyses of the datasets.

- A number of insects that caused the leaf damage on three *Nothofagus* tree species were collected, as well as a number of leaf mines and leaf galls. These are the focus of Chapter 5.

Chapter 5. Study of Modern Insect Traces in Chile

5.1. Herbivorous Insects

5.1.1. Introduction

The insects on *Nothofagus* trees in Chile that had their feeding traces recorded were collected and identified. The insect traces were allocated to the same categories as those used for the fossil traces in Chapter 2, namely general leaf chewing, skeleton feeding, leaf mines and leaf galls. All leaf mines and leaf galls are described in Sections 5.2 and 5.3 respectively.

For the majority of insect species in Chile there is limited information on their biology, as most previous work has focused on taxonomy, and therefore little is documented about plant-insect associations and known distributions within Chilean or other South American forests. Thus this study presents new information on plant-insect interactions in Chilean forests. The diversity of distinctive trace types is examined further and the range of insects that were observed creating them is discussed.

5.1.2. General Leaf Chewing

General leaf chewing was the most frequently observed trace and included two main types: marginal and non-marginal chewing (described previously in section 3.3.1). The complete range of observed traces is documented in Appendix 5, and for the remainder of this section, only traces created by insects which show specific geographical distributions are considered further. The summary table for the insect species highlights their distributions (Table 5.1).

Of the adult insects whose feeding traces were recorded, a total of 23 different species belonged to the order Coleoptera (Appendix 5). One species, *Hybreoleptops aureosignatus* (Blanchard 1851) was collected only from Nah (location map, section

4.2.1), where it occurred on both *N. pumilio* and *N. antarctica* trees. The genus *Hypreoleptops* (Coleoptera: Curculionoidea: Entiminae) is endemic to southern South America and all six species within the genus have been found only in Chile (Santiago, Maule and Valdivian Provinces), except for one species, *H. tuberculifer*, which was also recorded in Argentina (Pérez & Posadas, 2006). Little is known about the life history or habitat requirements of *H. aureosignatus*, although *H. tuberculifer* has been noted to use *Nothofagus* as a host plant (Morrone & Roig-Junent, 1995). *Hybreoleptops aureosignatus* was observed feeding in three different ways: continuous marginal leaf chewing (the most common trace) (Figure 5.1A), non-marginal chewing that created a small singular hole, and chewing that left a small bite mark on the leaf margin.

Another interesting species is *Polydrusus nothofagi* (Kushcel) (Coleoptera: Curculionidae: Brachyrhiniae), a species that occurred on both tree species and was most commonly found at Torres (Table 5.1B). It was also collected from Nah and Tolh, but only on *N. antarctica*, with just one insect specimen at each site. This species was observed making marginal chewing traces in both a continuous and a discontinuous manner. The discontinuous form of marginal feeding was the most common trace and included several areas removed from the margin of one leaf (Figure 5.1B). An adult *P. nothofagi* is shown in Figures 5.2A and 5.2B.

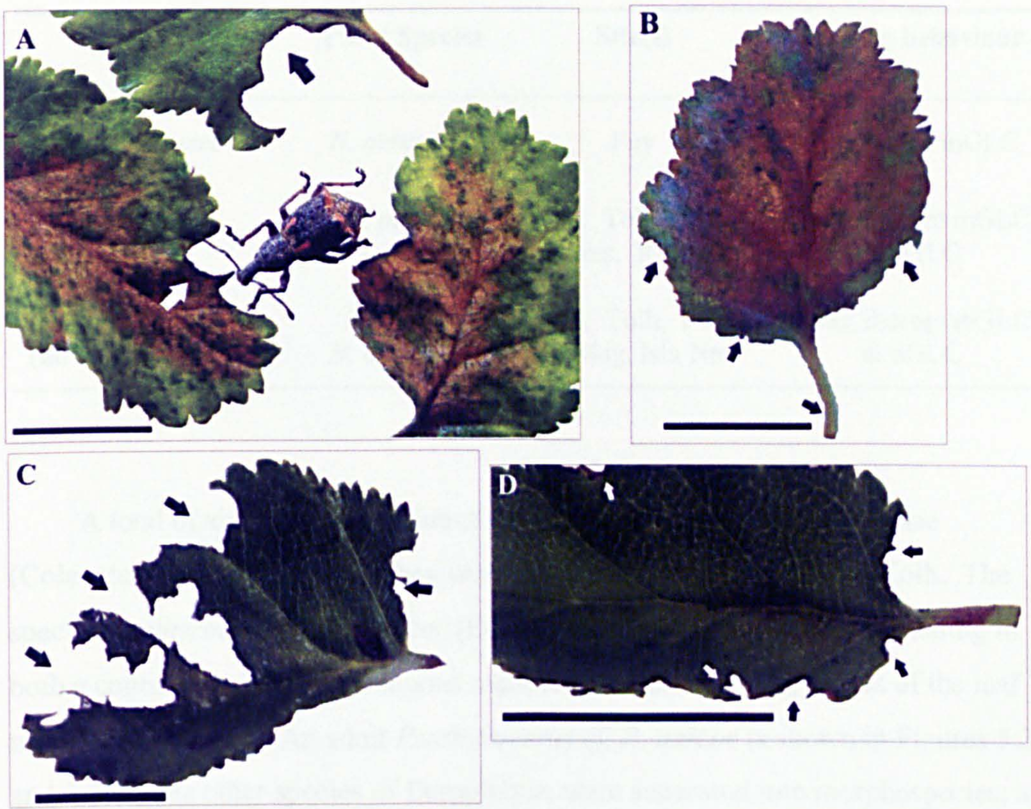


Figure 5.1. Insect traces collected from Chile. A) Continuous marginal chewing by *Hybreoleptops aureosignatus*. B) Discontinuous marginal chewing by *Polydrusus nothofagi*. C) Marginal general chewing by *Psathyrocerus cf. P. unicor*. D) Discontinuous marginal chewing by Eumolpinae species 4. Scale bar 1cm. Arrows highlight insect traces.

Table 5.1. Distributions of the different insect species collected in Chile that were described in section 5.1. The most common feeding behaviour and the sites from which the insects were mostly collected are in bold. Nah = Parque Nacional Nahuelbuta, Tolh = Parque Nacional Tolhuaca, Puy = Parque Nacional Puyehue, Torres = Parque Nacional Torres del Paine, RNMag = Reserva Nacional Magallanes and Isla Nav = Isla Navarino. Cont = continuous, discont = discontinuous, mGLC = marginal general leaf chewing, nGLC = non-marginal general leaf chewing. Continued on p103.

Insect Species	Plant Species	Site(s)	Feeding behaviour
Hybreoleptops aureosignatus	*N. pumilio* *N. antarctica*	Nah	**Cont**, discont mGLC & nGLC
Polydrusus nothofagi	*N. pumilio* *N. antarctica*	**Torres**, Nah, Tolh	Discont mGLC
Psathyrocerus cf. P. unicor	*N. pumilio* *N.antarctica*	Nah, Tolh	Cont & **discont** mGLC
Eumolpinae species 4	*N. antarctica*	Nah	Discont mGLC

Insect Species	Plant Species	Site(s)	Feeding behaviour
Alticinae species	*N. antarctica*	Puy	Rough cont mGLC
Sawfly sp. 2 (larvae)	*N. pumilio* *N. antarctica*	Nah, Tolh, **Puy,** Torres, RNMag	**Cont**, discont mGLC & nGLC
Geometridae spp. (larvae)	*N. pumilio* *N. antarctica*	Nah, Tolh, Puy, RNMag, Isla Nav	**Cont**, discont mGLC & nGLC

A total of six species were identified from the subfamily Eumolpinae (Coleoptera: Chrysomelidae); they were collected only from Nah and Tolh. The species *Psathyrocerus cf. P. unicor* (Eumolpinae) damaged the leaf by feeding in both a continuous and discontinuous manner and removing large areas of the leaf tissue (Figure 5.1C). An adult *Psathyrocerus cf. P. unicor* is shown in Figures 5.2C and 5.2D. The other species of Eumolpinae were separated into morphospecies; all were collected from Nah and only on *N. antarctica*. One of the morphospecies, Eumolpinae species 4, was represented by four specimens. They created small discontinuous marginal traces on the leaves (Figure 5.1D). Three individuals, identified as belonging to the same species in the subfamily Alticinae (Spinola 1844) (Coleoptera: Chrysomelidae), had a distinctive feeding trace on *N. antarctica* and were only collected at the Puyehue site. They chewed the leaf margin continuously, however, the vein endings at the margin were still intact, creating an uneven leaf edge (Figure 5.3A).

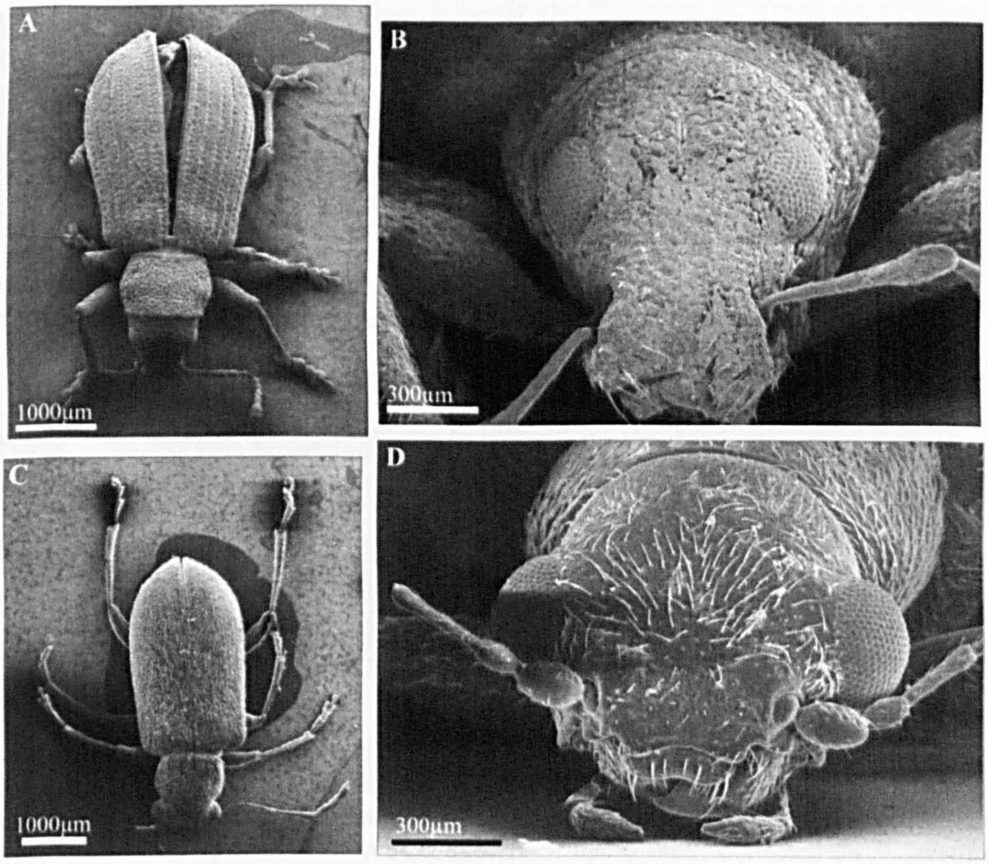


Figure 5.2. Insect species that created general leaf chewing traces on modern leaves. A) Dorsal view of *Polydrusus nothofagi*. B) Close up of head region of *P. nothofagi*. C) Dorsal view of *Psathyrocerus cf. P. unicor*. D) Close up of head region of *Psathyrocerus cf. P. unicor*.

Many insects were in the larval stage of development when they were collected. Of these, the feeding traces of 43 specimens were recorded and 21 different morphospecies were identified, including species of Hymenoptera, Lepidoptera and Coleoptera. The larvae created traces on the leaves in several ways, ranging from continuous marginal chewing traces to several small non-marginal holes. Also, larvae of the same species often created multiple types of traces. For example, three species were identified as hymenopteran larvae, all of which were species of sawfly (Hymenoptera: Symphyta). They were found at all sites and on both tree species. The most common morphospecies, sawfly 2, had distinct clusters of black hairs on the dorsal surface arranged in pairs. Although this species was mostly observed feeding continuously from the leaf margin, the larvae also made multiple small holes in the centre of the leaf, as well as creating small patches of skeletonization (Figure 5.3B).

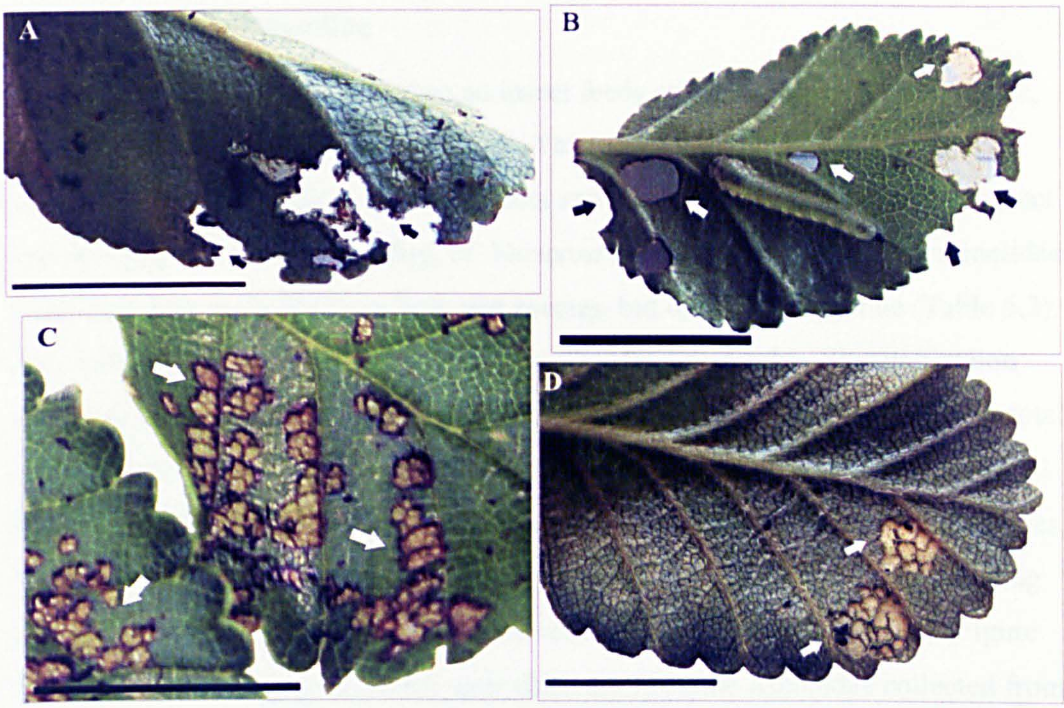


Figure 5.3. Insect traces collected from Chile. A) Small discontinuous marginal leaf chewing by the Alticinae. B) Continuous marginal chewing with rough edges by morphospecies sawfly 2. C) Skeleton feeding by *cf. Varicoxa* (Alticinae). D) Skeleton feeding of the lower epidermis only by a species of micro-moth collected on *N. pumilio*. Scale bar 1cm. Arrows highlight insect traces.

A total of 27 lepidopteran species were identified, including 19 species of Geometridae (Lepidoptera: Geometroidea), however, only 18 of the 27 lepidopteran species were observed feeding. They were found throughout the sampling sites and on both *Nothofagus* species. Both continuous and discontinuous marginal leaf chewing were common and four species were observed eating the entire leaf during feeding experiments. Identification of traces made solely by one species was difficult because for the majority of the lepidopteran morphospecies, only single specimens of each were observed feeding. Examination of the range of trace types made by the same family, such as the Geometridae however, indicates that one trace type could have been made by several species.

5.1.3. Skeleton Feeding

Skeleton feeding occurs when an insect feeds on and removes the leaf tissue, but the vein network remains untouched. Thirty two individuals were observed skeleton feeding, including 25 adult insects and 7 larvae. The most abundant insect species observed skeleton feeding, cf. *Varicoxa* species (Coleoptera: Chrysomelidae: Alticinae), was collected from both tree species, but only from Puyehue (Table 5.2). This species was not observed making any other feeding marks. Skeletonization occurs in small patches within close proximity to one another (Figure 5.3C). A total of 21 individuals of cf. *Varicoxa* species were observed (Figure 5.4A and 5.4B), 11 females and 10 males, distinguished by the striped pattern on the elytra of the males. Three individuals of Apionidae (Coleoptera: Curculionoidea) were collected using the beating technique and were also observed skeleton feeding (Table 5.2) (Figure 5.4C and 5.4D). These specimens were different from the Apionidae collected from the leaf galls described in Section 5.2.2.

Amongst the larvae collected, a species of micro-moth (Lepidoptera) was found only on *N. pumilio* trees (Table 5.2). The microlepidopterans are a non-monophyletic grouping which is very diverse and typically contains moths of a very small size. Micro-moths are distinguished from other moths due to the larvae having a prothoracic shield (the segment directly behind the head is made of chitin). One species of micro-moth had a more distinct method of skeleton feeding in which only the lower epidermal layer of the leaf was removed, leaving the vein network and the upper epidermis intact (Figure 5.3D).

Table 5.2. Distribution and feeding traces of the insect species collected in Chile and observed skeleton feeding. Nah = Parque Nacional Nahuelbuta, Puy = Parque Nacional Puyehue, RNMag = Reserva Nacional Magallanes.

Insect Species	Plant Species	Site(s)	Feeding behaviour
Cf. *Varicoxa* sp.	*N. pumilio* *N. antarctica*	Puy	Skeleton feeding
Apionidae sp.	*N. pumilio* *N. antarctica*	Nah Puy	Skeleton feeding
Micro-moth sp. (larvae)	*N.pumilio*	Nah RNMag	Lower epidermis skeleton feeding

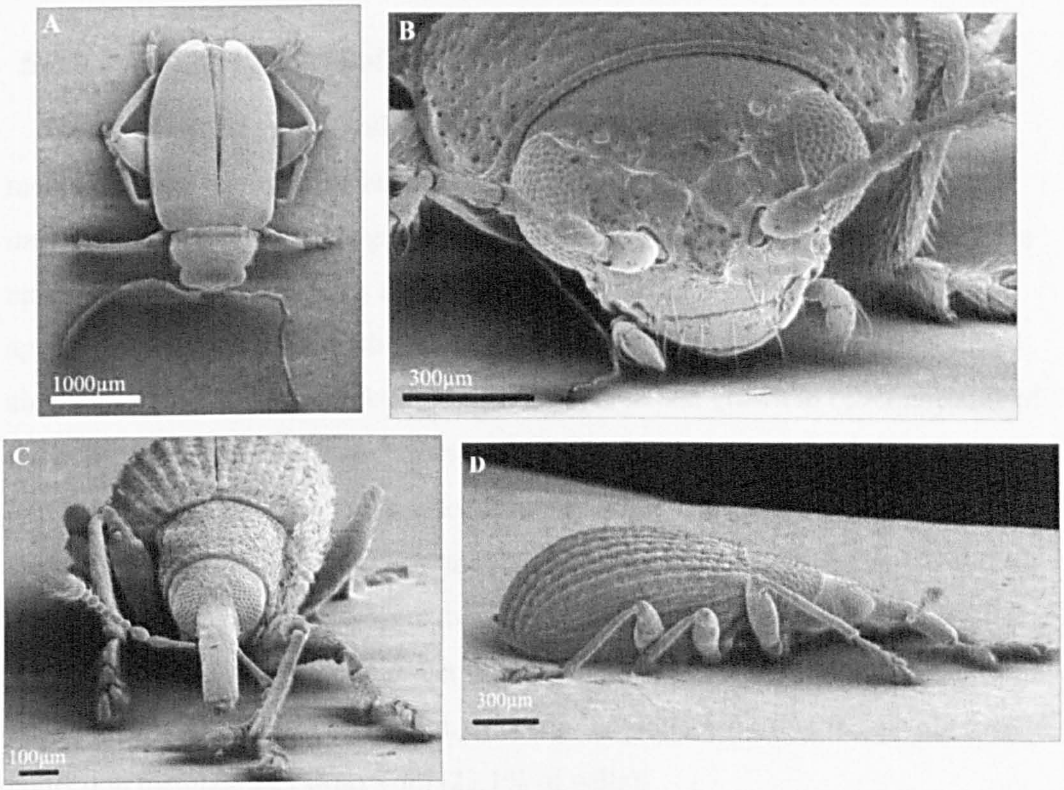


Figure 5.4. A) Cf. *Varicoxa* male that was observed skeleton feeding. B) Magnification of the head and mouth area of Cf. *Varicoxa* male. C) Frontal view of Apionidae species 6 observed skeleton feeding. D) Side view of Apionidae species 6.

5.2. Leaf Galls

The leaf galls collected from all sites visited in southern Chile are highlighted in Section 4.4.2 and 4.4.3.3. The most abundant galls will now be examined further by comparing their distribution across the sites visited. Other gall types were included in the statistical analyses, but could not be the focus of distribution studies due to their rarity. Rearing success from the galls was limited, therefore the gall causers could not be identified to species level and were solely distinguished by trace type. The galls are given descriptive names based on their shape, size and position on the leaf. All of the galls described in this section are types of covering gall, which, as the name suggests, leads to the inducer being enclosed within the gall.

5.2.1. Gall Type 1 – 'Ball Gall'

A total of 112 leaves with this gall type were collected (using all collection methods), most having been collected from *N. pumilio* and only four from *N. antarctica* (Table 5.3). This gall is a large circular protrusion, mainly on the upper epidermis of the leaf, but it is also evident on the lower epidermis where the gall appears as a thickened smooth protrusion (Figure 5.5A). Galls were only found singly on a leaf, having developed on the basal area and grown to cover one side of the upper epidermis of the leaf. When the insect emerged from the gall a circular exit hole was made in the gall surface (Figure 5.5B). Galls were found at several sites including Tolh, Nah, Puy (during first and second visit), Torres and RNMag. The galls from *N. antarctica* were, however, only found during the first visit to Puyehue. Although a larger number of ball galls were collected from Puyehue than any other site, the largest proportion of ball galls (collected using the quantitative collection method) was from Tolh (23.1% of galls).

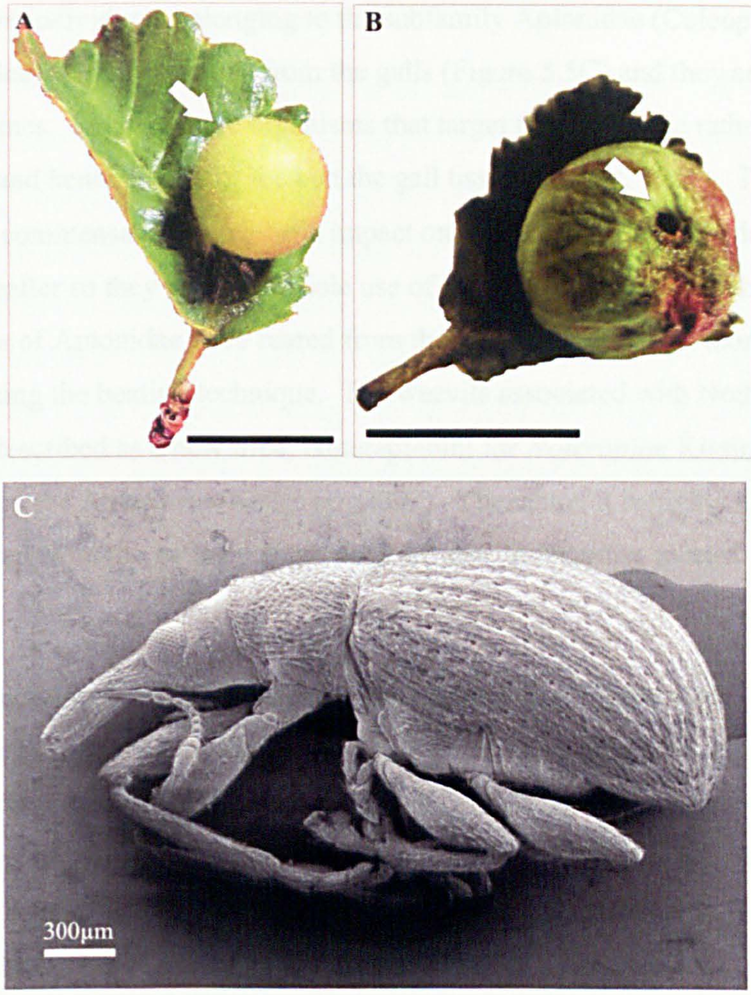


Figure 5.5. A) Gall type 1, ball gall, on *N. pumilio* leaf. B) Circular exit hole created by an emerging parasitoid on gall type 1. C) Apionid sp. 2 (inquiline) reared from the ball gall. Scale bar 1cm. Arrows indicate the trace.

Out of the 112 ball galls collected, 55 insects were reared successfully and the adults identified. Most of the adult insects reared were parasitoids or inquilines. A parasitoid is the name given to an insect which lays its eggs inside another insect (the host). During or after the parasitoid development the host is killed. The parasitoids reared from the ball galls were all species within the Chalcidoidea and Ichneumonoidea (Hymenoptera). Seven different species were reared, with only one species of Ichneumonoidea (Braconidae). The other six species were within the families Pteromalidae, Eulophidae and Torymidae (all Chalcidoidea).

Twelve individuals belonging to the subfamily Apionidae (Coleoptera: Curculionidea) were also reared from the galls (Figure 5.5C) and they are believed to be inquilines. Inquilines are organisms that target the gall tissue rather than the gall maker and hence live in or feed on the gall tissue (Askew, 1961). They range from being commensals (having little impact on the galler) to actually damaging or killing the galler so they can obtain sole use of the gall (Sanver & Hawkins, 2000). Two species of Apionidae were reared from the ball galls, with five more species collected using the beating technique. The weevils associated with Nothofagaceae have been described as a new tribe, Noterapionini for *Noterapion* Kissinger (type genus) within the Apioninae (Kissinger, 2002). Therefore, it is highly likely that the species reared from the galls on *N. pumilio* are also *Noterapion* species.

Five individuals reared were gall wasps (Cynipidae: Cynipoidea, Hymenoptera), the gall-causing insect. They were reared only from the galls collected from Puyehue. The cynipid is possibly a species in the genus *Paraulax*, as many species of this hymenoptera have been reared from galls on several *Nothofagus* species, such as *N. antarctica* and *N. dombeyi* (De Santis *et al.*, 1993; Kissinger, 2002), but there are no records for *N. pumilio*.

Table 5.3. Distribution of two types of leaf galls collected in Chile that were described in section 5.2. The sites in which the galls were most commonly found are in bold. Nah = Parque Nacional Nahuelbuta, Tolh = Parque Nacional Tolhuaca, Puy = Parque Nacional Puyehue, Torres = Parque Nacional Torres del Paine, RNMag = Reserva Nacional Magallanes and Isla Nav = Isla Navarino.

Leaf Gall	Description	Site (s)	Tree Species	Gall causing species
Gall type 1 (Ball gall) (Figure 5.3)	Large (1cm diameter), single protrusion, dominant on upper epidermis	Nah, Tolh, **Puy**, Torres, RNMag	*N. pumilio* *N. antarctica* (only Puy)	*Paraulax* sp. (Cynipidae)
Gall type 2 (Small gall)	Small multiple galls (0.2 - 0.5mm) at the secondary and primary vein junctions.	Nah, Tolh, **Puy**, Torres, RNMag, Isla Nav	*N. pumilio* *N. antarctica*	Eriophyidae sp. (Acari)

5.2.2. Gall Type 2 – 'Small gall'

Gall type 2, small gall, was found at all sample sites and on both *N. pumilio* and *N. antarctica* (Table 5.3). It is a small gall (0.2-0.5mm) found at the secondary and primary vein junctions of the leaf, usually with multiple galls per leaf (Figure 5.6). The gall is evident on both upper and lower epidermal leaf layers, although projecting more on the upper epidermis. As many as 379 leaves with gall type 2 were collected from *N. pumilio* and 480 leaves with the gall from *N. antarctica*. For leaves collected from *N. pumilio* using both collection methods, the proportion of leaves collected with this gall type was greatest at Puyehue, particularly from the first and second sampling time, and Nahuelbuta (Table 5.4). Isla Navarino had a very small proportion of type 2 galls (Table 5.4).

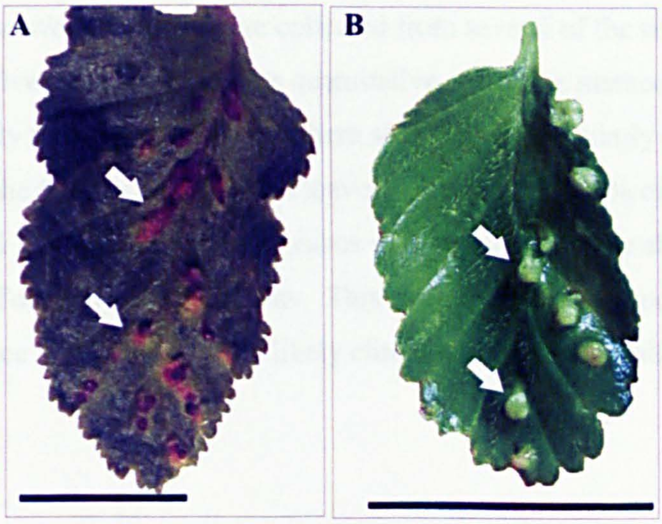


Figure 5.6. A) Gall type 2, small gall, on *N. antarctica*. B) Gall type 2 on *N. pumilio*. Scale bar 1cm. Arrows indicate the trace.

Table 5.4. The proportion of type 2 galled leaves collected at several sites using all collection methods. The most and least abundant sites are given. Nah = Parque Nacional Nahuelbuta, Tolh = Parque Nacional Tolhuaca, Puy = Parque Nacional Puyehue, Torres = Parque Nacional Torres del Paine, RNMag = Reserva Nacional Magallanes and Isla Nav = Isla Navarino.

Site	Total number of type 2 galls collected	% of type 2 galls collected per site
Puy all visits	241	63.6
Puy 1	120	31.6
Puy 2	108	28.5
Puy 3	13	3.4
Nah	80	21.1
Isla Nav	245	2.0

For *N. antarctica*, the galls were collected from several of the sites sampled using the qualitative method. Using the quantitative collection method, gall type 2 was however, only found in the more northern sites being particularly abundant at Puyehue during the first visit (349 galled leaves collected). 75.95% of the galls collected at Puy 1 were of gall type 2. Leaves with small galls were absent from *N. antarctica* from Tolh, Puy 3 and Isla Nav. This could be due to seasonal affects in the case of absence at Puy 3, but more likely climatic influences at Isla Nav and Tolh.

No insects were successfully reared from this gall type during the fieldwork. After microscope dissection of the galls on return to the laboratory, however, the origin of the galls was clarified. Each type 2 gall had a chamber containing many Eriophyidae mites (Acari: Eupodina: Eriophyoidea), a family which contains many hundreds of gall-inducing species (Westphal, 1992). The Eriophyoid mites have a reduced body structure with the loss of four rear legs and few body setae compared to other mite families. They have an elongated fusiform body with a cephalothoracic shield and have only 2 pairs of segmented legs located at the front of the body.

5.2.3. Gall Type 3 – 'Golf Gall'

Gall type 3, very similar in shape and size to gall type 2, is named "golf gall" due to a pointed projection on the gall on the upper side and a rounded projection on the underside of the leaf, resembling a golf tee (Figure 5.7A). This gall type is usually present as small, multiple galls found across the leaf area, but they are mostly concentrated at the primary and secondary vein junctions. In total, 256 leaves of *N. pumilio* with golf galls were collected from all sampling sites, although the galls were absent at Puyehue during the first visit. The largest number of golf galls was collected from Torres (Table 5.5). The largest proportion of golf galls were collected from the southern sites (70.31% of total leaves with golf galls), due to the abundance of golf galls at Torres (85.12% of the total galled leaves were golf galls).

Table 5.5. The proportion of type 3 galls collected at each site from *N. pumilio* using all collection methods. Nah = Parque Nacional Nahuelbuta, Tolh = Parque Nacional Tolhuaca, Puy = Parque Nacional Puyehue, Torres = Parque Nacional Torres del Paine, RNMag = Reserva Nacional Magallanes and Isla Nav = Isla Navarino.

Site	Total number of type 3 galls collected	% of total type 3 galls collected
Nah	46	18.0
Tolh	12	4.7
Puy 1	0	0
Puy 2	16	6.3
Puy 3	2	0.8
Torres	116	45.31
RNMag	23	9.0
Isla Nav	19	7.4

A total of 39 *N. antarctica* leaves with golf galls were collected from all sites, but they were absent from Nah, Tolh and RNMag (Table 5.6). Although this was only a small sample, and therefore the results have to be interpreted with caution, *N. antarctica* leaves with golf galls have a more even distribution between the northern and southern sites than *N. pumilio*. There was only a small difference in the percentage of golf galls collected from the northern (53.85% of golf galls) and southern sites (46.15% of golf galls).

Although golf galls have a similar morphology to gall type 2, upon dissection a species of nematode was found inside. The nematode species belongs to the Tylenchida (Nematoda: Secernentea: Tylenchia), which are the largest group of plant-parasitic nematodes. They have been found to exploit all plant organs, including flowers, seeds and most often roots (Siddiqi, 2000). There are limited reports of plant nematodes on the Nothofagaceae, particularly from Chile. One leaf-gall nematode was, however, reported on leaves of *Nothofagus obliqua*, a deciduous species in Chile (Moreno *et al.*, 1999). The leaf galls on *N. obliqua* are quite different in both size and shape from the golf galls and are described as convoluted spheroid thickenings on the leaf. The nematode on *N. obliqua* was later identified as *Subanguina chilensis* (Tylenchida: Anguinidae), the only species in the genus *Subanguina* because it is the only nematode species to induce galls on a deciduous host plant (Volvas *et al.*, 2000). The type 3 leaf galls were located across the leaf surface, but occurred proximally along the primary or secondary veins. They contained several individuals of nematode at different life stages within the single central cavity. The nematodes from gall type 3 were identified as belonging to the same genus as *Subanguina chilensis*, but the species differed with respect to the morphology of the tail and head regions (A. Troccoli, pers. comm.).

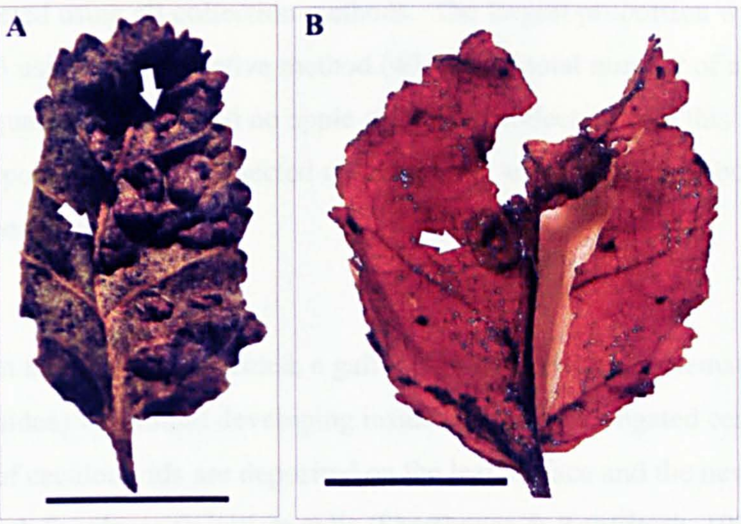


Figure 5.7. A) Gall type 3, golf gall, on *N. antarctica*. B) Gall type 4, apple gall on *N. antarctica*. Scale bar 1cm. Arrows indicate the trace.

Table 5.6. Distribution of two types of leaf gall collected in Chile that were described in section 5.2. The most common sites from which the galls were collected are in bold. Nah = Parque Nacional Nahuelbuta, Tolh = Parque Nacional Tolhuaca, Puy = Parque Nacional Puyehue, Torres = Parque Nacional Torres del Paine, RNMag = Reserva Nacional Magallanes and Isla Nav = Isla Navarino.

Leaf Gall	Description	Site (s)	Tree Species	Species causing the gall
Gall type 3 (Golf gall) (Figure 5.5A)	Pointed projection on upper epidermis and more rounded projection on lower epidermis.	Nah, Tolh, Puy, **Torres,** RNMag, Isla Nav	*N. pumilio* *N. antarctica*	Tylenchida (Nematoda)
Gall type 4 (Apple gall) (Figure 5.5B)	Single circular protrusion, found on secondary veins at basal area of the leaf	Nah, Tolh, **Puy**	*N. antarctica*	Cecidomyiidae

5.2.4. Gall Type 4 – 'Apple Gall'

Gall type 4 (apple gall) is of particular interest as it was only present on *N. antarctica* and only found at the northern sites of Nah, Tolh, Puy 2 and Puy 3 (Table 5.6). This gall type has a similar shape to gall type 1 (ball gall) with a circular protrusion to both the upper and lower epidermis of the leaf (Figure 5.7B). It is however, smaller and restricted to growing on the secondary veins at the basal area of the leaf. Only one gall was found per leaf. A total of 108 leaves with apple galls were collected using all collection methods. The largest proportion was collected from Puy 3 using the qualitative method (40.74% of total number of apple galls), but using the quantitative method no apple galls were collected from this site. The largest proportion of galls collected quantitatively was from Nahuelbuta (58.06% of total number of apple galls).

When the gall was dissected, a gall midge larva (Diptera: Nematocera: Cecidomyiidae) was found developing inside each large elongated central cavity. The eggs of cecidomyids are deposited on the leaf surface and the newly-hatched larvae search for places to initiate galls (Shorthouse & Rohfritsch, 1992). Previous studies have identified 19 genera and 53 species of gall midges from Argentina, Chile and Paraguay (Kieffer & Herbst, 1905). Only one species of cecidomyid had a *Nothofagus* species as a host and that was *Rhopalomyia nothofagi* (Cecidomyiidae), found in galls on *N. obliqua* (Gagné, 1994). Thus, further taxonomic work, beyond the scope of this project, is required to identify the gall midge collected from the apple galls on *N. antarctica*. In addition, one species of Apionidae (not the same species that emerged from gall type 1) was found in these galls and could have been an inquiline species.

5.2.5. Leaf Gall Diversity

There are specific associations and relationships within the leaf gall communities studied. For example, ball galls are only found on *N. pumilio* and apple galls are only found on *N. antarctica*. In the case of *N. pumilio*, gall types were found at all sites, but ball galls and golf galls show some distribution bias (Figure 5.8). Ball galls were more abundant in the northern sites and golf galls more abundant in the southern sites. The proportion of each gall type collected from each site, using the qualitative method, highlights differences between sites (Figure 5.8). Only 6 galled leaves were collected during the third visit to Puyehue, and these were all small galls. The 'other galls' category shown in Figure 5.8 includes gall types collected in small quantities and which could not be analysed further in terms of their distribution.

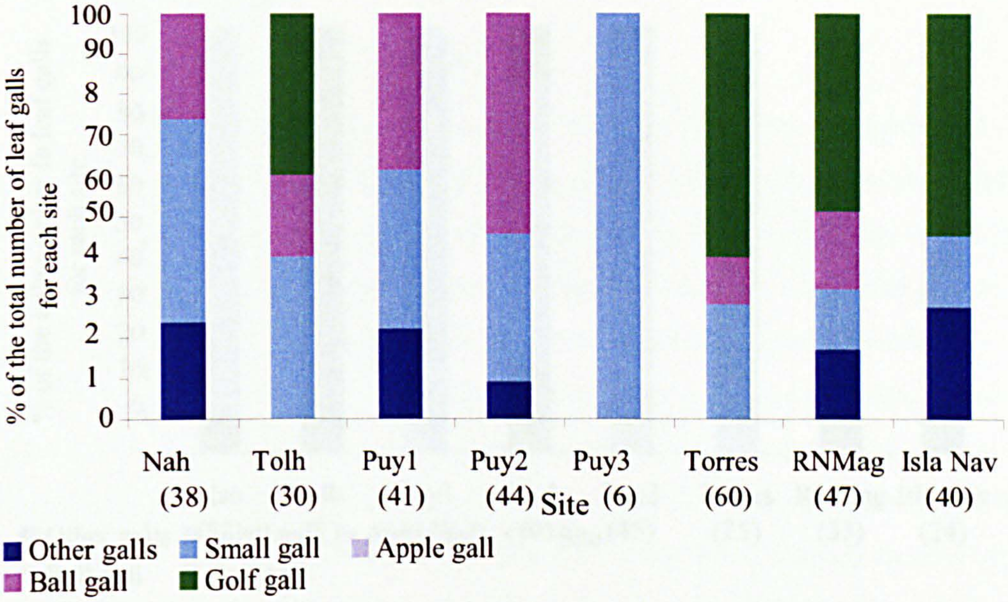


Figure 5.8. The relative proportions of types of leaf galls collected across the sampling sites from *N. pumilio* using the qualitative collection technique (December 2006 – March 2007). The total number of galls for each site is given in parentheses beneath each site name. Sites are in order of increasing latitude from left to right.

The distribution of the gall types sampled from *N. antarctica* also highlights site differences (Figure 5.9). The apple galls were only collected in the northern sites and dominated the collection of galls during the third sampling period at Puyehue (97.7% of galled leaves). Also of note is the absence of golf galls at the more northern sites of Tolhuaca and Nahuelbuta and their abundance at Torres. Many of the leaf galls from Isla Navarino were within the “other galls” category. A high proportion of galls at Isla Navarino were of one type of gall not collected at any other site (86.3% of other galls). Only a single leaf gall was collected from *N. antarctica* at Tolhuaca.

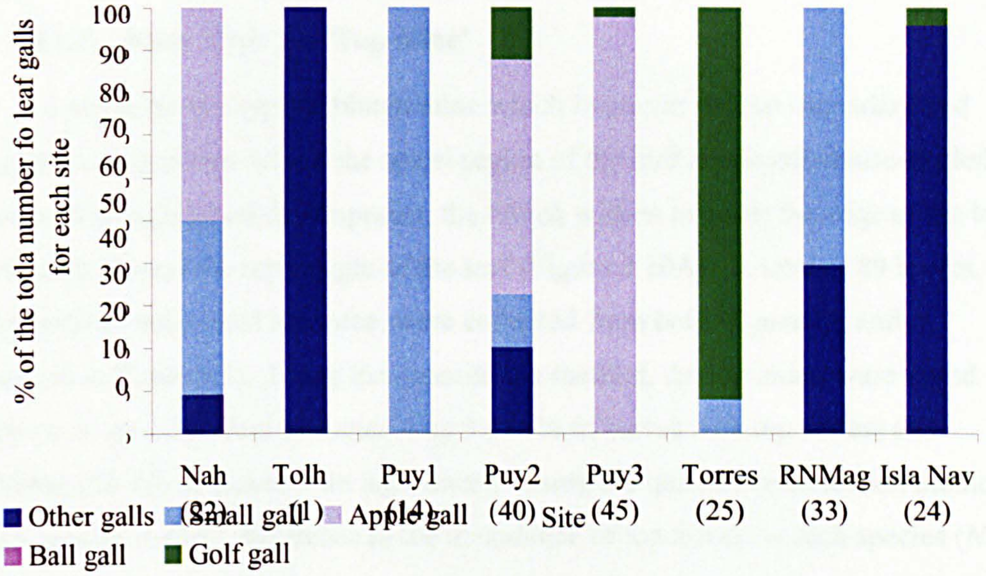


Figure 5.9. The relative proportions of types of leaf galls collected from the sampling sites from *N. antarctica* using the qualitative collection technique (Dec 2006 – March 2007). The total sample size for each site is given in parentheses beneath each site name. Sites are in order of increasing latitude from left to right.

5.3. Leaf Mines

Leaf mines collected from Chile were described briefly in Section 4.4.2 and Section 4.4.3.3. Several of the more common mines will now be examined in more detail. Identification of the leaf mining insect is often possible due to the characteristic appearance of the mine. A description of each type of mine is given in the following sections and their distribution within the sampling sites examined.

The majority of insects that emerged from the leaf mines were not successfully reared to adults, therefore identification of the leaf mining insect was based on mine type and larval stage. Parasitoids were, however, successfully reared from 10 leaf mines that represented 5 types of mine. The parasitoids that emerged were all species of Eulophidae (Chalcidoidea: Hymenoptera).

5.3.1. Blotch Mines

5.3.1.1. Mine Type 1 – 'Top mine'

A top mine is a type of blotch mine which begins at the last secondary and primary vein junction within the apical region of the leaf as a small obtuse-angled blotch. Through larval development, the blotch widens to cover the edge of the leaf in an arch around the top margin of the leaf (Figure 5.10A). A total of 89 leaves, each with an individual top mine, were collected from both *N. pumilio* and *N. antarctica* (Table 5.7). Using the quantitative method, the top mines were found only on *N. pumilio* from two sites: Puy 3 (5.6% of leaves with top mines) and RNMag (94.4% of leaves with top mines). Using the qualitative collection method, there was no distinct difference in the abundance of top mines on each species (*N. antarctica* 54.7% of the top mines, *N. pumilio* 45.3% of top mines). Also, all top mines were found at RNMag using the qualitative collection method.

The identification of the leaf-mining insects proved difficult due to limited success in rearing of the larvae. Regardless of this, nine individual larvae were identified as coleopteran leaf miners. The larvae were collected from both *N. pumilio* and *N. antarctica.* Parasitoids were also collected from this type of mine including a species of Entedontinae (Hymenoptera: Chalcidoideae: Eulophidae).

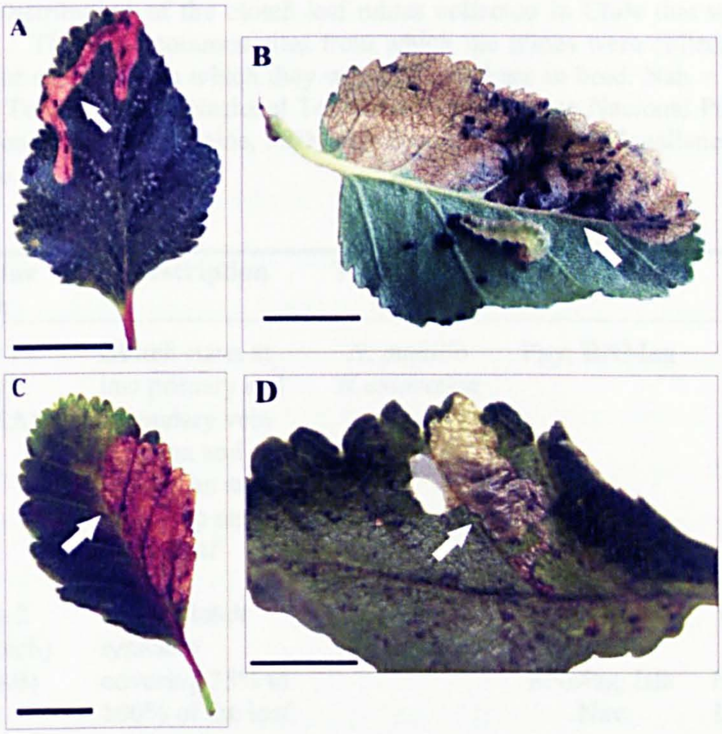


Figure 5.10. The diversity of leaf mine types collected from Chile. A) Mine type 1, top mine on *N. antarctica*. B) Mine type 2, large blotch on *N. antarctica* with lepidopteran larva. C) Mine type 3, standard blotch on *N. pumilio*. D) Mine type 4, blotch cocoon on *N. pumilio*. Scale bar 1cm. Arrows indicate insect traces.

5.3.1.2. Mine Type 2 - 'Large blotch'

Mine type 2 (large blotch) was collected on leaves that demonstrated different stages of larval development. When full size, the mine typically covered a large portion of the leaf area, ranging from 25% to 100% of the leaf area. The mine is visible from both lower and upper epidermal layers of the leaf and faecal pellets can easily be seen within the mine (Figure 5.10B.). A total of 119 large blotch mines were collected from both *N. pumilio* and *N. antarctica* and were found at all sites except at Tolh (Table 5.7). The quantities of leaves with large blotch mines varied greatly, with the largest number collected from Torres (57 mines, 55.0% of mines) and the smallest from Puy 1 (1 large blotch mine, 0.84% of mines). Also, a larger proportion of mines were collected on *N. pumilio* leaves (76 mines, 63.9% of top mines) than on *N. antarctica* (43 mines, 36.1% of top mines).

Table 5.7. Distribution of the blotch leaf mines collected in Chile that were described in section 5.3.1. The most common sites from which the mines were collected and the most common plant species from which they were collected are in bold. Nah = Parque Nacional Nahuelbuta, Tolh = Parque Nacional Tolhuaca, Puy = Parque Nacional Puyehue, Torres = Parque Nacional Torres del Paine, RNMag = Reserva Nacional Magallanes and Isla Nav = Isla Navarino.

Leaf mine name	Description	Tree Species	Site(s)	Leaf miner
Mine type 1 (Top mine) (Figure 5.8A)	Blotch starts at last primary and secondary vein junction and ends in an arch at the top margin of the leaf	*N. pumilio* *N.antarctica*	Puy, **RNMag**	Coleoptera
Mine Type 2 (Large Blotch) (Figure 5.8B)	Large blotch typically covering 25% to 100% of the leaf at full size.	***N. pumilio*** *N.antarctica*	Nah, Puy, **Torres**, RNMag, Isla Nav	Coleoptera (on *N. pumilio*) Hymenoptera & Lepidoptera (on *N. antarctica*)
Mine Type 3 (Standard Blotch) (Figure 5.8C)	Blotch covering at most 25% of leaf surface.	***N. pumilio*** *N.antarctica*	Nah, Tolh Puy, Torres, **RNMag**, Isla Nav	Distinct Coleopteran sp. (on *N. pumilio*)
Mine Type 4 (Blotch cocoon) (Figure 5.8D)	Small serpentine at start, changing into a blotch. The insect finally forms a cocoon using the leaf layers.	***N. pumilio*** *N.antarctica*	**Puy**, Torres, RNMag, Isla Nav	Nepticulidae (Lepidoptera)

Two larvae were identified from mines collected on *N. antarctica*, both from trees sampled at Puy 2. One was a lepidopteron leaf miner and the other a sawfly leaf miner (Hymenoptera). From the mines collected from *N. pumilio*, nine individual larvae were successfully reared to adults (4 individuals from Torres, 3 individuals from Puy, 1 individual from Isla Nav and 1 individual from RNMag), all of which were identified as coleopteran leaf miners. Only two of the nine coleopteran leaf miners were further identified as weevil leaf miners (Coleoptera: Curculionidae). Therefore, several different insect species produce mines of this form.

5.3.1.3. Mine Type 3 – 'Standard Blotch'

The standard blotch (mine type 3) is very similar to the large blotch, however, the mine only covers a maximum of 25% of the leaf surface (Figure 5.10C.). This could be because the mine was collected at an earlier stage in larval development. Identification of the larvae (see below) which were reared out of this mine type, showed that they were different from mine type 1 larvae. In total, 119 standard blotch mines were collected from both tree species. The largest number of this mine type was collected from RNMag (25.2% of standard blotch mines). Many were also found at Nah, Tolh and Torres. Standard blotch mines were present at all sites, albeit in small numbers at Puy 1 and Isla Nav (Table 5.7).

Six leaf miners were reared successfully from this mine type, three from each tree species. The larvae from *N. pumilio* were identified as a morphospecies of a coleopteran leaf mining insect, distinct from other types of leaf miners reared, whereas the larvae from *N. antarctica* were identified as lepidopteran leaf miners (also of a distinct morphospecies from other lepidopteran leaf miners).

5.3.1.4. Mine Type 4 – 'Blotch cocoon'

The blotch cocoon (mine type 4) first appears as a very small serpentine mine (approximately 2 mm long) that then enlarges into a blotch-shaped mine covering several inter-secondary vein areas (Figure 5.10D). During the final stages of the leaf miners development, the insect forms a cocoon at the end of the blotch, using the leaf layers as an outer protective casing. The cocoon is then attached to an available surface by the insect, which inside the Petri dish is the lid or side of the dish. In early instars the larva is visible through the mine (the leaf layer) and appears orange when examined in direct light. A total of 143 mines were collected from both tree species, although 81.8% of the blotch cocoon mines were found on *N. pumilio* (Table 5.7). The greatest proportion of blotch cocoon mines were collected during the third visit to Puyehue (41.3% of blotch cocoon mines) and Torres had the second largest proportion of this mine type (30.8% of blotch cocoon mines). Therefore, although there is no simple latitudinal trend to explain the distribution of this mine type, there may be a climatic influence because no mines were collected from Nah or Tolh, the most northerly sites.

Only three individuals were identified from blotch cocoon mines and they were Nepticulidae leaf miners (Lepidoptera: Nepticuloidea). A further three nepticulid purses (cocoons containing the developing larva) were also collected from this mine type. Nepticulidae are part of a superfamily which contain some of the smallest adult Lepidoptera with wing length of 1.5mm (Grimaldi & Engel, 2005) and they retain many primitive features in the adult form. The larvae are known to tunnel into the mine directly from the egg, forming a slender tortuous mine, which later expands either gradually or abruptly into a blotch mine (Common, 1970).

5.3.2. Line Mines

5.3.2.1. Mine Type 5 – 'Serpentine Mine'

The serpentine mine (mine type 5) is a type of line mine which starts very small within the secondary vein area. It has a very distinct linear frass pattern in the centre of the channel. As the larva develops, the frass channel widens and the leaf colour lightens adjacent to the mine (Figure 5.11A). The frass channel is unidirectional, but curves and winds several times throughout the leaf area. The largest mine occupied approximately 50% of the leaf area, but was more commonly found to cover 25% of the leaf area. In total, 93 serpentine mines were collected from both tree species and were found at most sites, apart from during the first two visits to Puyehue (Table 5.8). There were more serpentine mines on *N. pumilio* leaves (61.3% of mined leaves) than on *N. antarctica* leaves. The greatest abundance of serpentine mines were found at Torres (40.9% leaves with mines) and the smallest number were at Isla Nav and Nah (1.1% of leaves with serpentine mines). No larvae were successfully reared from this type of mine, therefore the causal insect could not be identified.

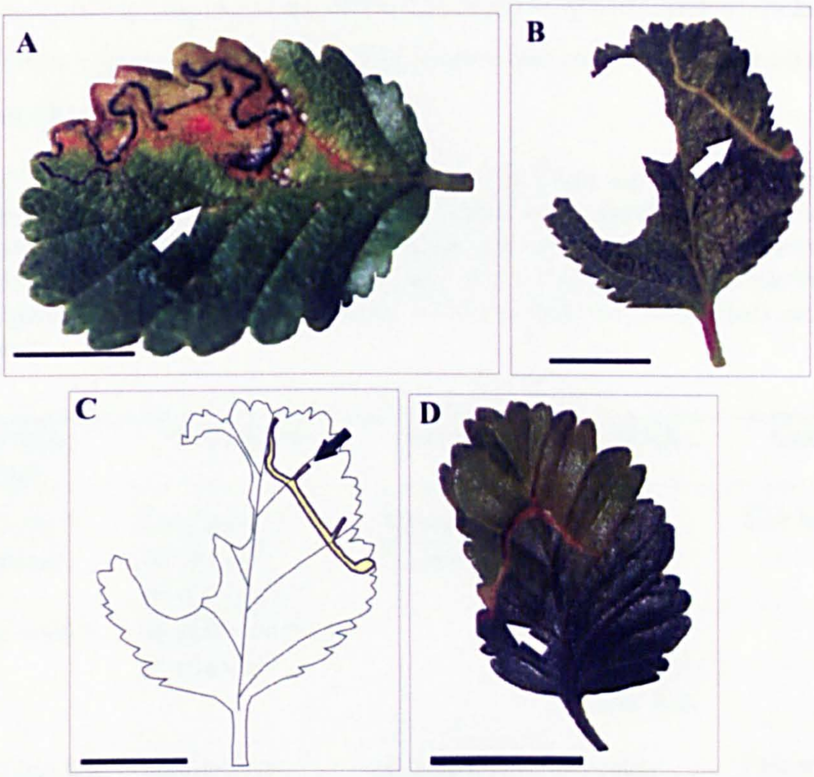


Figure 5.11. A range of leaf mine types collected from Chile. A) Mine type 5, Serpentine mine on *N. pumilio*. B) Mine type 6, ladder mine on *N. antarctica*. C) Drawing of mine type 6 on *N. pumilio* to show linear projections. D) Mine type 6, ladder mine on *N. pumilio* with terminal blotch. Scale bar 1cm. Arrows indicate insect trace.

5.3.2.2. Mine Type 6 – 'Ladder Mine'

Mine type 6 (ladder mine) is a linear mine which starts as a small line running across the upper epidermis of the leaf from one margin to the other. As larval development progressed, small sections project out at the secondary and primary vein junctions and then rejoin the main linear mine (Figure 5.11B & C). When the line reached the margin on the other side it changed into a long blotch that follows the edge of the leaf (Figure 5.11D). It is typically found at the apical region of the leaf. From the linear part of the mine to the apical margin the leaf colour changed from green to light brown. The small projection at the secondary veins could be beneficial to the developing larva by increasing food supply, as there is an active movement of nutritious solutions in the leaf veins (Hering, 1951). A total of 179 ladder mines were collected from all sampling sites and from both tree species (Table 5.8). A large proportion was collected at Nah (27.93% of total number of ladder mines), and at Puy 2 (29.61% of total number of ladder mines), but the largest proportion of mines was collected at Torres (36.87% of total number of ladder mines). Once again, rearing success was limited and only one parasitoid emerged from a mined leaf.

Table 5.8. Distribution of line leaf mines collected in Chile that were described in section 5.3.2. The most common sites from which the mines were collected and the most common plant species from which they were collected are in bold. Nah = Parque Nacional Nahuelbuta, Tolh = Parque Nacional Tolhuaca, Puy = Parque Nacional Puyehue, Torres = Parque Nacional Torres del Paine, RNMag = Reserva Nacional Magallanes and Isla Nav = Isla Navarino.

Leaf mine Type	Description	Tree Species	Site(s)	Leaf miner
Mine Type 5 (Serpentine Mine) (Figure 5.9A)	Line shaped mine with distinct frass pattern in centre of channel	***N. pumilio*** *N. antarctica*	Nah, Tolh, Puy, **Torres**, RNMag, Isla Nav	Not identified
Mine Type 6 (Ladder mine) (Figure 5.9A to C)	Linear mine running horizontally across the leaf from one margin to the other. Small mine projections at secondary vein.	***N. pumilio*** *N. antarctica*	Nah, Tolh, Puy, **Torres**, RNMag, Isla Nav	Not identified
Mine Type 7 (Serpentine-to-Blotch Mine) (Figure 5.10)	Starts as a serpentine mine and ends in a widened blotch. Exit hole is v-shaped at the end of the blotch	***N. pumilio*** *N. antarctica*	Nah, Tolh, Puy, **Torres**, **RNMag**, Isla Nav	Lepidoptera
Mine Type 8 (Balloon Mine)	Narrow channel gradually widening and abruptly broadens into a large blotch near the leaf margin.	***N. pumilio*** *N. antarctica*	Nah, Puy, Torres, RNMag	*Heterobathmia* sp. (Lepidoptera)

5.3.2.3. Mine Type 7 – 'Serpentine-to-Blotch Mine'

The "serpentine-to-blotch" mine type is a very distinctive mine, which, as the name suggests, starts off as a serpentine mine and ends in a widened blotch before the insect exits the mine. Faecal pellets are visible in the mine and are constrained to the middle of the serpentine part (Figure 5.12). The larva exits the mine through a distinct v-shaped flap at the end of the blotch section. A total of 515 serpentine-to-blotch mines were collected from both *N. pumilio* and *N. antarctica* (Table 5.8). The majority of mines were collected in the southern sites (77.7% of leaves with serpentine-to-blotch mines), 33.4% of the total number of serpentine to blotch mines was collected from RNMag and 24.5% from Torres. The smallest proportion of leaf mines was collected from Tolh (0.97% of the total number of serpentine to blotch mines). Therefore, there may be a latitudinal preference of the insects that make the serpentine-to-blotch mines towards the more southern sites than the northern sites. Seven larvae emerged from the serpentine-to-blotch mines and they were identified as lepidopteran leaf miners, one of which belonged to the family Nepticulidae (Lepidoptera: Nepticuloidea). The others could not be further identified due to poor quality preservation.

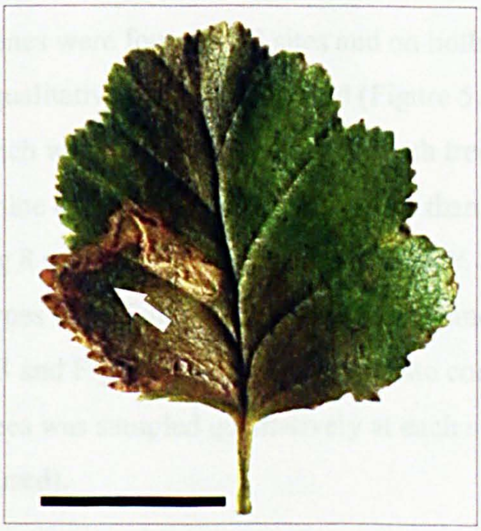


Figure 5.12. Mine type 7, Serpentine-to-blotch mine collected on *N. antarctica* in Chile. Scale bar 1cm. Arrow indicates insect trace.

5.3.2.4. Mine Type 8 – 'Balloon Mines'

Mine type 8 (balloon mine) was collected from both *N. pumilio* and *N. antarctica*, but only from Nah, Puy, Torres and R.N. Magallanes (Table 5.8). The mine begins as a narrow, almost linear channel, gradually widening and abruptly broadening into a large blotch near the margin of the leaf. A total of 19 individual mines were collected. The mine is interesting due to the causal insect. This mine is created by larvae in the genus *Heterobathmia* (Lepidoptera: Heterobathmiina: Heterobathmiidae), a genus associated with the Nothofagaceae (Kristensen & Nielsen, 1983). This is the sole genus in a family that occurs only in southern temperate South America (Grimaldi & Engel, 2005). The balloon mine is similar to the mine created by *H. pseuderiocrania (Kristensen & Nielsen, 1979)*, one of the most common species in the genus. For this species, the change in mine shape from linear to blotch coincides with the first moult of the head capsule of the first instar larva. The mature larva of *H. pseuderiocrania* is also similar in shape to the larva from balloon mines and has a distinct Y-shaped ridge on the dorsal surface of the head, and the thoracic and abdominal segments have a largely uniform shape.

5.3.3. Summary of Leaf Mine Diversity

A range of leaf mines were found at all sites and on both species of *Nothofagus*, using the qualitative collection method (Figure 5.13 & Figure 5.14), except the top mine which was limited to RNMag for both tree species. In general, there was greater leaf mine diversity in the southern sites than in the northern sites. For example, at RNMag 8 mine types were collected from *N. antarctica* but in contrast, only 3 mine types from Tolh. Although the leaf mine sample sizes presented in Figure 5.13 and Figure 5.14 vary, making site comparisons is difficult (the same number of trees was sampled qualitatively at each site and the same amount of search time used).

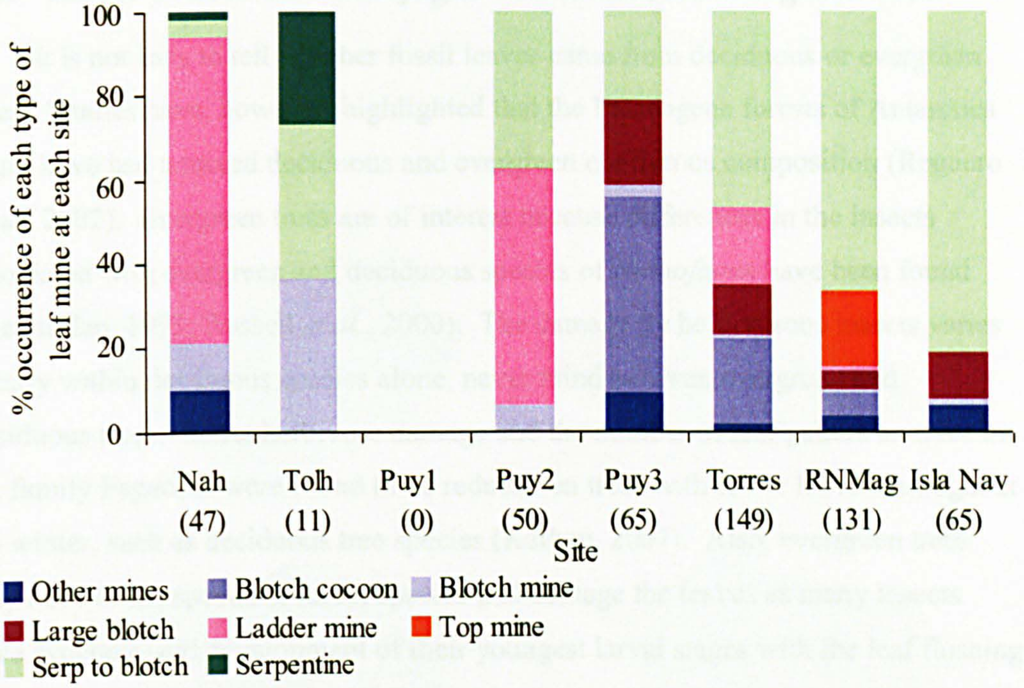


Figure 5.13. Proportion of different mine types for *N. pumilio* (using the qualitative collection method). The total number of mines collected at each site is given in parentheses. The category "other mines" includes other mine types collected in small numbers. Sites are in order of increasing latitude from left to right.

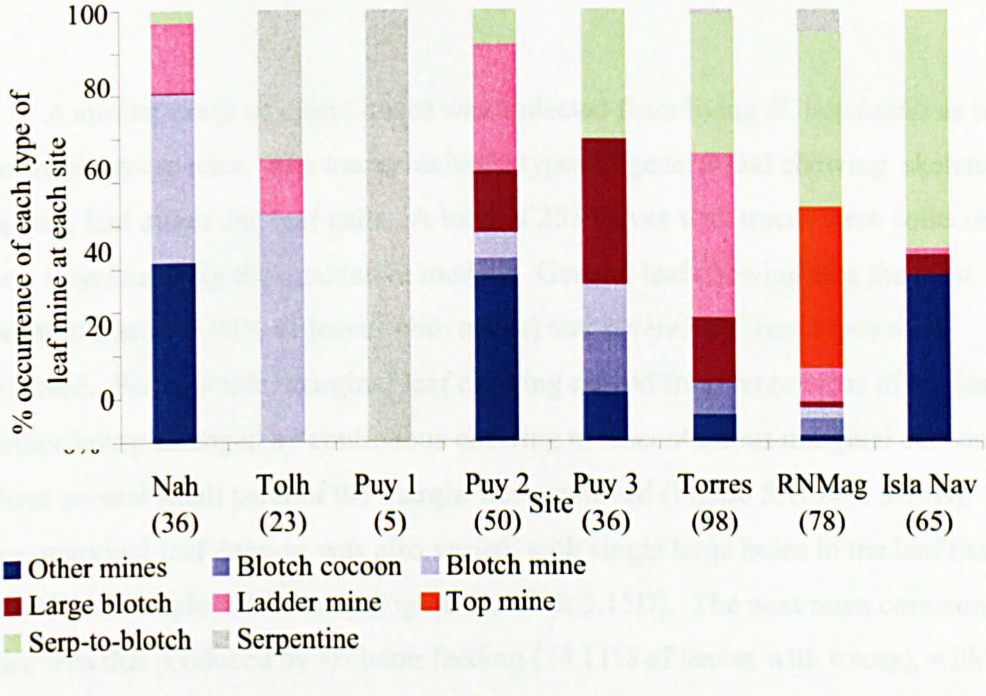


Figure 5.14. Proportion of mine types collected at each site for *N. antarctica* (using the qualitative collection method). The total number of mines collected from each site is given in parentheses. The category "other mines" includes other mine types collected in small numbers. Sites are in order of increasing latitude from left to right.

5.4. Insect Traces on *Nothofagus betuloides*, an evergreen tree.

It is not easy to tell whether fossil leaves came from deciduous or evergreen trees. Studies have, however, highlighted that the Palaeogene forests of Antarctica could have had a mixed deciduous and evergreen coniferous composition (Reguero *et al.*, 2002). Evergreen trees are of interest because differences in the insects associated with evergreen and deciduous species of *Nothofagus* have been found (McQuillan, 1993; Russell *et al.*, 2000). The damage by herbivorous insects varies greatly within deciduous species alone, never mind between evergreen and deciduous trees. Insect herbivore damage and the number of leaf gallers on trees in the family Fagaceae were found to be reduced on trees with fewer leaves throughout the winter, such as deciduous tree species (Karban, 2007). Also, evergreen trees may have fewer specialist insect species that damage the leaves as many insects have synchronized development of their youngest larval stages with the leaf flushing which occurs in deciduous species (Goolsby *et al.*, 2000). Hence, to obtain a comparable range of trace types from Chile that could be compared with the fossil traces (if from evergreen trees), *N. betuloides*, an evergreen species of *Nothofagus*, was sampled.

A similar range of insect traces was collected from living *N. betuloides* as to the deciduous species. The traces included types of general leaf chewing, skeleton feeding, leaf mines and leaf galls. A total of 253 leaves with traces were collected from Puyehue using the qualitative method. General leaf chewing was the most common trace (43.08% of leaves with traces) and several different types were collected. For example, marginal leaf chewing ranged from large areas of the leaf surface being damaged by continuous chewing to discontinuous marginal chewing where several small parts of the margin were removed (Figure 5.15A & 5.15B). Non-marginal leaf damage was also varied, with single large holes in the leaf tissue as well as multiple small holes (Figure 5.15C & 5.15D). The next most common trace was that produced by skeleton feeding (24.11% of leaves with traces), with examples of both top and bottom epidermal layers removed, as well as leaves on which only the top or bottom layers are removed (Figure 5.15E & 5.15F). The evergreen leaves differed from the deciduous species for both general leaf chewing and skeleton feeding. The main difference was that the ridge around the area of damage on the evergreen species was more distinct, due to the obvious colour change to yellow. The edge was also more rigid (Figure 5.15G & 5.15H).

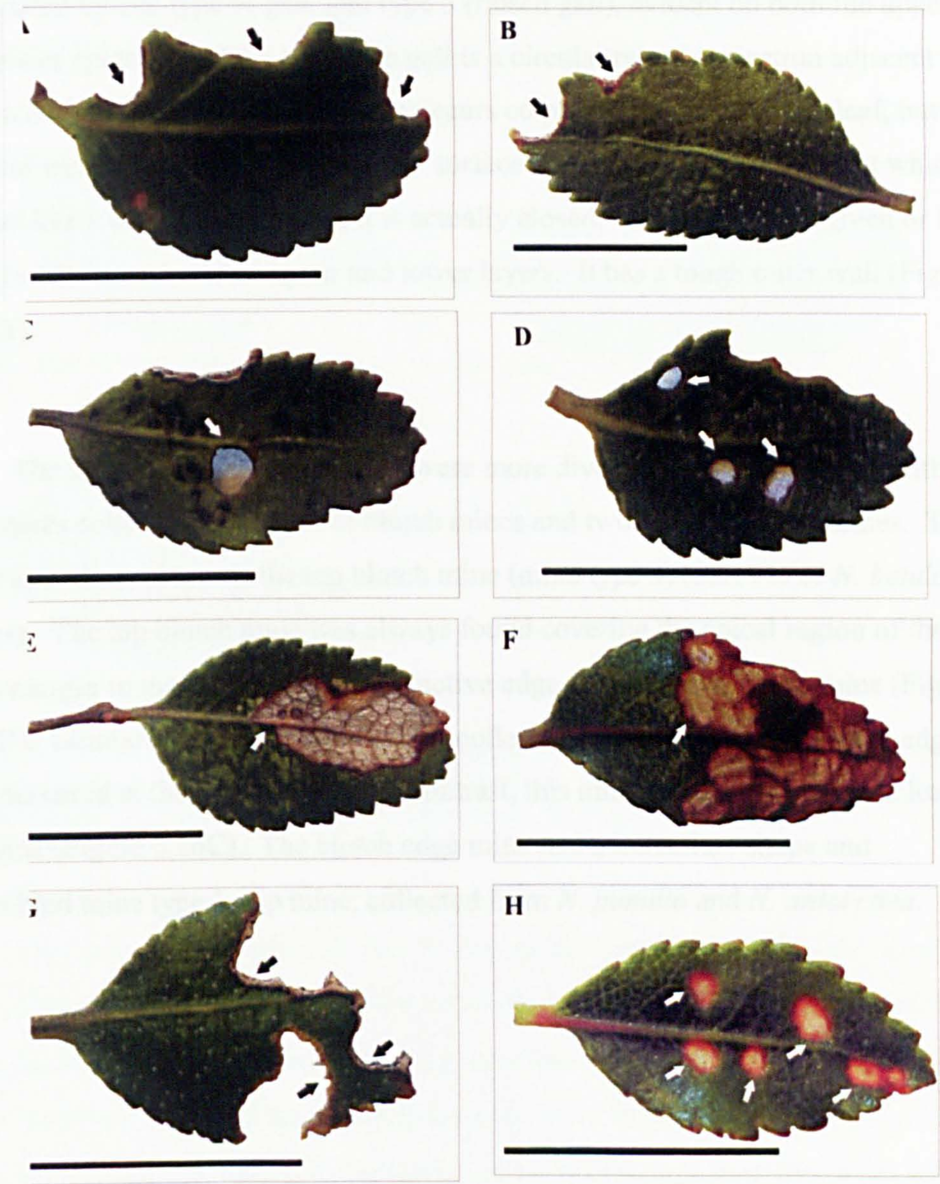


Figure 5.15. The range of insect traces collected from the evergreen Nothofagaceae, *N. betuloides*. A) Continuous marginal general leaf chewing. B) Discontinuous general leaf chewing. C) Single large non-marginal general leaf chewing. D) Multiple small non-marginal general leaf chewing. E) Large patch of skeleton feeding on upper epidermis only. F) Large patch of skeleton feeding on both upper and lower epidermal layers of the leaf. G) Distinctive defence ridge around the edge of the continuous marginal leaf chewing shown as yellowish white. H) Distinctive orange defence reaction around multiple areas of skeleton feeding. Scale bar 1cm. Arrows indicate traces.

Leaf mines and leaf galls were found on the 253 leaves collected (18.57% of traces were mines and 15.02% were galls). No insects were reared from any of the mines or galls so descriptions are based on the trace type alone. Leaf galls were dominated by one type of gall, gall type 5 (raised gall), evident on both the upper and lower epidermis of the leaf. The gall is a circular raised projection adjacent to the secondary veins. This gall type is occurs commonly as one gall per leaf, but two per leaf were also found. On the upper surface the gall had a central indent which looked like a hole in the gall, but it is actually closed. The gall is light green or light grey in colour on both the upper and lower layers. It has a tough outer wall (Figure 5.16A).

The leaf mines on *N. betuloides* were more diverse than the leaf galls, with four types collected; two types of blotch mines and two types of linear mines. The most abundant mine was the top blotch mine (mine type 9) (53.19% of *N. betuloides* mines). The top blotch mine was always found covering the apical region of the leaf from margin to margin, leaving a distinctive edge at the bottom of the mine (Figure 5.16B). Similarly, the other blotch mine collected (mine type 10) the blotch edge, also occurred at the leaf apex, but, in contrast, this mine was restricted to the leaf margins (Figure 5.16C). The blotch edge mine had a horseshoe shape and resembled mine type 1, top mine, collected from *N. pumilio* and *N. antarctica.*

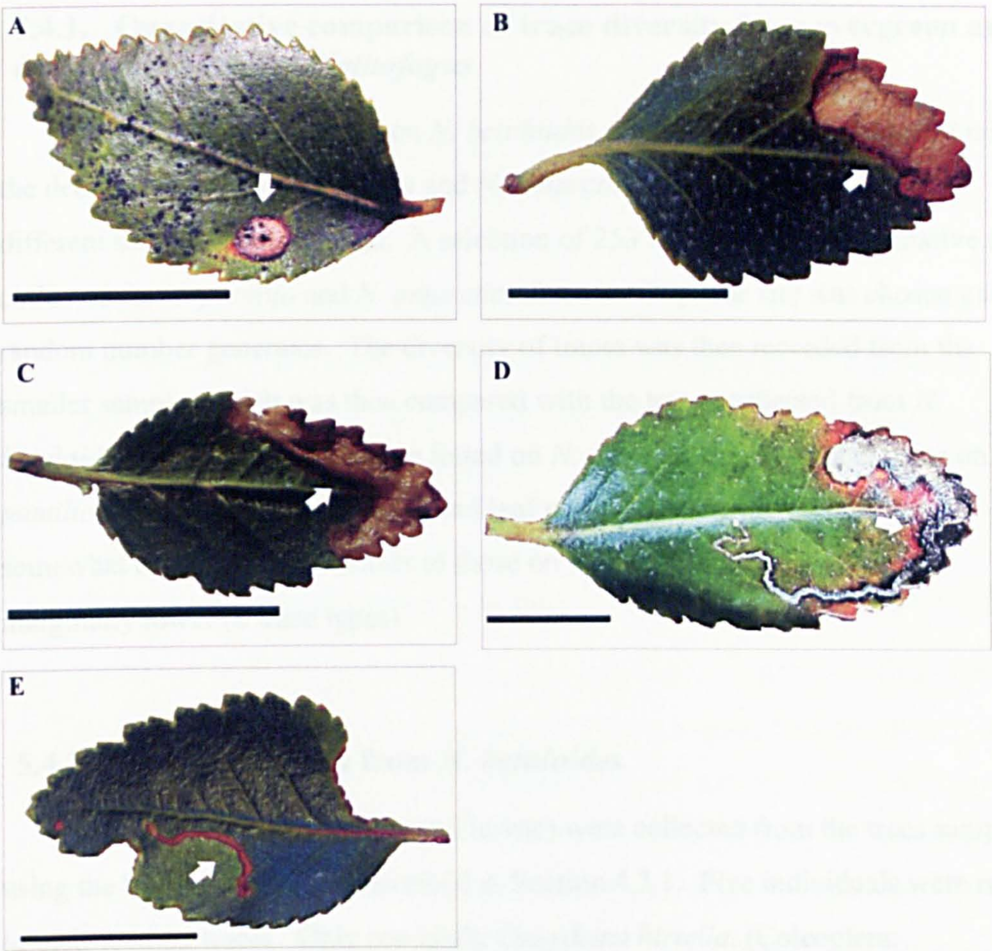


Figure 5.16. The range of leaf galls and leaf mines collected from *N. betuloides* in Puyehue. A) Gall type 5, raised gall. B) Mine type 9, top blotch mine. C) Mine type 10, blotch edge. D) Mine type 11, large serpentine. E) Mine type 12, standard serpentine. Scale bar 1cm. Arrows indicate insect traces.

The linear mines collected from *N. betuloides* were quite distinctive, although less of these types were collected than of the blotch mine type (14.9% of the mines were linear mines). Mine type 11 (large serpentine mine) occupies a large amount of the leaf surface as it is quite a long channel. The mine begins very small close to the primary vein and then, as it gradually increases in size, it migrates to the leaf margin, almost in a zigzag pattern, and continues around the edge of the leaf to the opposite side from where it started. The mine ends in a small round blotch where the larva exits the mine through a small circular flap (Figure 5.16D). The final mine, mine type 12 (small serpentine mine) is uniform in size (0.5mm wide) throughout its trace. The channel is s-shaped, curving along the leaf surface. It starts and ends in a very small blotch and has a distinct ridge on either side of the mine (Figure 5.16E).

5.4.1. Quantitative comparison of trace diversity from evergreen and deciduous species of *Nothofagus*

The insect trace diversity on *N. betuloides* cannot be directly compared with the deciduous species, *N. pumilio* and *N. antarctica*, without considering the different sample sizes obtained. A selection of 253 leaves from the qualitative data collected for *N. pumilio* and *N. antarctica* from the Puyehue site was chosen using a random number generator. The diversity of traces was then recorded from the smaller sample, which was then compared with the traces collected from *N. betuloides*. Nine trace types were found on *N. antarctica* and 12 trace types on *N. pumilio*. The number of leaf mine and leaf gall types on *N. betuloides* was somewhat comparable in number to those on the deciduous trees, although marginally lower (8 trace types).

5.4.2. Insects collected from *N. betuloides*

In total, 102 insects (adults and larvae) were collected from the trees sampled using the beating technique described in Section 4.3.1. Five individuals were related to their feeding traces. Only one adult, *Dasydema hirtella*, (Coleoptera: Curculionoidea: Entiminae) and four larvae were observed feeding. The larvae were all different species of Lepidoptera and two were further identified as Geometridae. Only continuous marginal general leaf chewing by live insects was observed.

5.4.3. Summary of insect traces on evergreen, *N. betuloides*

Although only one species of evergreen *Nothofagus* was sampled, the diversity of traces did not increase dramatically with each tree sampled. There are similarities between the range of traces collected from evergreen and deciduous species, however, the diversity is lower in the former. The leaf mines and leaf galls are different from those collected on the deciduous species despite the broader mine categories being the same. This indicates that a comparison with fossil traces, such as leaf mines and leaf galls, may clarify whether the fossil leaf was from an evergreen or deciduous tree species.

5.5. Summary

- The diversity of insects collected from two deciduous species of *Nothofagus* was recorded and the types of traces they create on the leaves discussed. Both the adult and larval insect specimens made a variety of general leaf chewing marks on the leaves. One trace made by a species of Alticinae was found on only one species (*N. antarctica*) and at one site (Puyehue). The majority of the insect species made similar traces. *H. aureosignatus* was site specific and only found in Nah and one insect species was specific to the more northerly sites of Nah and Tolh (*cf. P. unicor*).
- The most common species observed skeleton feeding was *cf. Varicoxa* (Coleoptera: Chrysomelidae: Alticinae). It was only collected from Puyehue.
- Of the leaf galls, 4 types occurred most commonly. Gall type 1 (ball gall) was more commonly found on *N. pumilio* and more abundant in the northern sites. Gall type 3 (small gall) was more abundant in the southern sites on *N. pumilio*. Gall type 4 (apple gall) was only found on *N. antarctica*. Gall types 2 and 3 highlight the complexity in forest community structure because both gall mites and leaf gall nematodes were identified as the gall makers. There is also evidence of complex interactions, such as the leaf galler-parasitoid-inquiline interaction seen in gall type 1.
- Eight types of leaf mine were most commonly found. Mine type 1 (top mine) is limited to the southern site, RNMag. In general there was higher leaf mine diversity in the southern sites than in the northern sites. The leaf mining insect fauna includes species of Lepidoptera, Hymenoptera and Coleoptera. Their associated parasitoid fauna was also reared.
- The range of traces collected from the evergreen *Nothofagus* species, *N. betuloides*, highlights the differences between evergreen and deciduous species, specifically the leaf mines and leaf galls. The different traces may be used to determine whether a fossil leaf was from an evergreen or deciduous species.

- Similarities exist in general leaf chewing and skeleton feeding in both evergreen and deciduous species of *Nothofagus*, but the plant defence reaction is more distinctive in the evergreen species. There was little difference in the diversity of leaf mines and galls between deciduous and evergreen species, although the evergreen species had a marginally smaller range of types.

Chapter 6. Analysis of the factors affecting insect herbivory in Chile

6.1. Introduction

Plant-insect interactions are influenced by many factors, both biotic and abiotic, which can affect the diversity and the intensity of the interactions in any one environment. In the case of insect associations with trees, the biotic factors may include leaf phenology, tree age, insect reproduction and development, and the parasitoids and predators within the system (Lawton, 1983; Leather, 1986; Basset, 1992; Denno *et al.*, 1995; Riihimäki *et al.*, 2006; Brehm, 2007; Forkner *et al.*, 2008). The abiotic factors that could affect the insect associations with plants include climatic components such as temperature, day length, wind speed, moisture and the latitude and altitude at which the plants survive. All abiotic factors can affect interactions at both a large and fine scale. An example is the creation of microclimates within a tree, which in turn are affected by the height of the leaf, the orientation of the leaves on a tree, as well as the forest structure (Strong *et al.*, 1984).

This complex array of factors may influence different types of plant-insect interactions to various extents. For example, leaf mining and leaf galling insects are buffered against climatic extremes more than are the external foliage feeders; biotic factors have a greater effect on their distribution than abiotic factors (Brown *et al.*, 1997; Goolsby *et al.*, 2000; Riley *et al.*, 2001; Pincebourde, 2007). Factors may also interact differently depending on an ecosystem, whether tropical or temperate, and on the species composition of the system, in both plants and insects. Therefore, it is difficult to predict the factors that may have the most important effect on both the insect diversity and the intensity of damage in the forests studied in Chile, and even more challenging to interpret how such factors would have affected the Palaeogene forests in Antarctica.

The data collected from Chilean forests, the nearest modern analogue to the Palaeogene forests in Antarctica, are thus examined to understand such complexities. If the factors studied influence the presence of damaged leaves or the number of leaf mines and leaf galls in the modern Chilean forests, then they may also have had an effect on the abundance of insect traces in Antarctica. This could enable the original position of the trace within the tree to be determined, especially for the leaf mine and leaf gall traces. For example, if a modern trace type was only found at the top of the trees then the comparable fossil trace may also have only been found at the tops of the trees in the fossil forest. The distribution of trace types within the fossil trees would otherwise be unknown.

The factors that are related to insect feeding examined in this chapter include: height within the tree, orientation on the tree, season of collection, the latitude at which the tree grew, the altitude of the tree, age of the leaf, canopy differences and differences between plant species. The effect of factors on the number of insect damaged leaves, on the number of leaf mines and on the number of leaf galls is examined using statistical analyses.

6.1.1. Data Analysis

All analyses were carried out using the statistics programme R 2.6.1 (R: A Language and Environment for Statistical Computing, The R Development Core Team, 2008). An explanation of the statistical analyses performed and how they are used to analyse the data is given in Appendix VI.

6.2. Do different species of *Nothofagus* have different numbers of insect-damaged leaves?

The presence or absence of insect-damaged leaves for both species of *Nothofagus* was highly skewed (Figure 6.1). Due to the variation in the total number of leaves collected within the samples, as mentioned previously in Chapter 4, proportions of insect-damaged leaves were calculated for a valid comparison to be made. In the programme R proportion data can also be examined by creating a weighted binomial response variable, which in this case is a two column matrix consisting of the number of leaves that were damaged and the number of leaves that were not damaged within each sample. This allows the analysis to take into account that the overall sample size can influence data interpretation. For example, a measurement of 50% of leaves being damaged from a total of 10 leaves and a measurement of 50% of leaves being damage from a total of 100 leaves is very different and the same conclusions should not be made (Crawley, 2002).

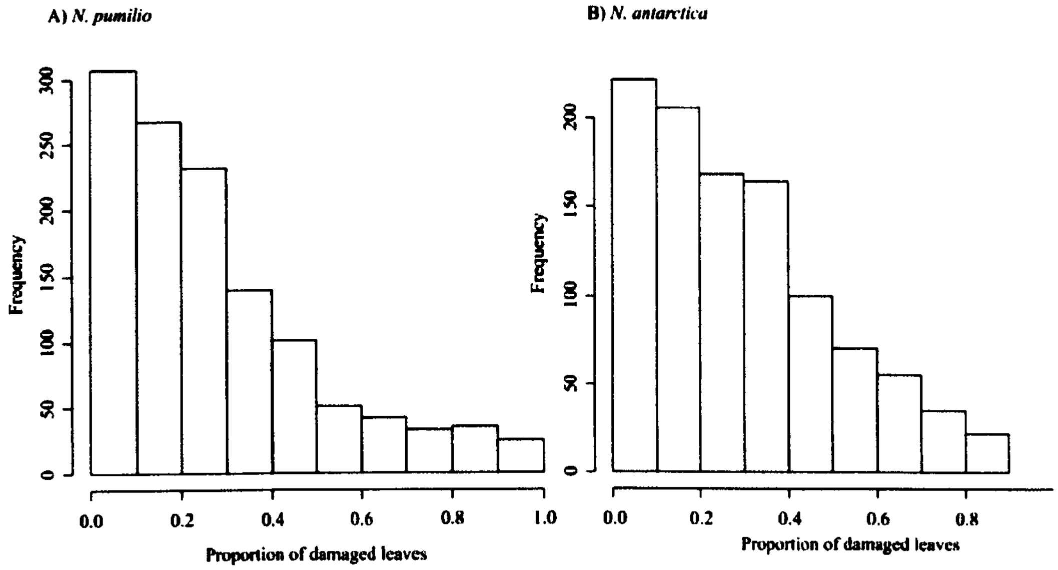


Figure 6.1. The proportion of insect-damaged leaves per sample for A) *N. pumilio* and B) *N. antarctica*. Both datasets are skewed to the left.

The difference in the presence or absence of insect-damaged leaves between *N. pumilio* and *N. antarctica* was examined for each site using linear mixed-effects models and generalized linear mixed-effects models. Linear mixed-effects models were used when the arcsin square root transformation of the proportion of damaged leaves data created a normal distribution of the dataset. Otherwise, generalized linear mixed-effects models were used with a weighted binomial response variable of the number of damaged leaves and the number of leaves that were not damaged as mentioned above. Only RNMag could not be included in the analysis as no quantitative data was collected for *N. antarctica*. Only the data from Isla Nav were analysed using generalized linear mixed-effects model. All models included a two level factor, tree species, as the fixed effect. The random effects were tree number and height within tree to account for several samples being collected from the same tree and the same height within each tree.

There was a significant difference in the proportion of insect-damaged leaves on *N.pumilio* and *N. antarctica* for most sites, except for Nah (Table 6.1). In the northern sites of Tolh and Puy, *N. antarctica* had a significantly greater proportion of damaged leaves than *N. pumilio*, but in the southern sites (Torres and Isla Nav) *N. pumilio* had the greater proportion of damaged leaves. Due to this difference, the effect of external factors on the occurrence of insect damaged leaves was analysed separately for each species in the following sections.

Table 6.1. The back transformed proportion data generated from generalized linear mixed-effects models and linear mixed-effects models (Isla Nav data only) to test for differences in the presence of insect-damaged leaves between *N. antarctica* and *N. pumilio*. Significant differences at the 95% confidence level are in bold. S.E. = standard error and CI = confidence interval.

	N. antarctica		*N. pumilio*	
Site	Mean % damaged leaves	S.E / 95% CI	Mean % damaged leaves	S.E / 95% CI
Nah	39.4	0.3	38.0	0.5
Tolh	**49.2**	**0.4**	**30.7**	**0.7**
Puy	**18.0**	**0.07**	**22.0**	**0.2**
Torres	**36.0**	**0.3**	**63.7**	**0.8**
Isla Nav	**5.0**	**2.0-12.0**	**22.0**	**8-48.0**

6.3. What factors affect the presence or absence of damaged leaves?

The effect of abiotic factors on the presence of insect-damaged leaves, such as height and orientation within a tree, as well as latitudinal distribution of the trees, was examined using one model. Initially, this was carried out by creating a maximal model which contained all sites (a 6 level variable), height as a 5 level variable and orientation as a 2 level variable. Model simplification was performed on both variables and variable levels. If two or more levels of a variable were not significantly different they were combined. For example, if height 1 and height 2 were not different, they would be combined to form a new level "low". The minimal adequate model was obtained and the results are shown (Section 6.3.1. to 6.3.3.). Sites were also analysed individually to understand the variation at each site in greater detail. The type of model used, the variables, the number of factor levels of each variable and the variables which show significant differences are summarised in Tables 6.2 and 6.7 for all minimal adequate models investigating the presence of damaged leaves.

6.3.1. Site comparison of presence or absence of damaged leaves

The presence or absence of insect-damaged leaves was highly skewed (Figure 6.1) and therefore the proportion data were examined by creating a weighted binomial response variable, which in this case is a two column matrix consisting of the number of leaves that were damaged and the number of leaves that were not damaged within each sample. Table 6.2 shows the different models used for this analysis and the factors that show significant difference in the presence or absence of damaged leaves between each factor level.

Table 6.2. Summary of the different minimal adequate models used to interpret the number of damaged leaves collected across the sites visited in Chile. All are generalized linear mixed-effects models (lmer). Continued on p141.

Model No.	**Plant Species**	**Response variable**	**Fixed Effects (factor levels)**	**Random effects**	**Significant results**
1	*N. pumilio*	Binomial with number of leaves damaged and number not damaged	Site (6) Height (5) Orientation (2)	Individual tree, height	Site -Torres greater than Isla Nav
2	*N. pumilio*	Binomial with number of leaves damaged and number not damaged	Site (2) Height (5) Orientation (2)	Individual tree, height	Orientation Height - 4m and 1m different

Model No.	Plant Species	Response variable	Fixed Effects (factor levels)	Random effects	Significant results
3	*N. antarctica*	Binomial with number of leaves damaged and number not damaged	Site (5) Height (7) Orientation (2)	Individual tree, height	Site - Nah, Tolh and Torres greater than Isla Nav
4	*N. antarctica*	Binomial with number of leaves damaged and number not damaged	Site (2) Height (7) Orientation (2)	Individual tree, height	No significant effects

6.3.1.1. Model results using *Nothofagus pumilio* data

a) Model 1 - All sites included

A generalized linear mixed-effects model was created with a weighted binomial response variable for the number of damaged and number of undamaged leaves. The fixed effect variables in the model were site, height and orientation; site was characterised as a six level factor that includes the three site visits to Puy as one, plus Tolh, Nah, Torres, RNMag and Isla Nav; height was a five level factor and orientation was a two level factor. The random effects in the model were tree number and height because several samples were included from the same tree and at the same height within the tree. A quasibinomial error structure was applied and, therefore a logit link function was used for the response variable transformation. Quasibinomial error is used in the model when a binomial error distribution does not explain the data due to overdispersion, which means that there is extra unexplained variation in the proportion of damaged leaves.

The results of the analysis with the minimal adequate model indicate that there are significant differences in the parameter estimates for orientation within the tree, height at 4m and also Torres (Table 6.3). When the standard errors for the model are taken into account however, the only significant difference is between the Torres and Isla Nav sites. Torres has a significantly greater proportion of chewed leaves (70.6% leaves damaged, 95% confidence interval (CI) = 11.7% - 34.0%) than Isla Nav (21.4% leaves damaged, 95% CI = 31.3 - 91.7%) (Figure 6.2). The overall variation in the data is marginally explained by random effects because variation between individual trees accounted for only 22.8%. A large component of the variation is due to the unexplained differences between the data points and the mean of each group (residual variance 62.8%). This means that a factor not included in the analysis could explain the variation in the data around the mean value. Therefore, the proportion of damaged leaves on *N. pumilio* is significantly greater at Torres, a site in the south, although the lowest level of damage is at RNMag also in the south.

Table 6.3. Minimal adequate model for the number of damaged leaves on *N. pumilio* trees using a generalized linear mixed-effects model. Overall sample size of leaves n = 1233. Height levels within individual tree n = 206. Number of individual trees n = 47. 95% confidence intervals generated using Markov Chain Monte Carlo (MCMC) sampling methods with a runtime of 10,000. Significant effects at alpha level of 0.05 are in bold with *. Precise probability values were not calculated.

	Random Effects			
	SD			
Height within individual tree	0.866			
Individual tree	1.092			
Residual	1.809			
	Fixed Effects			
	Estimate	SE	t-value	95% CI
Intercept	1.30	0.56	2.32	0.64 - 2.03
Height 2m	0.21	0.23	0.92	-0.05 - 0.48
Height 3m	0.26	0.22	1.17	0.012 - 0.53
Height 4m	0.46	0.22	**2.05***	0.212 - 0.70
Height 5m	0.36	0.23	1.56	0.12 - 0.61
Orient South	0.14	0.04	**3.49***	0.10 - 0.19
Nah	-1.18	0.72	-1.65	-2.05 - 0.41
Puy	-0.14	0.60	-0.23	-0.85 - 0.65
RNMag	0.81	0.72	1.13	-0.04 - 1.68
Tolh	-0.86	0.73	-1.18	-1.71 - -0.02
Torres	-2.18	0.72	**-3.03***	-3.04 - -1.24

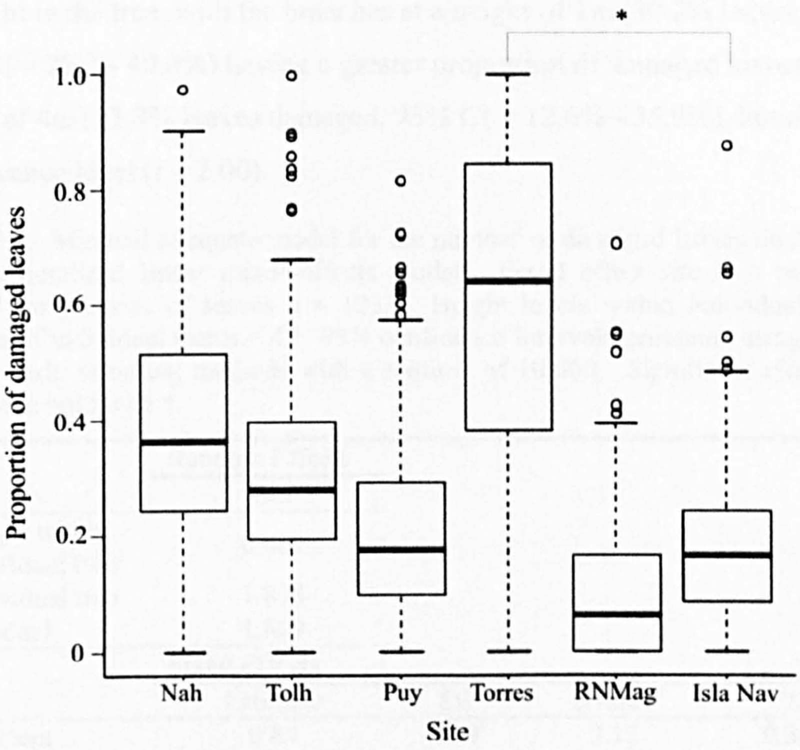


Figure 6.2. Mean proportion of damaged leaves on *N. pumilio* collected at each site, with the most northerly site on the left-hand side of the x-axis and the most southerly on the right-hand side. Outliers are represented as circles and significant differences are highlighted with a *.

b) Model 2 - Northern vs. Southern Sites

The data were also analysed to examine the relationship between the proportion of damaged leaves and the latitudinal gradient from Nahuelbuta (Nah) in the north to Isla Navarino (Isla Nav) in the south. The same variables as those in model 1 were used, but the fixed effect of site was changed to a two level factor consisting of northern and southern sites. Northern sites included Nah, Tolh and all Puy visits and the southern sites included Torres, RNMag and Isla Nav. The results are presented in Table 6.4. There was no difference in the number of damaged leaves on *N. pumilio* in the northern and southern sites, but there was a difference in the number of damaged leaves between the north-facing and south-facing branches within a tree (Table 6.4). There was a greater proportion of damaged leaves in the north-facing sections of the tree (30.2% leaves damaged, 95% C.I = 22.2 - 40.0%) than in the south-facing sections (27.0% leaves damaged, 95% CI = 19.1% - 38.6%). The north-facing part is the warmer part of a tree in the Southern Hemisphere. There was also a significant difference in the number of damaged leaves in relation to height in the tree, with the branches at a height of 1m (30.2% leaves damaged, 95% CI = 22.2 - 40.0%) having a greater proportion of damaged leaves than at a height of 4m (21.7% leaves damaged, 95% CI = 12.6% - 35.9%), but only at a low significance level (*t* = 2.00).

Table 6.4. Minimal adequate model for the number of damaged leaves on *N. pumilio* trees using generalized linear mixed-effects model. Fixed effect site is a two factor level. Overall sample size of leaves n = 1233. Height levels within individual tree n = 206. Number of individual tree n = 47. 95% confidence intervals generated using Markov Chain Monte Carlo sampling methods with a runtime of 10,000. Significant effects at alpha of 0.05 are in bold with *.

	Random Effects			
	SD			
Height within individual tree	0.867			
Individual tree	1.898			
Residual	1.809			
	Fixed Effects			
	Estimate	SE	t-value	95% CI
Intercept	0.84	0.39	2.12	0.38 - 1.26
Height 2m	0.21	0.23	0.90	-0.04 - 0.47
Height 3m	0.25	0.22	1.11	0.02 - 0.50
Height 4m	0.45	0.22	**2.00***	0.20 - 0.680
Height 5m	0.35	0.22	1.51	0.08 - 0.580
Orient South	0.14	0.04	**3.48***	0.10 - 0.19
Southern Sites	-0.02	0.59	-0.04	-0.68 - 0.69

6.3.1.2. Model results using *Nothofagus antarctica* data

a) Model 3 - All sites included

A generalized linear mixed-effects model was used to examine the data for *N. antarctica* with a weighted binomial response variable of number of damaged leaves and the total number of leaves collected per sample. The minimum adequate model included the fixed effects (explanatory variables) of height and orientation with an interaction term. Height is a seven level factor between 0.5m and 5m measured at regular intervals and orientation is a two level factor of north and south. Site is a fixed effect consisting of five levels (no quantitative sampling carried out at the RNMag site). The random effects are the individual trees sampled and height within individual trees. The model was fitted with a quasibinomial error structure and a logit link function (Table 6.2).

There was no relationship between height or orientation and the number of damaged leaves for *N. antarctica*, but there were differences in the number of damaged leaves per site (Table 6.5). Tolh, Torres and Nah had a significantly higher average number of damaged leaves (Table 6.6) than Isla Nav (Figure 6.3). Once again the variation between individual trees accounts for variation in the proportion of damaged leaves (34.86%), although a large proportion of the variation is unexplained by the random factors included (57.73%). Although the interaction between height and orientation is not significant and neither is the orientation variable, removing them from the model increased the Akaike's Information Criteria (AIC) value (Model with interaction: AIC = 4083.7, model with site alone AIC = 4185.9). The lower the AIC value, the better the model is at explaining the data.

Table 6.5. Minimal adequate model for the proportion of damaged leaves on *N. antarctica* trees using a generalized linear mixed-effects model. Overall sample size of leaves n = 1048. Height levels within individual tree n = 175. Number of individual tree n = 42. 95% confidence intervals generated using Markov Chain Monte Carlo sampling methods with a runtime of 10,000. Significant effects at alpha of 0.05 are in bold with *.

	Random Effects			
	SD			
Height within individual trees	0.655			
Individual trees	1.420			
Residual	1.828			
	Fixed Effects			
	Estimate	SE	t-value	95% CI
Intercept	2.48	0.70	3.56	1.70- 3.31
Height 1m	0.13	0.39	0.32	-0.30- 0.55
Height 1.5m	-0.02	0.50	-0.04	-0.54- 0.57
Height 2m	0.17	0.40	0.44	-0.27- 0.63
Height 3m	0.27	0.41	0.66	-0.17- 0.73
Height 4m	0.55	0.41	1.35	0.07- 0.99
Height 5m	0.34	0.42	0.82	-0.12- 0.82
Orient South	0.32	0.27	1.19	0.05- 0.62
Site Nah	-2.45	0.87	**-2.83***	-3.48- -1.48
Site Puy	-1.22	0.71	-1.71	-2.09- -0.43
Site Tolh	-2.76	0.87	**-3.20***	-3.75- -1.73
Site Torres	-2.22	0.87	**-2.56***	-3.29- -1.31
1m: Orient South	-0.46	0.29	-1.59	-0.75- -0.15
1.5m: Orient South	0.17	0.37	0.47	-0.24- 0.56
2m: Orient South	-0.13	0.28	-0.47	-0.46- 0.14
3m: Orient South	-0.14	0.28	-0.49	-0.41- 0.18
4m: Orient South	-0.46	0.28	-1.61	-0.77- -0.16
5m: Orient South	-0.04	0.29	-0.14	-0.38- 0.24

Table 6.6. Back transformed estimates from the generalized linear mixed-effects model for the proportion of damaged leaves on *N. antarctica* trees. Values significantly greater than the proportion of damaged leaves from Isla Nav are in bold with *.

Site	Mean % damaged leaves	95% CI
Nah	49.2	**13.8 - 85.6%***
Tolh	57.0	**11.9 - 83.0%***
Puy	22.1	5.33 – 59.4%
Torres	43.4	**11.9 - 83.0***
Isla Nav	7.7	3.5 - 15.6%

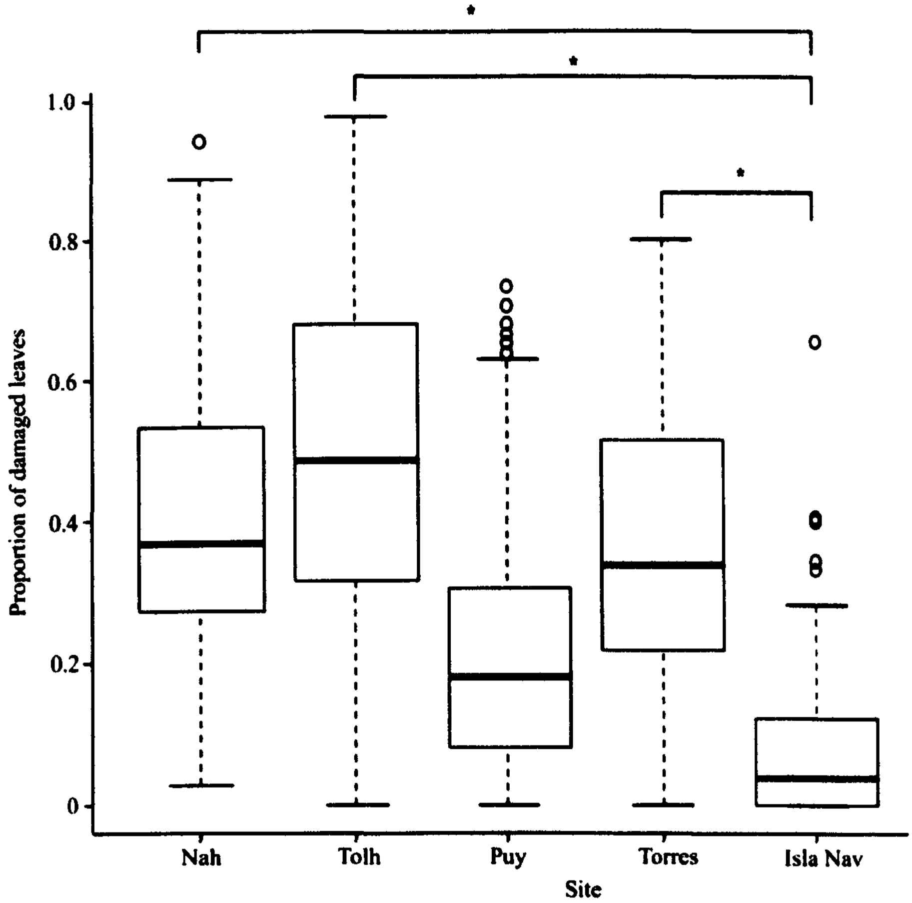


Figure 6.3. The median and inter-quartile range of the proportion of damaged leaves collected from *N. antarctica* from each site. The most northerly site is at the left side of the x-axis and the most southerly on the right. Significantly different results are highlighted with * and outliers are shown as circles.

b) Model 4 - Northern vs. Southern sites

The data for *N. antarctica* were also analysed by examining the latitudinal gradient along which the sites are located, from Nahuelbuta in the north to Isla Navarino in the south. Model 3 was applied to the data again, but the fixed effect of site was changed to a two level factor consisting of northern and southern sites (Model 4, Table 6.2). Northern sites included Nah, Tolh and all Puy sites and the southern sites included Torres and Isla Nav. There were however, no significant differences between the number of damaged leaves at any height level or orientation within the tree. The minimal adequate model remained a better fit to explain the variance in the data than the null model which has no explanatory variable incorporated (minimal adequate model AIC = 4108, null model AIC = 4184).

6.3.2. Individual site analysis of presence or absence of damaged leaves

The presence or absence of leaf damage was also analysed for each site. The overall significant effects in the generalized linear mixed-effects models for all sites together may have masked the within-site variability or a highly significant effect of a factor at one site may have influenced the overall outcome of the effect. To explore this, linear mixed effect models were applied to the data set for each site visited using transformed proportion data. This normalised the error structure and fitted the assumptions of the models applied. The standard transformation for proportion data was applied (the arcsin square root transformation). In the following sections on specific sites, only significant results are reported and summarised in Table 6.7.

Table 6.7. Summary of all minimal adequate models used in the analysis of the factors that could affect the number of damaged leaves at individual sites. All models were linear mixed-effects models (lme).

Model No.	Plant Species	Response variable	Fixed Effects (factor levels)	Random effects	Significant results
5	*N. pumilio*	Arcsine square root transformed proportions - Torres data only	Height (2) Orientation (2)	Individual trees, Height	Height and Orientation
6	*N. pumilio*	Arcsine square root transformed proportions - Isla Nav data only	Height (2)	Individual trees, Height	Height
7	*N. antarctica*	Arcsine square root transformed proportions - all Puy data	Height (4)	Individual trees, Height	Height
8	*N. antarctica*	Arcsine square root transformed proportions – Tolh data only	Height (6) Orientation (2) Interaction	Individual trees, Height	Interaction significant
9	*N. antarctica*	Arcsine square root transformed proportions - Nah data only	Orientation (2) Height (5)	Individual trees	Orientation significant

6.3.2.1. Individual site results using *Nothofagus pumilio* data

a) Model 5 – Presence or absence of damaged leaves from Torres del Paine

The minimal adequate model for Torres had the transformed proportion data as the response variable and the fixed effect variables were height and orientation. The variable height was a two level factor of "high" (2m, 3m, 4m and 5m levels combined) and 1m level. Orientation was also two levels, consisting of north and south. The random effects were individual trees and height within individual trees and the estimation method used was restricted estimate maximal likelihood (REML) (Table 6.7). The variation in the response variable can be explained by the random effects such as variation within individual trees (41.31%) and the variation between heights within individual trees (14.32%), but the most significant explanation is the variation between the individual samples and the mean number of damaged leaves irrespective of grouping (residual variation) (44.36%). There were significant effects using both height and orientation explanatory variables at Torres (Height; $F_{1,20} = 16.5$, $P = 0.0006$, orientation; $F_{1,131} = 29.1$, $P < 0.0001$). Therefore, at higher levels in the tree there were fewer damaged leaves than at the 1m level (From model: 1m = 92% leaves damaged ± 1.1SE, High = 67.04% leaves damaged ± 3.4SE). Sections in the south have a lower mean number of insect- damaged leaves (79.8% leaves damaged ± 2.0SE) than from sections in the north (Table 6.8).

Table 6.8. Minimal adequate model for Torres del Paine *N. pumilio* using a linear mixed-effects model on arcsine square root transformation of the proportion of leaves in each sample that were damaged by insects. Overall sample size of leaves n =159, height levels within individual trees n = 27, number of individual trees n = 6. Significance level for fixed effects height; $F_{1,20} = 16.530$, $P = 0.0006$, orientation; $F_{1,131} = 29.074$, $P < 0.0001$. Significant effects at alpha of 0.05 are in bold with *.

	Random Effects		
	SD	95% CI	
Height within trees	6.85	4.26 -11.02	
Individual trees	11.64	5.79- 23.38	
Residual	12.06	10.69-13.61	
	Fixed Effects		
	Estimate	SE	t-value
Intercept	73.65	6.28	11.73
Height High	-18.51	4.35	**-4.25***
Orient S	-10.38	1.93	**-5.39***

b) Model 6 - Presence or absence of damaged leaves from Isla Navarino

For examining the effect of the explanatory variables of height and orientation, on the number of insect damaged leaves on *N. pumilio* from Isla Nav, a linear mixed-effects model was created. The response variable was the transformed proportion of damaged leaves collected per sample. The minimum adequate model had the fixed effect of height with two levels, 1m and "high" (2m, 3m, 4m and 5m levels combined) and the random effects of individual trees and height within individual trees (Table 6.7). There was a significant height effect with the damage levels greater at 1m in the tree (37.4% leaves damaged ± 0.5SE) than at higher levels (13.4% leaves damaged ± 1.8SE) (Table 6.9).

Table 6.9. Minimal adequate model for *N. pumilio* data from Isla Nav: linear mixed-effects model with arcsine square root transformation performed on the proportion of damaged leaves in each sample. Overall sample size of leaves n = 126, height levels within individual trees n = 21, number of individual trees = 5. Significance level of height fixed effect ($F_{1,15}$ = 167.66, *P* = 0.0008). Significant effects at alpha of 0.05 are in bold with *.

	Random Effects			
	SD	95% CI		
Individual trees	3.20	0.70 - 14.54		
Height within trees	4.82	2.68 - 8.70		
Residual (within group)	9.09	7.94 - 10.41		
	Fixed Effects			
	Estimate	SE	d.f.	t-value
Intercept	37.72	3.89	105	9.71
Height high	-16.22	3.86	15	**-4.20***

6.3.2.2. Individual site results using *Nothofagus antarctica* data

a) Model 7 - Presence or absence of damaged leaves from Puyehue

The response variable used for the linear mixed-effects model was the arcsin square root transformed proportion of damaged leaves per sample per site. The minimal adequate model for examination of all samples from Puyehue included a fixed effect of height, plus the random effect of individual trees and height within individual trees. The fixed effect height was a four level factor with “low” (0.5m, 1m, 1.5m and 2m levels combined), 3m, 4m and 5m levels (Table 6.7). There was a significant effect of height ($F_{3,59}$ = 6.586, *P* = 0.0006) on the occurrence of damaged leaves (Table 6.10) with the mean damage level at the lower height levels being greater that at the 3m, 4m and 5m height levels within the tree (Table 6.11). The variance in the proportion of damaged leaves can be explained largely by the variance between individual trees sampled (52.66%).

Table 6.10. Minimal adequate model for *N. antarctica* data from Puyehue: linear mixed-effects model with arcsine square root transformation on the proportion of damaged leaves per sample. Overall ample size of leaves n = 480, height levels within individual trees n =18, number of individual trees n = 80. Significance level of the height fixed effect $F_{3,59} = 6.586$, $P = 0.0006$). Significant effects at alpha of 0.05 are in bold with *.

	Random Effects			
	SD	95% CI		
Individual trees	9.50	6.63 - 13.62		
Height within trees	3.14	2.09 - 4.72		
Residual	8.44	7.88 - 9.05		
	Fixed Effects			
	Estimate	SE	d.f.	t-value
Intercept	27.44	2.38	400	11.53
Height 3m	-3.36	1.51	59	**2.22***
Height 4m	-3.81	1.51	59	**-2.52***
Height 5m	-6.91	1.61	59	**-4.285***

Table 6.11. Back transformed estimates from the linear mixed-effects model applied to the proportion of damaged leaves on *N. antarctica* from Puyehue. The "low" height category is a combination of the data from 0.5m, 1m, 1.5m and 2m levels. Significantly greater proportion of damaged leaves than any other height level at the 0.05 alpha level is in bold with *.

Height within tree	Mean % of damaged leaves	SE
"low"	**21.0**	**0.2%***
3m	16.7	0.5%
4m	16.0	0.5%
5m	12.3	0.5%

b) Model 8 - Presence or absence of damaged leaves from Tolhuaca

The minimal adequate model for Tolhuaca included a two level factor of orientation (north and south) and the six level factor height (1m, 1.5m, 2m, 3m, 4m and 5m) (Table 6.7). The response variable was the transformed proportion of damaged leaves. The random effects in the model that could explain the changes in the variance within the response variable were the variation between individual trees (58.35%) and, marginally, the variation between different height levels within individual trees (3.39%). The interaction between height and orientation was the only significant factor (Likelihood-ratio = 22.46, $P = 0.00045$, F-test: $F_{5,157} = 4.39$, $P = 0.0009$). Therefore, the difference between the proportion of damaged leaves at each height level is only different when the orientation is also considered. The level of damaged leaves is higher in the north-facing sections of the tree than the south-facing sections when at a height level of 2m and 5m, but lower than in the south at 1m, 3m, and 4m (Figure 6.4).

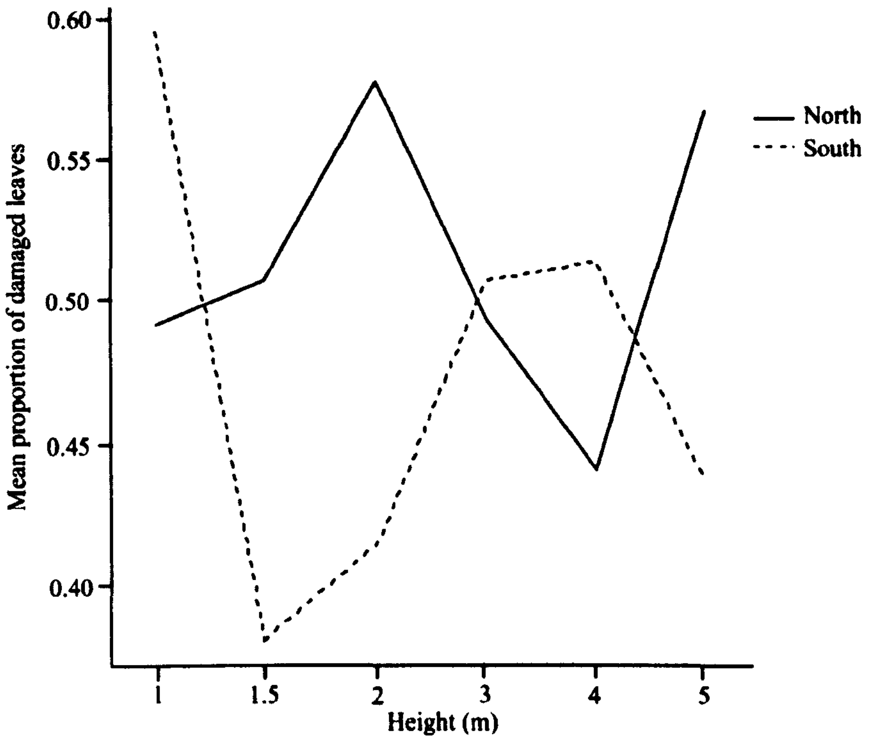


Figure 6.4. The mean proportion of damaged leaves counted on *N. antarctica* at Tolh at each height level and at the two orientations using the output of a linear mixed-effects model. This highlights the significance of the interaction effect in the minimal adequate model. The height level is categorical and the model extrapolates between the values at each level.

c) Model 9 – Presence or absence of damaged leaves from Nahuelbuta

The linear mixed-effects model for the data from Nahuelbuta had the explanatory variables (fixed effects) of orientation (two levels, north and south) and height, a five level factor with 1m, 2m, 3m, 4m and 5m levels (Table 6.7). Only the variance effect caused by individual trees was included as the random effect as there was no significant effect on the deviance of the model when height was removed as a random effect (Likelihood-ratio = $1.90\text{x}10^{-07}$, $P = 0.999$). For the explanatory variables there was a significant effect of orientation on the number of damaged leaves (Likelihood-ratio = 4.419, $P = 0.036$), with a greater proportion of damaged leaves in the north-facing branches than in the south-facing branches.

6.3.3. Summary of the presence/absence of damaged leaves

The level of insect damage present within a forest, whether focused within individual trees or between different sites, can be influenced by environmental factors. The effect of factors such as height and orientation is however, not straightforward because differences in damage levels can also occur due to both site variation and plant species. The results presented here indicate that variation in leaf damage on both species cannot be explained by latitudinal differences alone, implying that other factors have an effect. There was evidence that data from some sites may be influencing the significance level of the factors affecting the proportion of leaf damage. For example, for all sites, except that at Torres, the levels of leaf damage in *N. pumilio* were not related to the orientation within the trees. When the data from all of the sites were analysed together however, orientation was found to be a significant variable. The height and orientation of the leaves had a significant effect on the proportion of damage on *N. pumilio* leaves in the southern sites, Torres and Isla Nav (Section 6.3.1.1). For *N. antarctica*, the effects on the proportions of leaf damage were only significant in the more northerly sites of Puy, Tolh and Nah (Section 6.3.1.2). A highly significant difference in the number of damaged leaves related to a change in height within a tree was only apparent when sites were examined separately for both *Nothofagus* species. This suggests that the presence of damaged leaves at any particular part of a tree, whether the height or orientation within a tree, is both species and site dependent.

6.4. Does intensity of leaf damage change with latitude?

External factors may not only affect the presence of damaged leaves, but also the intensity of the damage. Therefore, the intensity of leaf damage was analysed to determine if there were differences with latitudinal variation. All sites were also examined separately due to the different effects of the factors (height and orientation within a tree and latitude) in Section 6.3 on the presence of damaged leaves. The data collected for each species were also analysed separately using a generalized linear mixed-effects model to compare the number of leaves within each damage category (the measure of intensity). The explanatory variables used were height and orientation and the minimal adequate models for each site had the same fixed effects. Damage category was the only fixed effect variable and had 4 levels: 0-25%, 26-50%, 51-75% and 76-100% of the leaf area damaged. To ensure that the counts for each damage category were grouped within the model, a sample identification number was included as a random effect. The response variable for all models was a weighted binomial consisting of the number of damaged leaves within each category and the total number of leaves damaged per sample. Due to a non-normal distribution, a quasibinomial error structure was defined to avoid overdispersion and the model was fitted using the Laplace approximation.

All models, for both species and all sites, indicated a significant difference in the number of leaves in each category, particularly between the lowest damage category of 0-25% and the higher damage categories (Figure 6.5). Ninety-five percent confidence intervals were generated for the model estimates using a Markov Chain Monte Carlo (MCMC) method with a runtime of 10,000. An example of the model and MCMC output for the data from Torres for both *N. pumilio* (Table 6.12) and *N. antarctica* (Table 6.13) indicates that the models accurately describe the real data as shown by the small confidence intervals. The lowest level of damage, 0-25% of the leaf area damaged, had a significantly greater proportion of damaged leaves than the other categories for both species of *Nothofagus* (Table 6.14).

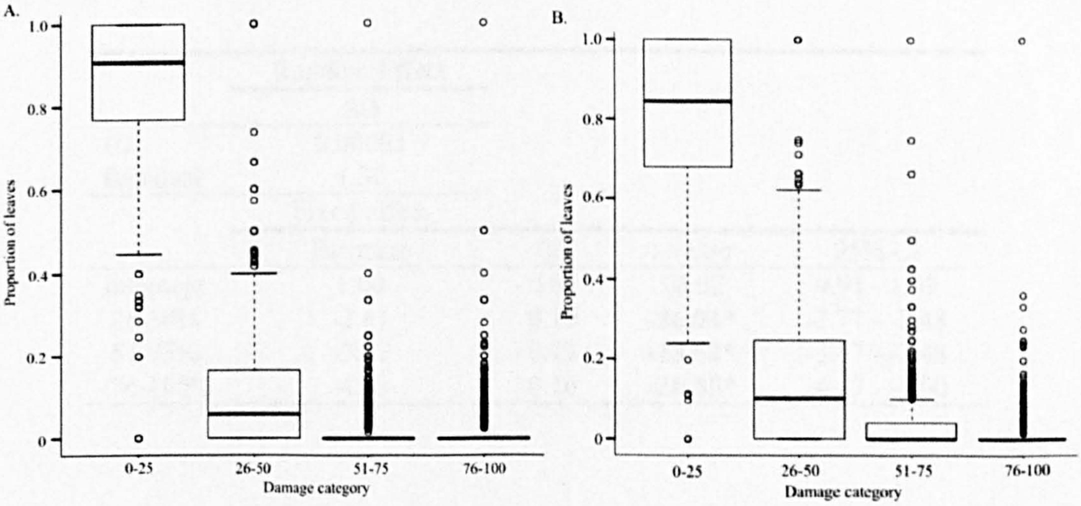


Figure 6.5. Box plot of the proportion of damaged leaves within each damage category level for A) *N. pumilio* and B) *N. antarctica* using a compilation of data from all sites.

Table 6.12. The minimal adequate model for examining the intensity of damage on *N. pumilio* leaves from Torres. The model has a weighted binomial response variable of damaged leaves in each category and total leaves damaged per sample. The fixed effect is damage category, a four level factor including 0-25%, 26-50%, 51-75%, and 76-100% of the leaf area damaged. The random effect is sample identification number (n = 158). The 95% confidence intervals were generated using Markov Chain Monte Carlo sampling with a run time of 10,000. Significant effects at alpha of 0.05 are in bold with *.

	Random Effect			
	SD			
ID	0.00004			
Residual	1.66			
	Fixed effect			
	Estimate	SE	t-value	95% CI
Intercept	1.37	0.07	19.36	1.28- 1.45
26-50%	-3.29	0.11	**-29.77***	-3.42 - -3.16
51-75%	-4.54	0.16	**-28.20***	-4.74- -4.37
76-100%	-4.65	0.17	**-27.74***	-4.86 - -9.69

Table 6.13. The minimal adequate model for examining the intensity of damage on *N. antarctica* leaves from Torres. The model has a weighted binomial response variable of damaged leaves in each category and total leaves damaged per sample. The fixed effect is damage category, a four level factor including 0-25%, 26-50%, 51-75% and 76-100% of the leaf area damaged. The random effect is sample identification number (n = 149) and the 95% confidence intervals were generated using Markov Chain Monte Carlo sampling with a run time of 10,000. Significant effects at alpha of 0.05 are in bold with *.

	Random Effect			
	SD			
ID	0.00003			
Residual	1.30			
	Fixed effect			
	Estimate	SE	t-value	95% CI
Intercept	1.00	0.06	16.02	0.91 - 1.09
26-50%	-2.61	0.10	**-26.91***	-2.77 - -2.48
51-75%	-3.67	0.13	**-28.52***	-3.87 - -3.48
76-100%	-4.23	0.16	**-26.85***	-4.47 - -4.00

Table 6.14. Back transformed estimates from a generalized linear mixed-effects model on the proportion of leaves within each damage category, % of leaf are damaged, for both *N. pumilio* and *N. antarctica*. Damage categories significantly greater than the others at an alpha of 0.05 are highlighted in bold.

	N. pumilio		*N. antarctica*	
Damage Category	Mean % damaged leaves	95% CI	Mean % damaged leaves	95% CI
0 - 25%	**79.7**	**78.2 - 81.0%**	**73.1**	**71.2 - 74.9%**
26 - 50%	12.7	10.5 - 15.3%	16.6	13.5 - 20.0%
51-75%	4.0	3.0 - 5.1%	6.5	4.9 - 8.5%
76 - 100%	3.6	0.02 – 2.7%	3.8	2.7 - 5.2%

6.4.1. Summary

The data collection and analysis indicates that the majority of leaves that were damaged had only a small amount of damage on each leaf. The proportion of damaged leaves decreased as the area of damage per leaf increases. This trend was observed for both *N. pumilio* and *N. antarctica* and at all sites. The intensity of leaf damage did not significantly differ with change in latitude.

6.5. Did the proportion of leaf damage vary with height in the tree canopy?

The statistical analysis of the difference in the number of damaged leaves on *N. pumilio* in canopy levels was carried out to determine whether leaf damage varied at different heights in the canopy. The linear mixed effect models used the arcsin square root transformed proportion of leaves damaged per sample as the response variable (to make the data normally distributed). The fixed effect was canopy section with three factor levels (top, middle and bottom) and the random effects were individual trees and the height within individual trees. The model was estimated using REML.

There was a significant difference in the proportion of damaged leaves at different levels in the canopy (Table 6.15). The proportion of damaged leaves was found to decrease towards the higher sections of the canopy (Figure 6.6). The final conclusions, however, can only be made after correcting the significance levels for multiple comparisons of the means using Tukey pair wise comparisons. Using the Tukey test, there was a significant difference in the proportion of insect-damaged leaves between the bottom and top canopy sections (Tukey test, top and bottom sections compared, d.f. = 10, $Z = -3.649$, $P < 0.001$) and a lower significance level between the middle and bottom sections (Tukey test, middle and bottom sections, d.f. = 10, $Z = -2.424$, $P = 0.041$). The intensity of damage in the four damage categories did not show any significant difference between canopy levels. Therefore, the proportion of damaged leaves decreased with an increase in height into the canopy, but the extent to which individual leaves were damaged did not vary.

Table 6.15. Linear mixed-effects model for the effect of canopy height on the occurrence of insect damage on *N. pumilio* leaves. The minimal adequate model has an arcsine square root transformed proportion response variable and has a quasibinomial error structure using REML. Overall sample size of leaves n = 120, height within tree n = 20, individual trees n = 8. Significant results at alpha level 0.05 are in bold with *.

	Random Effects			
	SD	95% CI		
Height within individual trees	5.19	0.84 - 8.12		
Individual trees	2.61	2.54 -10.62		
Residual	7.73	6.73 - 8.88		
	Fixed Effects			
	Estimate	SE	d.f.	t-value
Intercept	37.54	2.54	100	14.79
Middle	-5.06	2.28	10	**-2.22***
Top	-9.24	2.53	10	**-3.65***

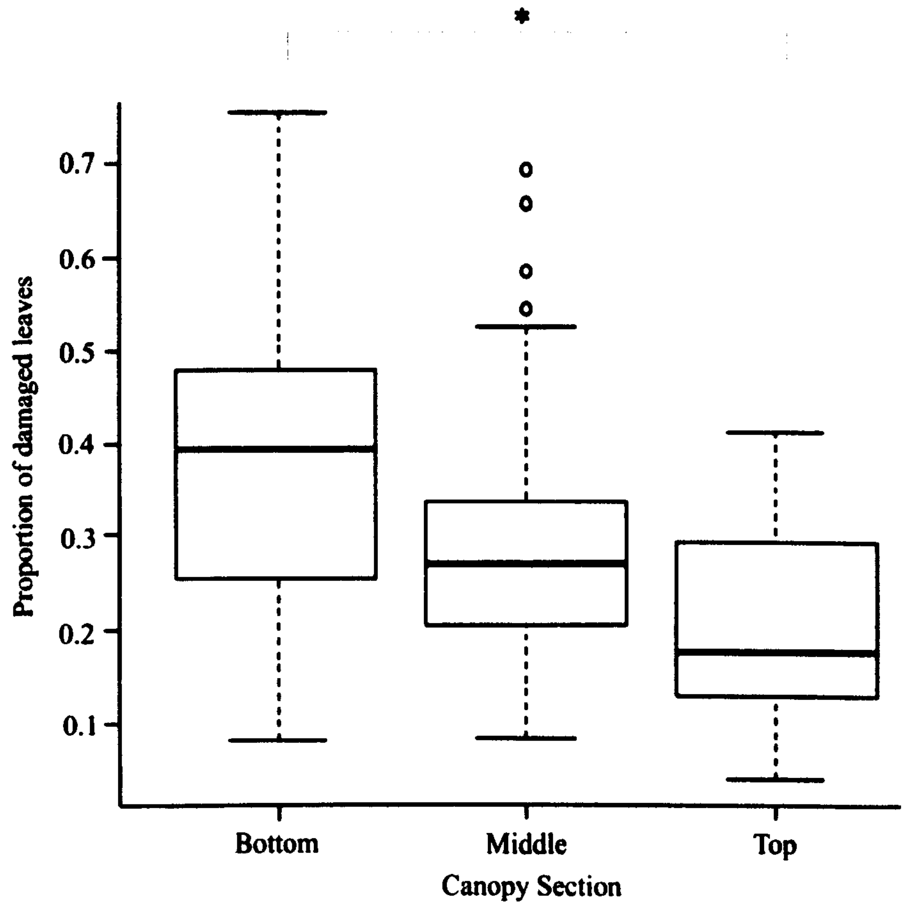


Figure 6.6. The median, 25% and 75% inter-quartile ranges of the proportion of damaged leaves per sample on *N. pumilio* trees collected in different canopy sections. Significant differences at the 0.5 alpha level are marked with *. Outliers are marked with circles.

6.6. Can other abiotic factors affect the occurrence of damaged leaves?

In Chapter 4, species accumulation curves were plotted to show that further sample collection would not have substantially increased the diversity of insect traces obtained. Other factors that can create unwanted variation within the data set or cause bias in the results also have to be considered. For example, the people undertaking the collecting may categorize the leaf damage intensity differently. Alternatively, sampling at different time periods within the study can lead to a temporal collection bias. Therefore, testing for the effect of such factors can increase confidence in the interpretation of the results.

Factors such as elevation, age of the leaf and sampling technique not only influence the interpretation of the modern insect damage levels, but can also help interpret the fossil herbivory traces. The fossil leaves were preserved as fossil leaf mats which could have accumulated over one or several seasons. They could have come from various parts within one tree, from various heights and orientations, and from trees that grew at different elevations. The original growing position of either the leaves or trees is unknown. Therefore, the level of damage could be due to the original position of the leaves or the tree and is not representative of the whole forest ecosystem. If however, such factors do not have a significant effect on herbivore levels in modern systems then an assumption may be made that they were not important factors in the geological past. Each of the factors mentioned above will now be assessed in the following sections using generalized linear mixed-effects models to analyse the data (Table 6.16).

Table 6.16. Summary of the different models used to test for differences in damage levels due to abiotic factors. Such factors also help to validate the collection methods applied. DCat ID- damage category identification. All are generalized linear mixed-effects models consisting of a response variable, one or more fixed effects and random effects. Continued on p160.

Model No.	**Plant Species**	**Response variable**	**Fixed Effects (No. of factor levels)**	**Random Effects**	**Significant Effects**
10	*N. pumilio*	Binomial - damaged leaves vs. not damaged	Season (3)	Individual trees Height	No effects
11	*N. antarctica*	Binomial - damaged leaves vs. not damaged	Season (3)	Individual trees Height	No effects
12	*N. pumilio*	Number of leaves in damage category	Observer (2) Damage (4) Interaction	Individual trees DCat ID Sample No.	No effects
13	*N. antarctica*	Number of leaves in damage category	Observer (2) Damage (4) Interaction	Sample No.	No effects
14	*N. pumilio*	Binomial- damaged leaves vs. not damaged	Collection (2)	Individual trees Height	No effects

Model No.	Plant Species	Response variable	Fixed Effects (No. of factor levels)	Random Effects	Significant Effects
15	*N. pumilio*	Binomial - damaged leaves vs. not damaged	Elevation (4)	Individual trees Height	No effects
16	*N. antarctica*	Binomial - damaged leaves vs. not damaged	Elevation (6)	Individual trees Height	Middle elevations greater damage than low and high elevations
17	*N. pumilio*	Binomial - damaged leaves vs. not damaged	Age (2)	Sample ID	More damage on older leaves
18	*N. antarctica*	Binomial - damaged leaves vs. not damaged	Age (2)	Sample ID	More damage on older leaves

6.6.1. Does the amount of leaf damage vary with season?

Generalized linear mixed-effects models were used to assess changes in the abundance of insect-damaged leaves that occurred within a single season at a single site by comparing the data collected during the three visits to Puyehue. The minimal adequate model for both *N. pumilio* and *N. antarctica* contained a weighted binomial response variable of the number of damaged leaves and the number of non-damaged leaves per sample (Model 10 & Model 11, Table 6.16). The fixed effect (explanatory variable) that was used to determine seasonal differences in the number of insect-damaged leaves was site, a three level factor of Puy 1, Puy 2 and Puy 3. The random effects were individual trees and the height level within individual trees. Both models used a quasibinomial error structure and were fitted using Laplace approximation methods. MCMC sampling was carried out to generate 95% confidence intervals for the model estimates of the fixed effects. From the models, there were no significant seasonal differences for either species (Table 6.17& Table 6.18), although some variation did exist (Figure 6.7). Therefore, it can be concluded that comparison of sites is valid when leaves are collected at different time periods within the one field season (late Spring (Dec) to early Autumn (March)).

Table 6.17. The minimal adequate model parameters for the effect of season on the occurrence of insect damaged leaves on *N. pumilio* at P.N. Puyehue. The 95% confidence intervals were generated using Markov Chain Monte Carlo sampling with a run time of 10,000. The random effects are height within individual trees (n = 87) and individual trees (n = 18) with an overall sample size of n = 522.

	Random Effects			
	SD			
Height within individual trees	0.73			
Individual trees	0.90			
Residual	1.71			
	Fixed Effects			
	Estimate	SE	t-value	95% CI
Intercept	-2.00	0.40	-5.06	-2.54 - -1.49
Puy 2	0.71	0.56	1.28	-0.02 - 1.47
Puy 3	0.81	0.56	1.46	0.08 - 1.56

Table 6.18. The minimal adequate model for the effect of season on the occurrence of insect damaged leaves on *N. antarctica* at P.N. Puyehue. The 95% confidence intervals were generated using Markov Chain Monte Carlo sampling with a run time of 10,000. The random effects are height within individual trees (n = 80) and individual trees (n = 18) with an overall sample size of n = 480.

	Random Effects			
	SD			
Height within individual trees	0.83			
Individual trees	1.04			
Residual	1.82			
	Fixed Effects			
	Estimate	SE	t-value	95% CI
Intercept	-1.35	0.50	-2.73	-2.02 - -0.77
Puy 2	-0.94	0.65	-1.43	-1.74 - -0.11
Puy 3	0.43	0.68	0.64	-0.40 - 1.33

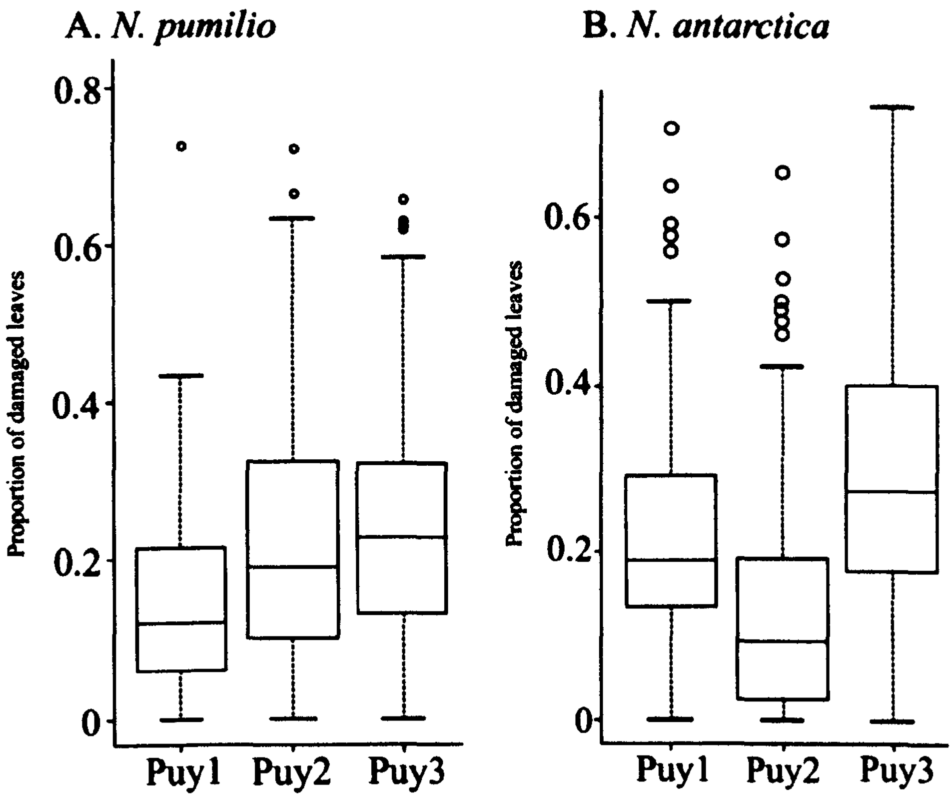


Figure 6.7. The median and inter-quartile ranges of the proportion of damaged leaves found at Puyehue at several sampling times in the season for both tree species. Puy1 = Start of Dec, Puy2 = Start of Jan, Puy3 = End of March. Outliers are shown as circles.

6.6.2. Can the observer create a bias in the results?

Due to the large volume of samples that were needed, two people counted and categorized the level of insect damage on the leaves. The leaves were divided into damage categories by each person. This could, however, lead to observer bias, especially with leaves that are borderline between two damage categories. Therefore samples were chosen at random at several different sites and counted by both observers. To test if observers were categorising damage using the same standards a generalized linear mixed-effects model was fitted to the data (Model 12 & 13, Table 6.16).

For *N. pumilio*, the minimal adequate model used the number of leaves identified in each damage category as the response variable. The fixed effects were the two level factor observer, observer 1 and observer 2, and the four level factor damage category. The random effects were individual trees and individual sample numbers within individual trees. The model was fitted using a Laplace approximation with a poisson error structure and hence a log link function, due to the response variable being count data. In this model it is the significance of the interaction between the observers that is of interest because differences between damage categories have already been assessed (Section 6.4). There was found to be no significant difference between the average number of leaves identified within each damage category by each observer.

For *N. antarctica*, the minimal adequate model had the same response variable and fixed effects as above, but the random effect was sample number alone and a quasipoisson error structure was used to avoid overdispersion in the model. Again, there was no significant difference between the counts for observers within each damage category. Thus, no observer bias exists within the data collected. The sources of error that such bias can introduce are minimised (Figure 6.8).

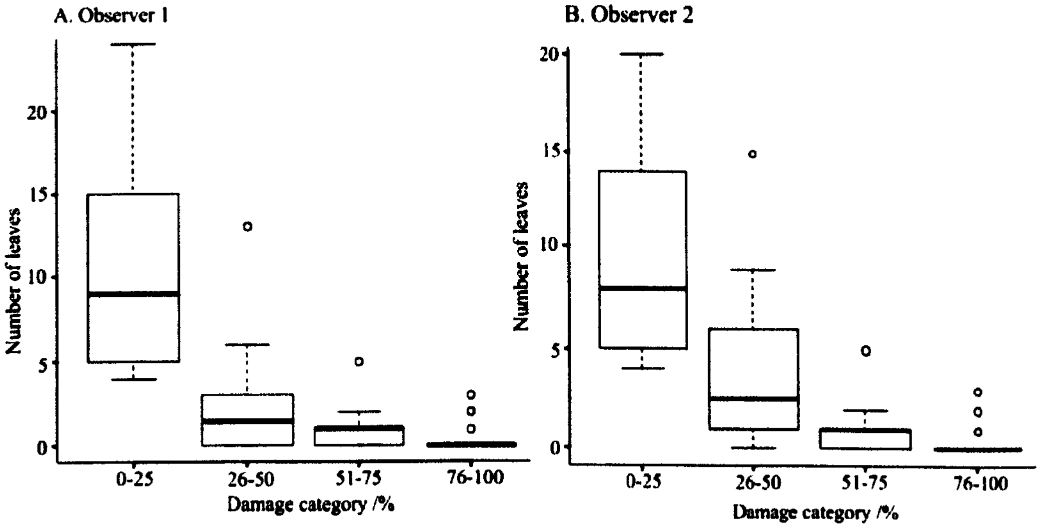


Figure 6.8. The median and inter-quartile range of the number of leaves placed in each damage category by two observers for data from a random selection of sites and for both tree species. Outliers are shown as circles.

6.6.3. Which collection technique is valid: collecting from the canopy or the ground?

The proportion of insect-damaged leaves from the canopy was collated and compared with the data from samples collected from ground level using the pruning poles, to test if sampling from the ground provided a representative sample of the insect damage levels within the study system. A generalized linear mixed-effects model was used to assess the difference in the presence of insect-damaged leaves for *N. pumilio* collected using the two techniques (Model 14, Table 6.16). A weighted binomial response variable, number of damaged leaves and number not damaged, was used with one fixed effect of collection type that had two levels to compare, the canopy and the ground. The random effects were individual trees and height within individual trees and a quasibinomial error structure was used.

There was no significant difference in the average proportion of damaged leaves between the ground samples and the canopy samples (Table 6.19). Therefore, leaf damage data collected solely from the ground are acceptable. Although this analysis was only possible with *N. pumilio* trees, the results can be applied to *N. antarctica* trees due to the height of the trees used during the ground sampling. *N. antarctica* trees were, on average, smaller than *N. pumilio* (*N. antarctica* mean height: 6.01m ± 1.21m 95% CI, *N. pumilio* mean height: 9.28m ± 1.42m 95% CI) and so if no difference existed between the ground and canopy samples for *N. pumilio* in this study, then the height gradient is likely to have been too small to affect the damage levels on *N. antarctica.*

Table 6.19. The minimal adequate model used to assess the effect of collection technique on the proportion of insect damaged leaves for *N. pumilio* at Puyehue. MCMC method used to generate 95% confidence intervals with a run time of 10,000. Response variable is a weighted binomial variable of the number of insect damaged leaves and number of undamaged leaves. The fixed effect variable has two levels, ground and canopy. Overall sample size of leaves n = 468, height levels within trees n = 20 and individual trees n = 20.

	Random Effects			
	SD			
Height within trees	0.36			
Individual trees	0.36			
Residual	1.48			
	Fixed Effects			
	Estimate	SE	t-value	95% CI
Intercept	-1.48	0.15	-9.88	-1.70 - -1.25
Canopy	0.24	0.24	0.10	-0.12 - 0.58

6.6.4. Does the presence of damaged leaves change with altitude?

Insect diversity is known to be linked with changes in elevation and normally decreases with increasing elevation. The coleopteran diversity in Puyehue was found to decrease with increasing elevation after examination of the fauna of several plant species (Ashworth & Hoganson, 1987). Hence, the effect of elevation on the proportion of insect-damaged leaves on the two species studied from Puyehue was assessed. The elevation of each tree sampled within Puyehue was estimated using a 3 arc-second (90m) digital elevation model (DEM) generated by NASA's Shuttle Radar Topography Mission (SRTM) (Farr *et al.*, 2000). The latitude and longitude co-ordinates were recorded using a handheld GPS unit during sampling and the elevation was obtained from the DEM. Due to the resolution of the DEM, coordinates were often in the middle of two adjacent pixels and so an average elevation was calculated from the data. Also, due to the limited resolution, several trees had the same elevation, and so altitude was considered a categorical variable and not a continuous variable for further analyses.

A generalized linear mixed-effects model was used to examine the data from both species. A binary response variable of number of leaves damaged and number not damaged was used and altitude was the fixed effect. The random effects were individual trees and height within individual trees and a quasibinomial error structure was applied. For *N. pumilio* (Model 15, Table 6.16), the elevational range was from 1010m to a maximum of 1404m, so the minimal adequate model had altitude as a four level factor: <1100m, <1200m, <1300m, and >1300m. There were no significant differences in the level of insect damage at different altitudes.

For *N. antarctica* (Model 16, Table 6.16), the altitudinal range was, however, from 1010m to 1319.5m, so the minimal adequate model had altitude as a six level factor, low (1010m and 1020.5m combined), 1025m, 1040m, 1046m, <1200m (1110m and 1129m combined) and high (1230m, 1288m and 1319.5m combined). The trees at 1046m had a significantly greater proportion of damaged leaves than at low, 1040m and high altitudes (Table 6.20 & Table 6.21). Therefore, there seems to be a difference in damage levels with change in altitude, with less damage found at the lowest altitude, but increasing damage with an increase in altitude, with ultimately a decrease in damage levels when the altitude becomes too high. The differences between the highest (27.89% of leaves damaged) and the lowest mean value (6.81% of leaves damaged) is 21.08% of leaves (Figure 6.9). Although there is a significant difference in the proportion of damaged leaves with altitude, the highest levels are at the medium altitude.

Table 6.20. The minimal adequate model used to assess the altitude effect on the proportion of insect-damaged leaves on *N. antarctica* at Puyehue. The fixed effect is the seven level factor altitude and the response variable is a weighted binomial two column matrix of the number of damaged leaves and number of undamaged leaves per sample. 95% confidence intervals were obtained using MCMC estimation with a run time of 10, 000. Overall sample size of leaves n = 480, height levels within individual trees n = 80, and individual trees n = 18. Significant results at alpha level 0.05 are in bold with *.

	Random Effects			
	SD			
Height within trees	0.83			
Individual trees	0.76			
Residual	1.82			
	Fixed Effects			
	Estimate	SE	t-value	95% CI
Intercept	-2.23	0.49	-4.50	-2.94 - 1.49
1025m	1.03	0.70	1.48	0.03 - 2.09
1040m	-0.33	0.10	-0.34	-1.74 - 1.15
1046m	1.28	0.61	**2.11***	0.45 - 2.23
<1200m	1.25	0.79	1.57	0.06 - 2.34
High	-0.39	0.72	-0.54	-1.40 - 0.69

Table 6.21. Back transformed estimates from the generalized linear mixed-effects model to assess the effect of altitude on the proportion of damaged leaves on *N. antarctica* at Puyehue. "Low" is a combination of data from 1010m and 1020.5m altitude and "high" is a combination of data from 1230m, 1288m and 1319.5m altitude. Factor levels significantly greater than the other factor levels are in bold with *.

Elevation Category	Mean % damaged leaves	95% CI
Low	9.7	0.0 - 98.0%
1025m	**23.2**	**5.1 - 97.3%***
1040m	7.2	2.0 - 25.0%
1046m	**27.9**	**0.0 - 82.0%***
<1200m	**27.3**	**5.3 - 97.8%***
High	6.8	3.0 - 90.0%

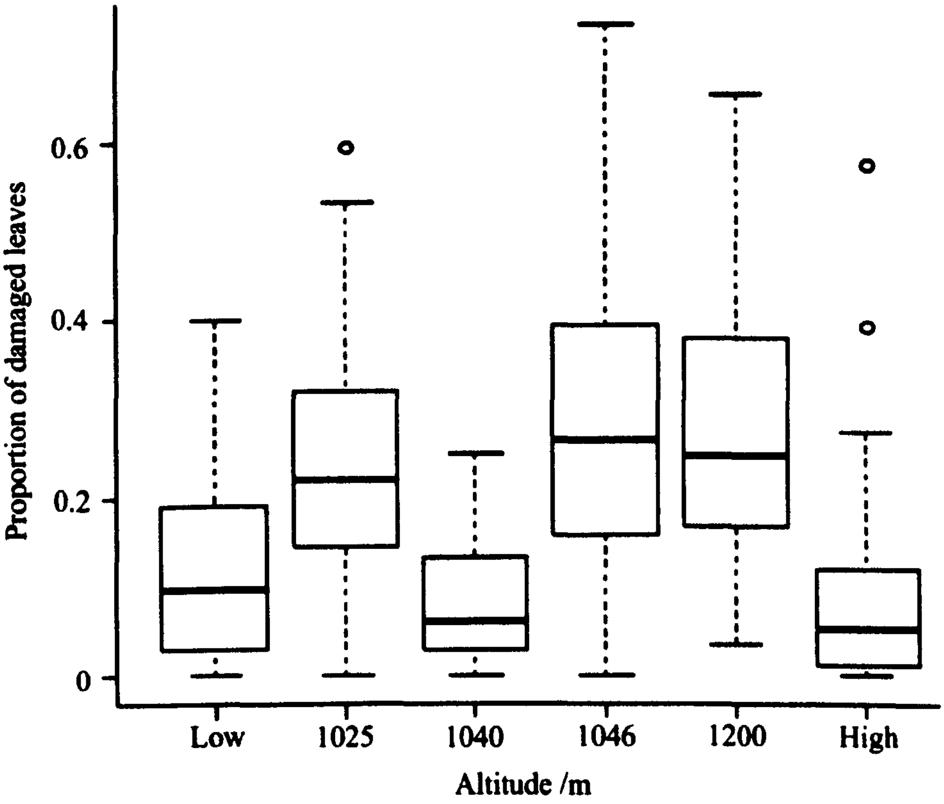


Figure 6.9. The proportion of damaged leaves on *N. antarctica* at different altitudes within Puyehue. Low is a combination of data from altitudes 1010m and 1020.5m, and high includes data from trees at altitudes >1200m. Outliers are represented as circles.

6.6.5. Does insect damage change with leaf age?

The intensity of herbivore damage caused by insects is known to vary with leaf age mainly because younger leaves, especially immediately after emergence, are softer and more palatable than the older leaves, which harden with time and often contain higher tannin concentrations (Feeny, 1970; Coley & Barone, 1996). Damage that occurred on the young leaves will still, however, be evident on the leaf even when it has matured. Thus, the mature leaves can represent an accumulation of insect damage over one season within deciduous tree species. This theory was tested by analysing the data across all sites to determine if the number of leaves that were attacked were different between two age categories, young and old. Young leaves were defined as the leaves that had recently emerged from the bud, had an enclosed venation and were pale green. Old leaves had thicker tissue than the young leaves and had fully opened venation.

The minimal adequate model for both *Nothofagus* species had a weighted binomial response variable of the number of damaged leaves and the number of undamaged leaves per sample and a fixed effect of age, a two level factor of old and young (Model 17 & Model 18, Table 6.13). The random effect was sample identification to link the counts of young and old leaves to the same sample. There was a significant difference between the level of damage in young leaves compared with that in old leaves for both *N. pumilio* and *N. antarctica* (Table 6.22). There were higher proportions of damaged leaves within the older leaf age category than the young (*N. pumilio*: mean damaged old leaves = 92.4% of damaged leaves, 95% CI = 91.9 - 92.1%; mean damaged young leaves = 7.6% of damaged leaves, 95% CI = 7.0 - 8.0%).

Table 6.22. Minimal adequate model to examine the difference in the proportions of damaged leaves that are young or old, for *N. pumilio* and *N. antarctica* across all sites studied. Estimates for the fixed effects are the level mean for the intercept (old leaves) and the difference between mean levels for the young leaves. 95% confidence intervals are obtained form MCMC sampling with a run time of 10,000. Significant differences at alpha level of 0.05 are in bold.

	N.pumilio			*N.antarctica*		
Random Effects	SD			SD		
Sample ID	0.00003			0.00003		
Residual	1.61			1.76		
Fixed Effects	Estimate	SE	95% CI	Estimate	SE	95% CI
Old	2.50	0.05	2.44 - 2.56	2.81	0.07	2.74 - 2.88
Young	**- 4.10**	**0.08**	-5.09 - -4.91	**-5.61**	**0.09**	-5.72 - -5.51

The results indicate that, as expected, leaf age had a significant effect on the extent of insect damage on leaves, with more damage on older leaves than younger leaves. This is likely to represent the accumulation of insect damage throughout a season on the older leaves, rather than a preference for older leaves by insects. For all analyses, both young and old leaves were included to prevent any bias in the insect damage levels. With regard to fossil leaf collections, the age of the leaf can be difficult to interpret, depending on the preservation of the leaves. Therefore, only if a fossil leaf collection was made of entirely young leaves would an underestimate of insect damage levels be obtained. This is highly unlikely, considering that many fossil leaf mats are an accumulation of leaves abscised from trees naturally or due to mechanical forces such as wind. They would contain leaves of various ages (Spicer, 1989).

6.6.6. Summary of factors affecting leaf damage

The difference in the presence of damaged leaves due to various factors has highlighted some interesting trends and validated the collection technique used. In conclusion:

- Samples can be obtained at different time periods within a season without significantly affecting the interpretation of the damage.
- Using different people to collect the data did not significantly affect the quality of the data.
- There was no bias on the distribution of damaged leaves using ground collection methods alone.
- The change in the number of damaged leaves with elevation is not a simple relationship; however, this study found that the trees at mid elevations had higher damage levels than the trees at low and high elevations. This pattern cannot be explained by a difference in the tree species abundance as from field observations there was no difference between the middle and top or middle and bottom elevations, only between the top and bottom elevations at the site.
- The low levels of insect damage at high altitudes were as expected due to the decline in insect diversity and abundance with increase in altitude. Only insects adapted to cope with low temperatures and high exposure will survive there. The low insect damage levels at the lower elevations could be due to a higher abundance of other vegetation types which provide an alternative source of leaves for generalist insects to feed on.

- Older leaves were more damaged than younger leaves, which is likely to represent the accumulation of damage over the whole period that the leaves are in bloom.

6.7. Does latitude affect leaf mine and leaf gall distribution?

Generalized linear models were used to examine the differences between the proportion of leaf mines and leaf galls found at each site and the effect of factors such as height and orientation. The data for all species were combined as the same species of leaf mine and leaf gall were not present at all sites. The same method as used in previous sections was chosen, but with the presence and absence of the leaf mine and leaf gall data as the response variable. The samples collected by the quantitative method were analysed only, due to the high variability in numbers from the qualitative collection method.

The analyses of the leaf gall data showed that there were no significant differences between sites, height levels or orientation levels that affected the frequency of leaf galls. Therefore, only the leaf mine results are discussed further. The aim of using the minimal adequate model was to examine the change, if any, in leaf mine proportions at the different sites (over different latitudes), but at the same time accounting for other factors such as height and orientation of the branch.

6.7.1. Differences in leaf mine proportion – site comparison

The occurrence of leaf mines collected from *N. pumilio* trees was examined using a generalized linear mixed-effects model. The leaf mine data had a highly skewed distribution which could not be normalised through data transformation. Therefore, a response variable with two parts, the number of leaves with mines and the number of leaves without mines, was used. Height, orientation and site were the fixed effects used in the model. The random effects, the variables that are expected to vary naturally, were individual trees and height within individual trees. Due to the type of response variable, a quasibinomial error structure and hence a logit link function was used and the model was fitted using a Laplace approximation method.

The minimal adequate model (Table 6.23) showed that there were significantly greater proportions of leaf mines at the lower heights in the tree than at the 4m and 5m height levels (Table 6.24). There was also a greater proportion of leaf mines in the southerly orientation within a tree than the northerly one (north: mean leaves with mines = 3.0% of leaves, 95% CI = 1.4 - 6.0%, south: mean leaves with mines = 3.6% of leaves, 95% CI = 1.4 - 8.4%).

Table 6.23. The minimal adequate model for the proportion of mines from *N. pumilio* and the effect of height, orientation and site. Overall observations n = 1233, height levels within individual trees n = 206 and individual trees n = 47. The 95% confidence intervals were estimated using MCMC methods with a run time of 10,000. Significant effects are highlighted in bold and with *.

	Random Effects			
	SD			
Height within individual trees	0.46			
Individual trees	0.82			
Residual	1.06			
	Fixed Effects			
	Estimate	SE	t- value	95% CI
Intercept	-3.46	0.42	-8.20	-4.28 - -2.72
Height 2m	-0.09	0.18	-0.48	-0.36 - 0.37
Height 3m	-0.30	0.18	-1.62	-0.59 - 0.03
Height 4m	-0.63	0.19	**-3.33***	-0.10 - -0.35
Height 5m	-0.54	0.19	**-2.74***	-0.80 - -0.17
Orient South	0.19	0.08	**-2.37***	0.06 - 0.33
Nah	-1.57	0.56	**-2.79***	-2.75 - -0.40
Puy 1	-3.47	0.64	**-5.42***	-4.87 - -2.30
Puy 2	-1.58	0.56	**-2.81***	-2.51 - -0.50
Puy 3	-1.02	0.55	-1.88	-2.12 - 0.00
RNMag	0.12	0.54	0.22	-0.84 - 1.24
Tolh	-1.56	0.58	**-2.68***	-2.59 - -0.23
Torres	0.04	0.54	0.07	-1.10 - 1.01

Table 6.24. Back transformed estimates from the generalized linear mixed-effects model to assess the effect of height within a tree on the proportion of leaves with leaf mines on *N. pumilio* across all sites. Height levels with significantly fewer leaf mines than the other levels (at alpha 0.05) are in bold with *.

Height level	Mean % of leaves with mines	95% CI
1m	3.0	1.4 - 6.0%
2m	2.8	1.0 - 8.7%
3m	2.3	0.8 - 6.4%
4m	**1.6**	**0.5 - 4.0%**
5m	**1.8**	**0.6 - 5.3%**

The leaf mine data from Isla Nav (mean 3.0% leaves with mines, 95% CI = 1.4 - 6.2%) was significantly different from the data collected at the northern sites of Nah, Puy 1, Puy 2 and Tolh. There was a greater proportion of leaf mines at Isla Nav than at the northern sites (Nah, Tolh, Puy1 and Puy2) (Table 6.25). However, leaves from Isla Nav, Torres, RNMag and Puy 3 all had similar occurrences of leaf mines (Figure 6.10).

Table 6.25. Back transformed estimates from the generalized linear mixed-effects model to assess the difference in the proportion of leaves with leaf mines on *N. pumilio* between sites. Sites with a significantly greater proportion of leaf mines than the others (at alpha 0.05) are in bold with *.

Site	Mean % of leaves with mines	95% CI
Nah	0.6	0.09 - 4.3%
Tolh	0.7	0.1 - 5.0%
Puy 1	0.1	0.01 - 0.7%
Puy 2	0.6	0.1 - 3.9%,
Puy 3	**1.1**	**0.2 - 6.2%***
Torres	**3.2**	**0.5 - 15.4%***
RN Mag	**3.4**	**0.6 - 18.6%***
Isla Nav	**3.0**	**1.4 - 6.2%***

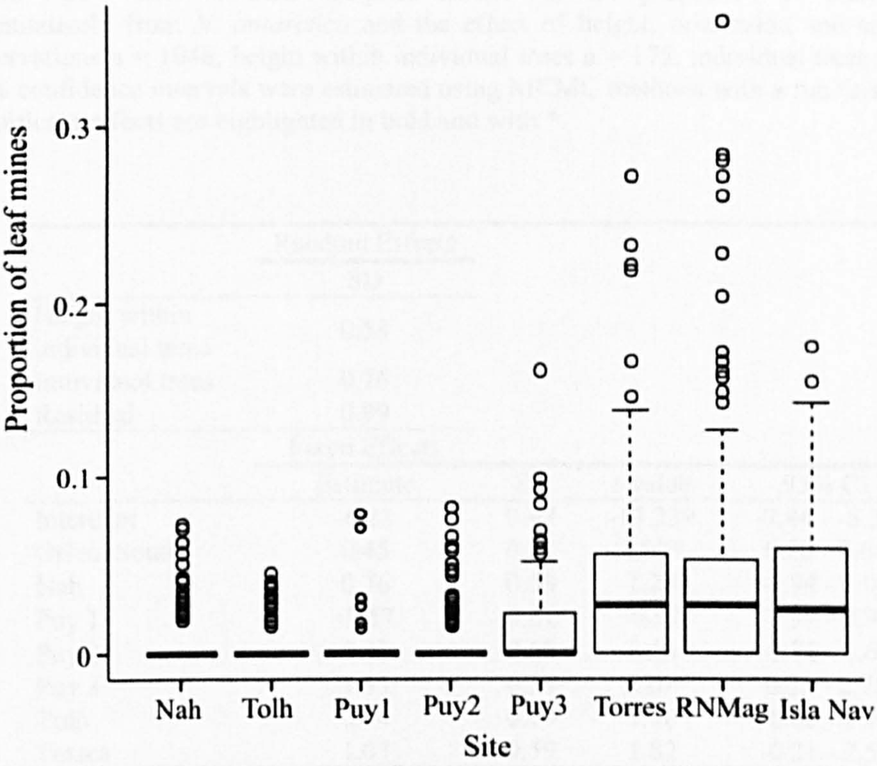


Figure 6.10. The proportion of leaf mines on *N. pumilio* at all sample sites. Medians (black line), inter-quartile range (rectangle) and outliers (circles) are shown.

The leaf mines collected from *N. antarctica* were analysed using a binary response variable, consisting of the number of leaves with leaf mines and number of leaves without leaf mines. The fixed effects were orientation, a two level factor, and site, a seven level factor. The fixed effect of height was removed during model simplification and did not cause the Akaike's Information Criteria (AIC) value to increase (model with factor height: AIC value = 963.81; model without factor height AIC value = 959.86). The random effects were the variation between individual trees and height within individual trees. The model highlighted that a significantly greater proportion of mines were collected in the south-facing parts of the tree (mean leaves with mines = 0.3% of leaves, 95% CI = 0.07 - 0.9%) than in the north-facing orientations (mean leaves with mines = 0.2% of leaves, 95% CI = 0.06 - 0.5%). The mean proportion of mines from Puy 3 (mean leaves with mines = 0.8% of leaves, 95% CI = 0.07 - 7.0%) was significantly greater than all of the sites apart from Torres and Nah (Table 6.26) (Figure 6.11).

Table 6.26. The minimal adequate model for the proportion of mines collected quantitatively from *N. antarctica* and the effect of height, orientation and site. Overall observations n = 1048, height within individual trees n = 175, individual trees n = 42. The 95% confidence intervals were estimated using MCMC methods with a run time of 10,000. Significant effects are highlighted in bold and with *.

	Random Effects			
	SD			
Height within individual trees	0.53			
Individual trees	0.76			
Residual	0.99			
	Fixed Effects			
	Estimate	SE	t-value	95% CI
Intercept	-6.21	0.47	-13.239	-7.44 - -5.30
Orient South	0.45	0.11	**4.07***	0.20 - 0.64
Nah	0.76	0.59	1.28	-0.94 - 2.02
Puy 1	-0.57	0.62	-0.92	-1.97 - 0.91
Puy 2	0.41	0.60	0.68	-0.71 - 1.69
Puy 3	1.33	0.59	**2.26***	0.23 - 2.78
Tolh	0.64	0.59	1.10	-0.68 - 1.76
Torres	1.07	0.59	1.82	-0.21 - 2.50

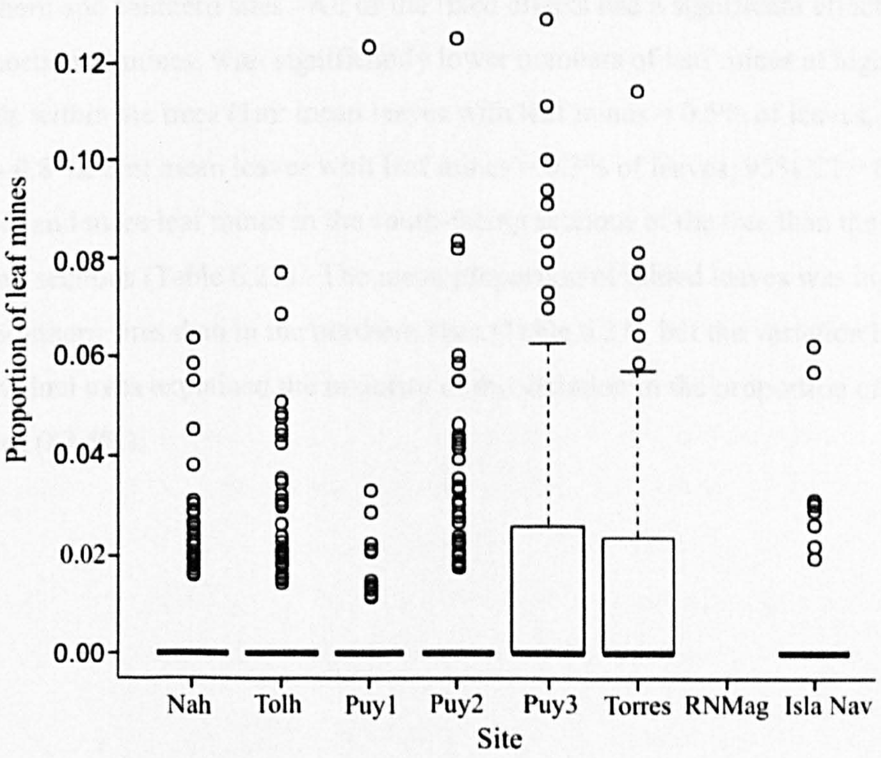


Figure 6.11. The proportion of leaf mines on *N. antarctica* from all sites. Medians (black line), inter-quartile range (rectangle) and outliers (circles) are shown.

6.7.2. Difference in leaf mine proportions - northern vs. southern sites

6.7.2.1. Leaf mine data from *Nothofagus pumilio*

The analysis of the proportion of leaf mines collected from all sites in 5.8.1 was repeated, but the sites were grouped into the northern sites, Nah, Tolh and all samples collected at Puy, and the southern sites, Torres, RNMag and Isla Nav. The proportion of leaf mines collected in the northern and southern sites was then examined for each tree species. The minimal adequate model for *N. pumilio* had a binary response variable (number of leaf mines and the number of leaves without mines) and the random effects were individual tree and height within individual tree. The fixed effects were orientation (north-facing and south-facing), a two level factor and height, a five level factor. A binomial error structure was, however, applied as the scale parameter was 1.056, showing that no overdispersion had occurred (ideal value for scale parameter being 1.00). The site fixed effect had only two levels, northern and southern sites. All of the fixed effects had a significant effect on the proportion of mines, with significantly lower numbers of leaf mines at higher height levels within the trees (1m: mean leaves with leaf mines = 0.5% of leaves, 95% CI = 0.3 - 0.8 %, 5m: mean leaves with leaf mines = 0.3% of leaves, 95% CI = 0.1 - 0.6%) and more leaf mines in the south-facing sections of the tree than the north-facing sections (Table 6.27). The mean proportion of mined leaves was higher in the southern sites than in the northern sites (Table 6.27), but the variation between individual trees explained the majority of the variation in the proportion of mined leaves (83.5%).

Table 6.27. The minimal adequate model for the effect of site as a two level factor of northern and southern sites on the proportion of leaf mines sampled from *N. pumilio*. Overall observations n = 1233, height levels within individual trees n = 206 and individual trees n = 47. The 95% confidence intervals were estimated using MCMC methods with a run time of 10,000. Significant effects are highlighted in bold and with *.

	Random Effects			
	SD			
Height within individual trees	0.43			
Individual trees	0.96			
	Fixed Effects			
	Estimate	SE	z-value	95% CI
Intercept	-5.28	0.39	-22.12	-5.75 - -4.86
Height 2m	-0.08	0.17	-0.48	-0.40 - 0.22
Height 3m	-0.28	0.17	-1.65	-0.61 - 0.02
Height 4m	-0.63	0.18	**-3.51***	-0.98 - -0.33
Height 5m	-0.53	0.18	**-2.92***	-0.84 - -0.12
Orient South	0.19	0.07	**2.51***	0.06 - 0.32
Southern Sites	1.84	0.32	**5.80***	1.38 - 2.64

6.7.2.2. Leaf mine data from *Nothofagus antarctica*

A minimal adequate model for examining the difference in the proportion of leaf mines between northern and southern sites for samples collected from *N. antarctica* was also created. The model had a binary response variable (number of leaves with leaf mines and the number of leaves without mines) and individual tree and height as the random effects. The fixed effects were site (northern and southern), and orientation (north-facing and south-facing). A binomial error structure and a logit link function were used as the scale parameter indicated no overdispersion (scale parameter = 0.990). There was a significant difference between the south-facing and north-facing orientation levels (north: mean leaves with mines = 3.0%, 95% CI = 2.0 - 4.8%; south: mean leaves with mines = 5.0%, 95% CI = 3.0 - 9.2%), but no significant difference between the northern and southern sites (Table 6.28). Again, the variation between individual trees explained the majority of the variation within data on the proportion of leaves with mines (78.19%).

Table 6.28. The minimal adequate model for the effect of site as a two level factor of northern and southern sites on the proportion of leaf mines sampled from *N. antarctica*. Overall observations n = 1048, height levels within individual trees n = 175 and individual trees n = 42. The 95% confidence intervals were estimated using MCMC methods with a run time of 10,000. Significant effects are highlighted in bold and with *.

	Random Effects			
	SD			
Height within individual trees	0.52			
Individual trees	0.98			
	Fixed Effects			
	Estimate	SE	z-value	95% CI
Intercept	-5.71	0.21	-26.72	-6.14 - -5.32
Orient South	0.46	0.11	**4.02***	0.25 - 0.65
Southern sites	0.06	0.39	0.14	-0.69 - 0.78

6.7.2.3. Summary of leaf mine & leaf gall distribution

In summary, there was a greater proportion of leaf mines in the more southerly sites than in the northerly sites for both tree species. An exception was for *N. pumilio*, when during the third visit to Puy a similar number of mines was observed as those in the southern sites. This could be due the seasonality of the leaf mines, as the final sampling session represented an accumulation of the overall seasonal spread of different leaf mining insects, resulting in the proportion of mined leaves being similar to the higher levels seen in the southern sites. In contrast, there was no simple latitudinal divide in the proportion of mined leaves on *N. antarctica*, with higher proportions of leaf mines at both northern and southern sites.

There was a greater proportion of leaf mines in the south-facing sections of the tree than the north-facing sections for both *N. pumilio* and *N. antarctica*. The north-facing parts of the tree are warmer than the south-facing parts of the tree in the Southern Hemisphere, which would be advantageous for the development of leaf mining larvae (Pincebourde, 2007). Therefore, it is expected that there would have been a greater proportion of leaf mines in the north-facing sections than south-facing. This possibly highlights other factors affecting their distribution within the tree such as parasitism or predation rates. The effect of orientation on leaf mine occurrence is also likely to be species specific, influenced by the tree species or the species of leaf mining insect.

6.8. Summary

- In this study it was observed that the number of leaves damaged by herbivorous insects can vary due to factors such as latitude, height within the tree and orientation. These factors will have different effects, depending on the tree species.
- The intensity of damage to *Nothofagus* was relatively unaffected by such factors, regardless of site or tree species. There was more variation between the damage category levels (the amount of damage) than due to between-tree or between-site differences.
- Although there was a significantly greater amount of damaged leaves in lower areas of the tree canopy than the middle or upper parts, there was still no significant difference in the amount of damage sampled from the ground and from the canopy. Therefore, sampling from ground alone will not lead to an under-estimation of the levels of insect-damaged leaves.
- There was also no difference in the number of damaged leaves throughout the season. Differences related to altitude were found, but not a simple linear relationship. There was no significant difference in the categorization of leaf damage between observers.
- A higher proportion of older leaves were damaged than younger leaves. Therefore, a greater number of older leaves included in the analysis represented damage over a longer time period than younger leaves.
- Therefore, there was no overall underestimation of the proportion of insect damage leaves within the sites.
- There was no effect of external factors such as latitude, height and orientation on the occurrence of leaf galls.
- There was a significantly greater proportion of leaf mines on *N. pumilio* in the southern sites than in the northern sites. There was no simple latitudinal gradient to explain the variation of leaf mines on *N. antarctica* even though site differences did exist.

- Orientation within a tree had a significant effect on the occurrence of leaf mines for both tree species. For both *N. pumilio* and *N. antarctica*, there were a greater proportion of leaf mines on the south-facing parts of the tree. The north-facing parts of a tree are likely to be warmer than the south-facing sides, which would be advantageous for the development of leaf mining insects. The contrasting position of leaf mines within the trees suggests the effect of orientation is more dependent on other factors as well as the insect species than on orientation within the tree.

Chapter 7. Comparison of the fossil herbivory traces with modern traces from Chile

7.1. Data Analysis

Statistical analyses were performed using the programme R and the results presented in Section 7.3. Generalized linear models (Appendix VI) were used to test the difference in proportions of trace types between the fossil and modern assemblages. This is equivalent to a χ^2 test for analysing contingency tables. Cluster analysis was performed (as explained in Chapter 3) and the results presented in Section 7.3.1.

7.2. Visual Comparisons

The range of feeding traces collected from the sampling sites in Chile were compared with the fossil insect traces. This involved examination of the specific trace types, for example several small non-marginal traces on the fossil leaves, and matching them with equivalent traces on a modern leaf. This was possible for samples found at both fossil localities. The insect traces were compared with leaves from all sites in Chile. The herbivory traces from each fossil locality were analysed separately because of the difference in both abundance and range of trace types (discussed previously in Chapter 3) and the possible different environments the traces were created in. Thirty-three trace types from the King George Island assemblages matched modern traces (44.7% of 142 traces were comparable), and 18 trace types from Seymour Island matched modern traces (83.9% of 31 traces were comparable). All fossil and modern trace comparisons are summarised in Appendix VII; for the remainder of this section examples will be given to highlight the similarities and differences observed.

7.2.1. King George Island Comparison

7.2.1.1. Marginal general leaf chewing

Many traces on the fossil leaves from King George Island were similar to the modern traces (25 leaves with traces) (Table 7.1), with every type of fossil marginal general leaf chewing trace represented in the modern collections from *N. pumilio* and *N. antarctica* (Figure 7.1 & Figure 7.2). Whilst the majority of marginal general leaf chewing traces collected in Chile were a type of continuous damage (54.3% of marginally damaged leaves), discontinuous marginal damage was also present (45.7% of marginally damaged leaves). Discontinuous marginal chewing was, however not observed on any fossil leaves, although multiple continuous chewing was observed (trace morphotype K1.10).

Table 7.1. Summary of the number of marginal general leaf chewing trace fossils collected from King George Island that are comparable to the modern traces collected in Chile and the fossil traces in each trace type that could not be matched directly. Fossil trace morphotypes are described in Chapter 3. References are given for the corresponding photographs within the chapter and are highlighted in bold. All other similar traces are in Appendix VII.

Trace Morphotype	**Number of fossils in morphotype similar to modern traces**	**Number of fossils of this morphotype not matched to a modern trace**
K 1.1	9 **(Figure 7.1A, p182),**	1
K 1.2	5 **(Figure 7.1B, C, D p182),**	1
K 1.3	1	0
K 1.4	1	0
K 1.5	2 **(Figure 7.2A, p183)**	0
K 1.6	2	1
K 1.7	1	0
K 1.8	1 **(Figure 7.2B, p183)**	0
K 1.9	2 **(Figure 7.2C, p183)**	0
K 1.10	1	0
Total trace fossils	**25**	**3**

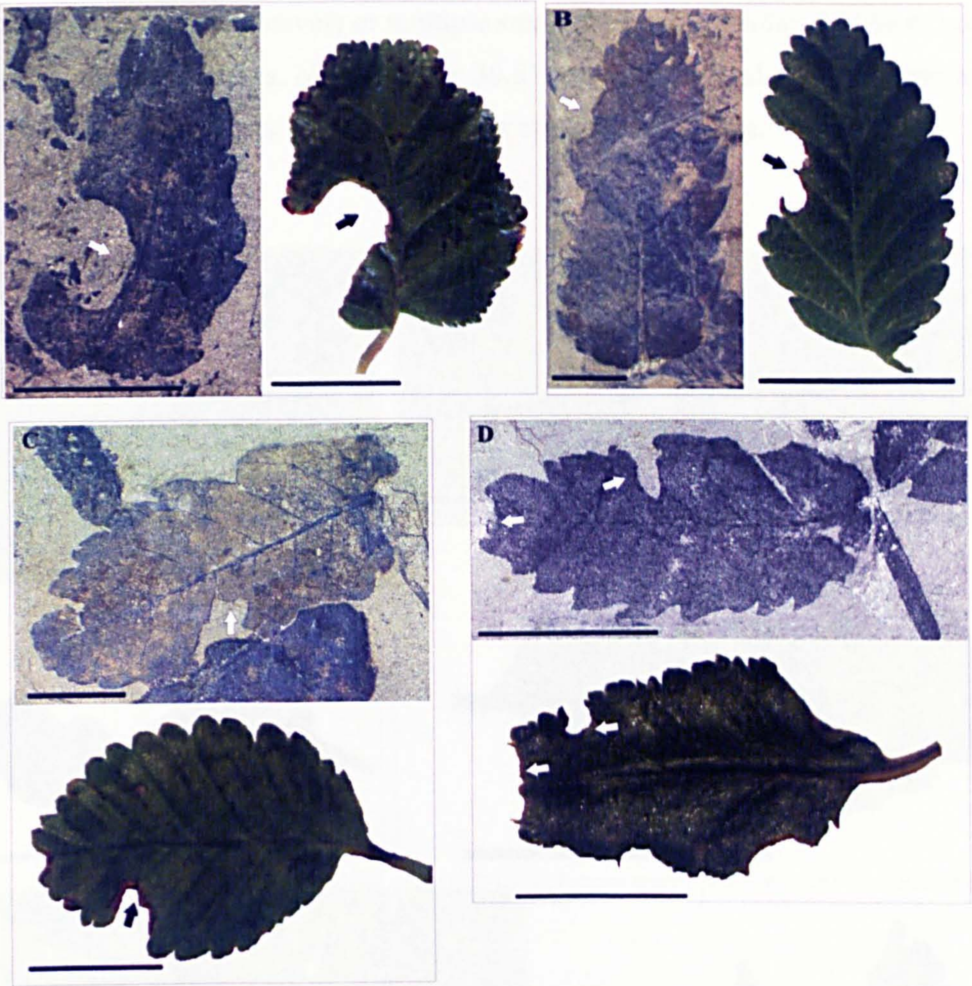


Figure 7.1. The range of marginal general leaf chewing traces on fossil leaves from King George Island and comparable traces on modern leaves from Chile; Trace K1.1: A) P.3001.139(1). Trace K1.2: B) P.3001.178(1), C) P.3001.47(2) and D) P.3001.46(2). Scale bar 1cm. Arrows highlight insect traces.

7.2.1.2. Non-marginal general leaf chewing

The modern non-marginal general leaf chewing trace types from King George Island were also similar to the fossil examples (Table 7.2) with both single large and multiple circular areas of leaf damage (Figure 7.3 & 7.4). Approximately half of the non-marginal fossil traces matched modern traces from Chile (42 fossil traces, 52.5% of non-marginal fossil traces), but ten fossil trace types (38.5% of 26 trace types) were not found in the modern systems. The trace types from King George Island not represented in the modern leaf herbivory types were all either single small or multiple small areas that were missing on different plant species. In contrast, many of the leaves from Chile with non-marginal insect traces had either single large (*N. pumilio*: 53.3% of non-marginal damaged leaves, *N. antarctica*: 56.1% of non-marginal damaged leaves) or multiple small marks (*N. pumilio*: 42.0% of non-marginal damaged leaves, *N. antarctica*: 30.8% of non-marginal damaged leaves), but the traces were not as small as the traces on the fossil leaves.

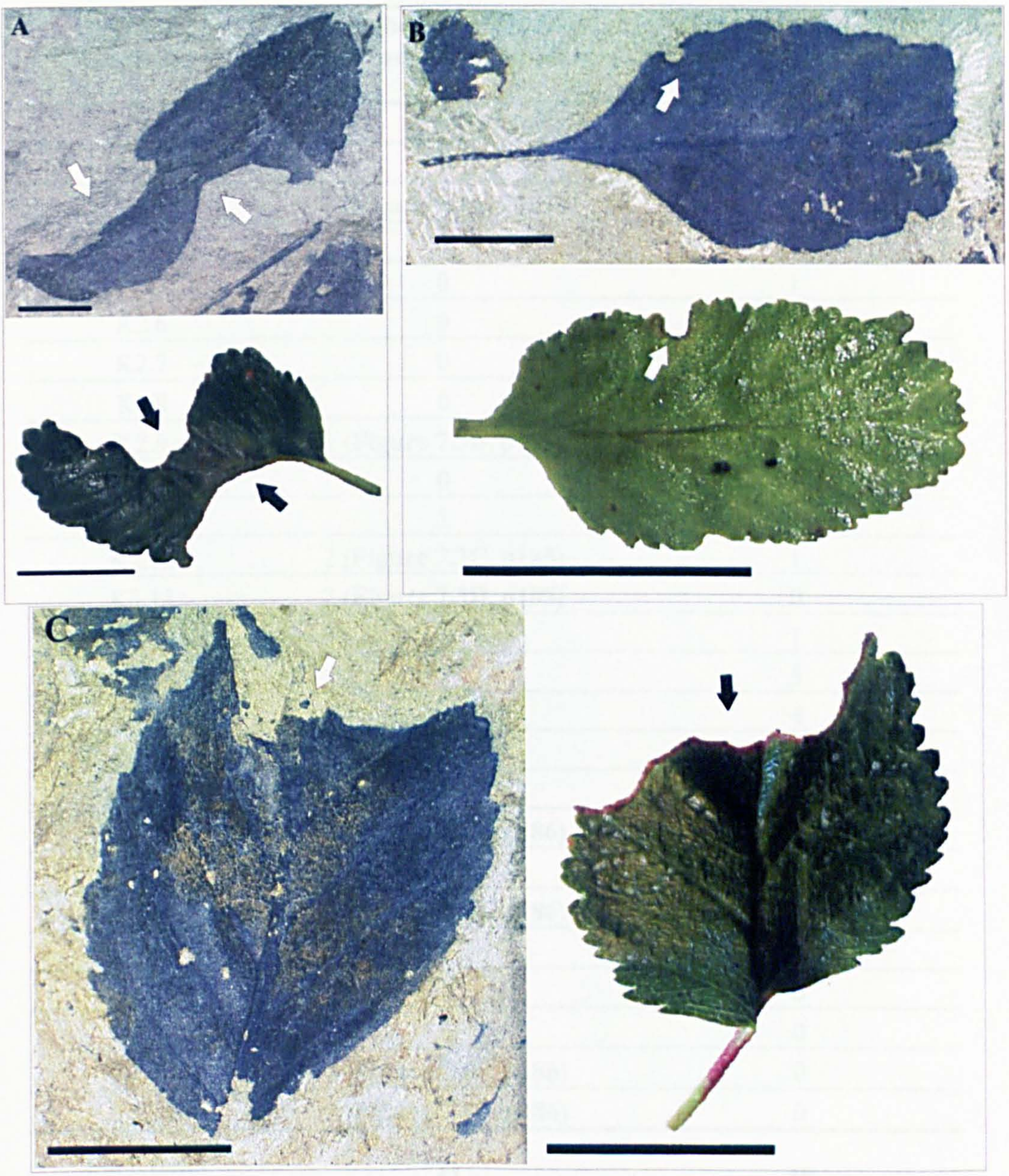


Figure 7.2. More marginal general leaf chewing fossil traces from King George Island (dark background) and comparable modern leaves with insect traces from Chile (white background). A) Trace K1.5, P.3001.169 (3), B) Trace K1.8, P.3001.58.1(1), C) Trace K1.9, G.9.3(3). Scale bar 1cm. Arrows indicate insect traces.

Table 7.2. Summary of the number of non-marginal general leaf chewing trace fossils on leaves from King George Island that are comparable to the modern traces collected in Chile and the number of fossils of that type that do not directly match the modern traces. Fossil trace morphotypes are described in Chapter 3. References are given for the corresponding photographs within the chapter and are highlighted in bold.

Trace Morphotype	**Number of fossils in morphotype similar to modern traces**	**Number of fossils of this morphotype not matched to a modern trace**
K2.1	0	2
K2.2	1	0
K2.3	1 **(Figure 7.3A, p185)**	0
K2.4	0	1
K2.5	0	1
K2.6	0	1
K2.7	0	1
K2.8	0	1
K2.9	1 **(Figure 7.3B, p185)**	0
K2.10	0	1
K2.11	1	0
K2.12	2 **(Figure 7.3C, p185)**	1
K2.13	2 **(Figure 7.3D, p185)**	0
K2.14	0	1
K2.15	1	5
K2.16	3	4
K2.17	4	4
K2.18	1	3
K2.19	2 **(Figure 7.4A, p186)**	2
K2.20	5	4
K2.21	1 **(Figure 7.4B, p186)**	3
K2.22	5	3
K2.23	7	0
K2.24	3	0
K2.25	1 **(Figure 7.4C, p186)**	0
K2.26	1 **(Figure 7.4D, p186)**	0
Total fossil traces	**42**	**38**

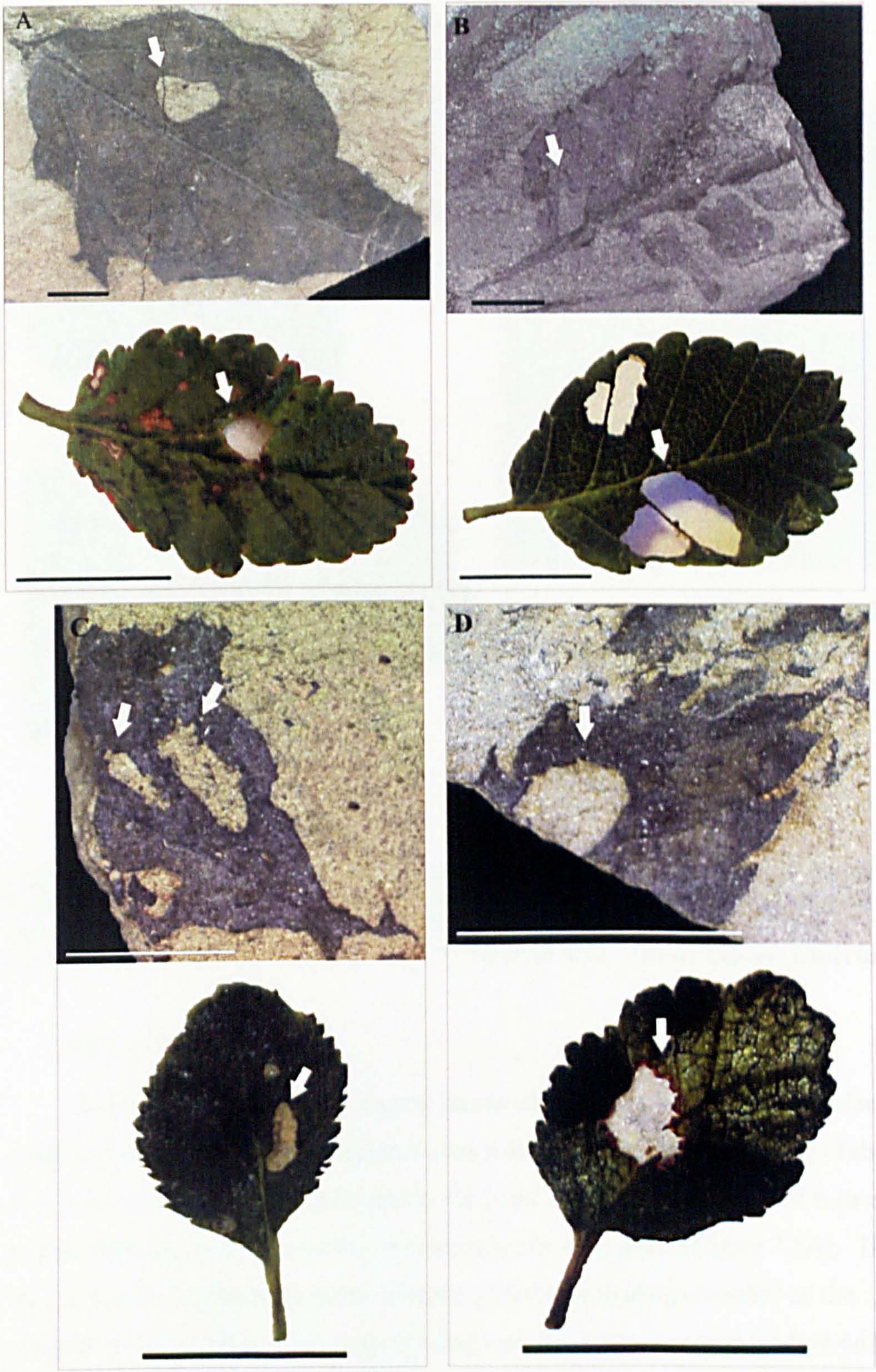


Figure 7.3. The range of non-marginal general leaf chewing fossil traces from King George Island (dark background) and comparable modern traces from Chile (white background). A) Trace K2.3, P.3001.36.1(1). B) Trace K2.9, G.312.15. C) Trace K2.12, P.236.4(3) and D) Trace K2.13, P.2810.18(3). Scale bar 1cm. Arrows indicate insect traces.

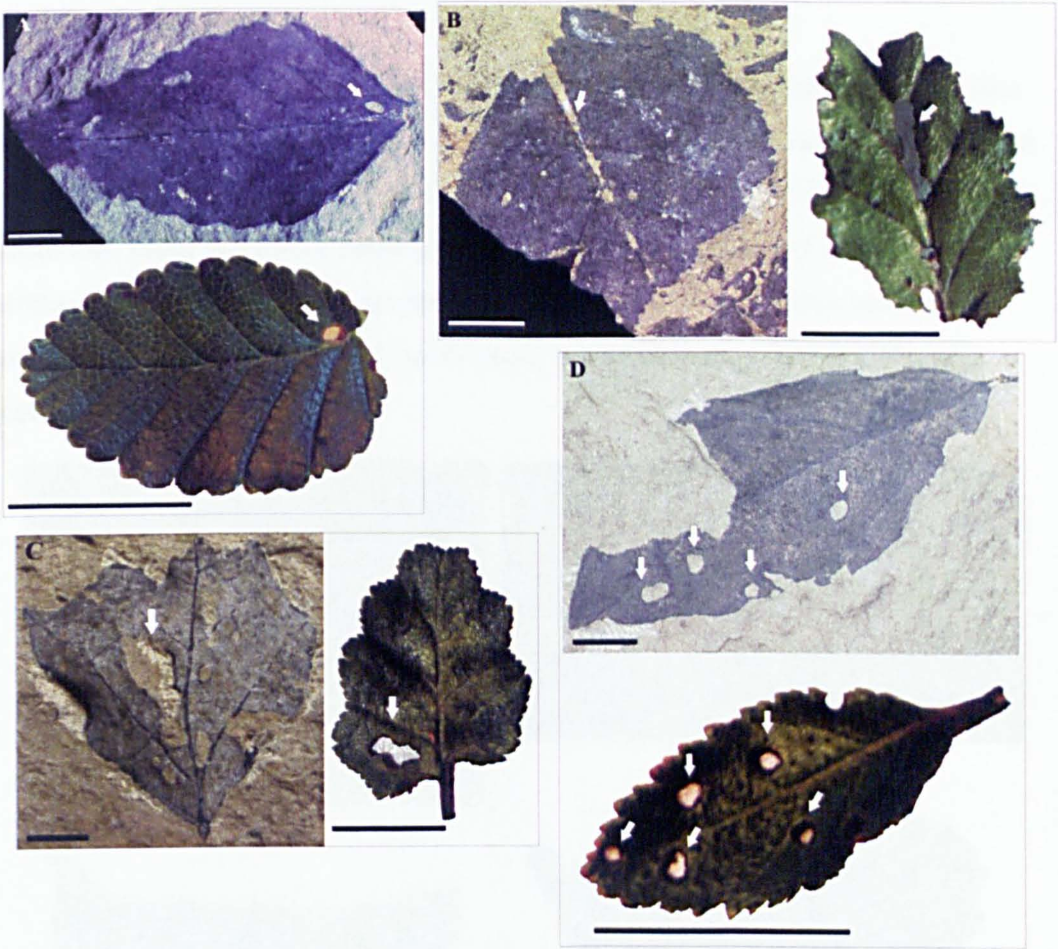


Figure 7.4. Examples of non-marginal, general leaf chewing traces on fossil leaves from King George Island (dark background) and comparable traces on modern leaves from Chile (white background). A) Trace K2.19, P.3001.108. B) Trace K2.21, P.236.4 (1). C) Trace K2.25, P.3013.9. D) Trace K2.26, P.3013.7. Scale bar 1cm. Arrows indicate insect traces.

7.2.1.3. Skeleton Feeding

The traces of skeleton feeding on leaves collected from King George Island were very similar to several modern leaves with traces of skeleton feeding (Table 7.3). On both fossil and modern leaves the plant defence reaction wound is present around the damaged area and the remaining leaf area is intact (Figure 7.5A). There was, however, a greater diversity of types of skeleton feeding recorded on the modern leaves (6 types of skeleton feeding and 32 insects observed feeding on both tree species). For example, types of skeleton feeding ranged from small multiple patches to the entire leaf being attacked. In addition, there were types on which only one leaf layer was chewed and some on which both the upper and lower leaf layers had been consumed.

7.2.1.4. Leaf galls

Two types of fossil leaf galls were found and equivalent galls found in Chile (Figure 7.5B-C). Fossil trace type K3.13 resembled gall type 1 and trace type K3.6 was similar to the gall collected from *N. betuloides*, gall type 5 (Table 7.3). Apart from this, fossil galls were either at different positions on the leaf or were of different size. For example, several gall trace types on fossil leaves included multiple galls on the same leaf, but the galls were larger than the multiple galls collected from Chile.

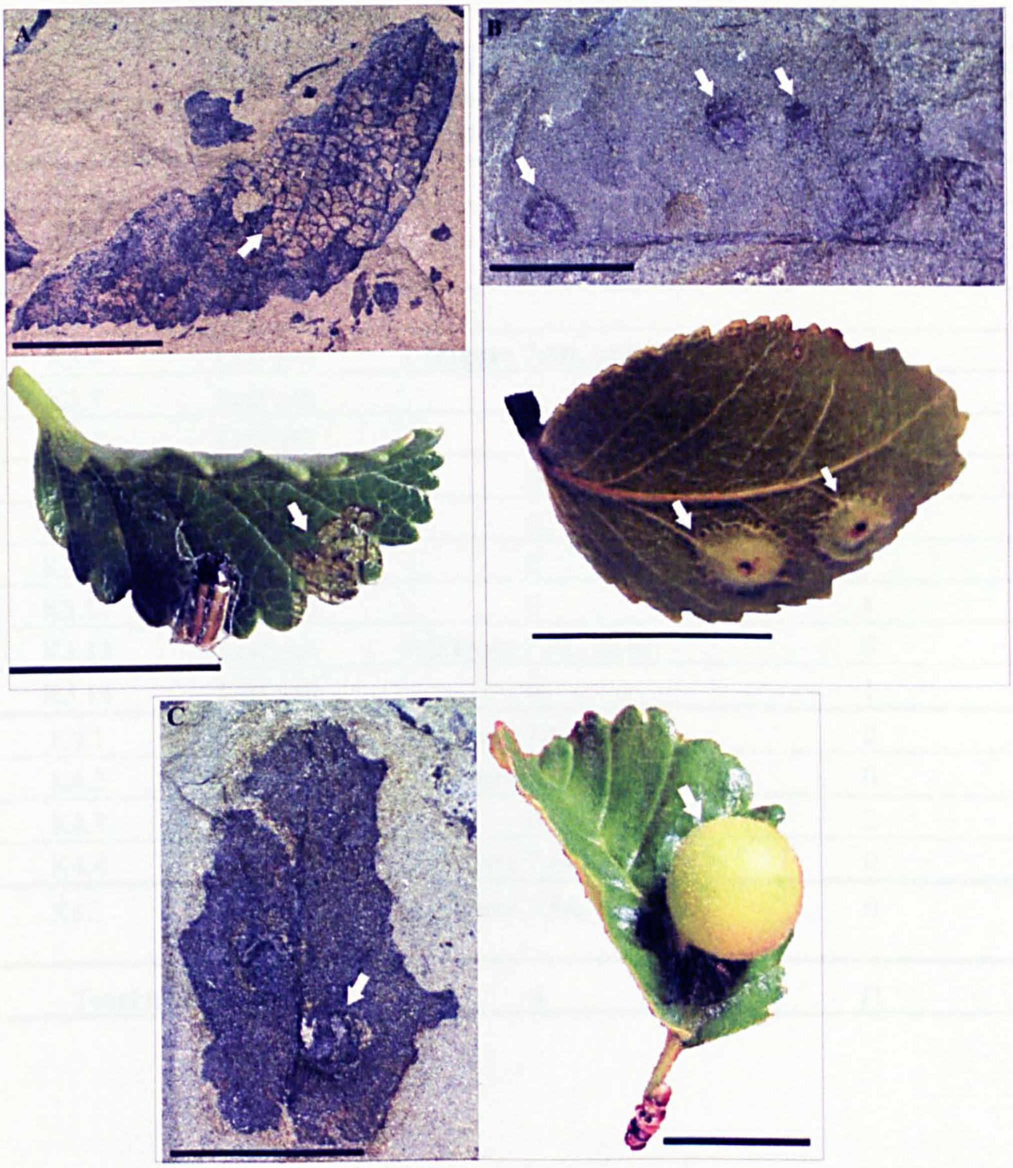


Figure 7.5. An example of fossil traces from King George Island (dark background) and comparable traces on modern leaves from Chile (white background). Skeleton feeding trace K5.1; A. P.3001.140 (2). Leaf galls: B) Trace K3.6., G.312.6 with gall type 5. C) Trace K3.13, P.3001.58.1 with gall type 1 (2). Scale bar 1cm. Arrows highlight insect traces.

7.2.1.5. Leaf mines

Three of the four leaf mine fossil traces had comparable traces on the modern leaves (Table 7.3), with both blotch and linear mines represented (Figure 7.6). A comparable trace for the very small circular blotch mine found on the fossil leaf (Trace type K4.3) was not found, possibly due to modern leaf mines only being collected when the insects that create them are at a later stage of development.

Table 7.3. Summary of the number of fossil traces collected from King George Island that are comparable with modern traces collected in Chile for leaf galls, leaf mines and traces of skeleton feeding. References are given for the corresponding photographs within the chapter and are highlighted in bold.

Trace Morphotype	Trace type	Number of fossils in morphotype similar to modern traces	Number of fossils of this morphotype not matched to a modern trace
K3.1	Leaf gall	0	3
K3.2	Leaf gall	0	1
K3.3	Leaf gall	0	2
K3.4	Leaf gall		
K3.6	Leaf gall	**1 (Figure 7.5B, p187)**	6
K3.7	Leaf gall	0	1
K3.8	Leaf gall	0	1
K3.9	Leaf gall	0	1
K3.10	Leaf gall	0	1
K3.11	Leaf gall	0	1
K3.12	Leaf gall	0	1
K3.13	Leaf gall	**1 (Figure 7.5C, p187)**	0
K3.14	Leaf gall	0	1
K4.1	Leaf mine	**1 (Figure 7.6A, p189)**	0
K4.2	Leaf mine	**1 (Figure 7.6B, p189)**	0
K4.3	Leaf mine	0	2
K4.4	Leaf mine	**1 (Figure 7.6C, p189)**	0
K5.1	Skeleton Feeding	**1 (Figure 7.5A, p187)**	0
Total trace fossils		**6**	**21**

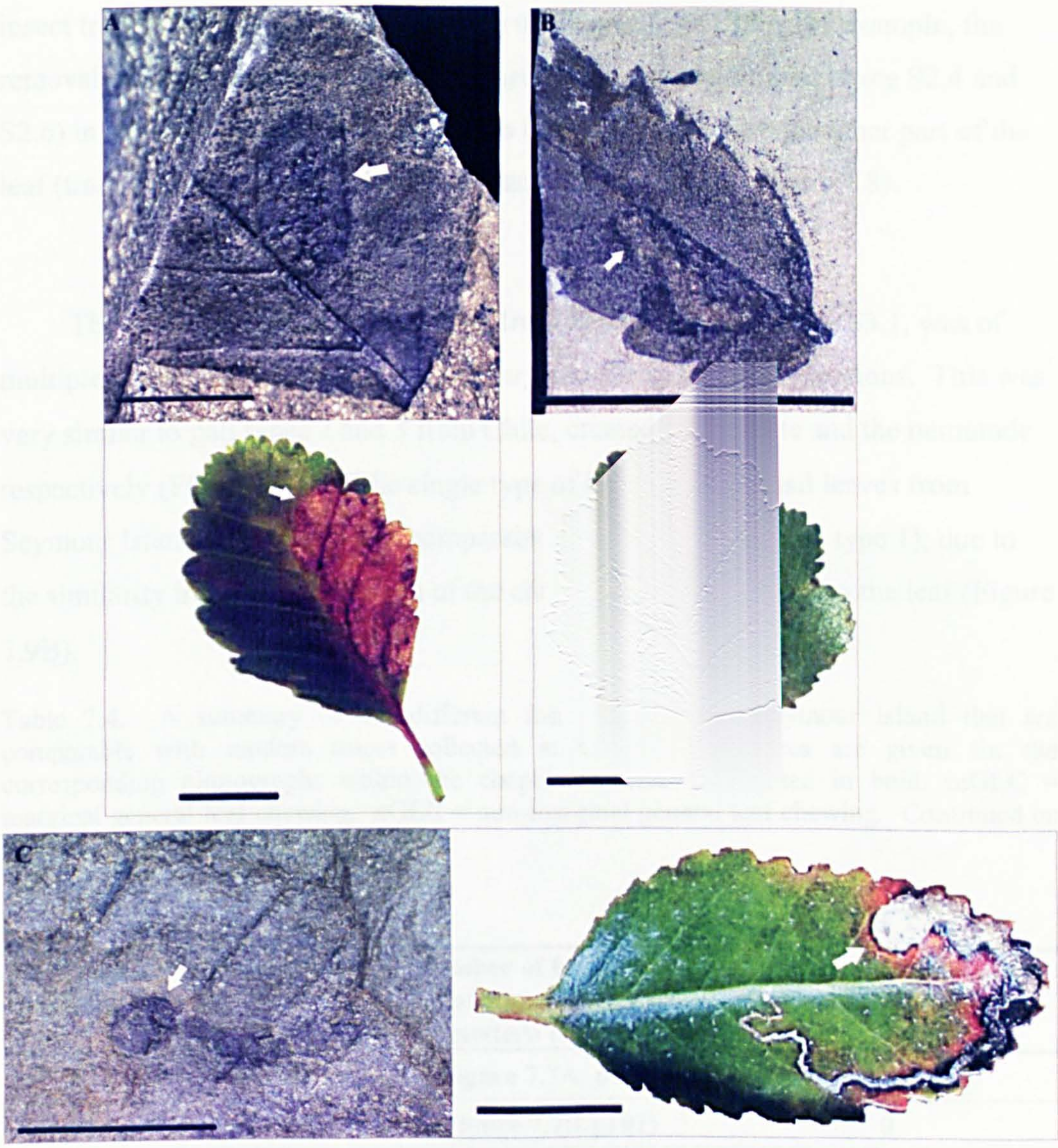


Figure 7.6. The range of leaf mine insect traces on fossil leaves from King George Island (dark background) and comparable traces on modern leaves from Chile (white background). A) Trace K4.1, P.1404.20(1) with Mine type 2. B) Trace K4.2, P.3001.81B with Mine type 7. C)Trace K4.4, G.53(20)(1) with Mine type 11. Scale bar 1cm. Arrows highlight insect traces.

7.2.2. Seymour Island Comparisons

From Seymour Island, 85.2% of the 31 fossil insect trace types had modern equivalents collected (Table 7.4). All of the general leaf chewing trace categories both marginal and non-marginal types were matched. For example, removal of a small section of the leaf margin at the basal area of the leaf in trace S1.1 was also present on several modern leaves (Figure 7.7A). The variation in non-marginal insect traces was also commonly seen on the leaves from Chile, for example, the removal of large, single, irregularly-shaped areas of the leaf tissue (trace S2.4 and S2.6) in addition to multiple small circles being removed from the inner part of the leaf (trace S2.1) was seen on both fossil and modern leaves (Figure 7.8).

The single trace of a leaf gall type from Seymour Island, trace S3.1, was of multiple small galls adjacent to the primary and secondary vein junctions. This was very similar to gall types 2 and 3 from Chile, created by the mite and the nematode respectively (Figure 7.9A). The single type of leaf mine on fossil leaves from Seymour Island, trace S4.1, was comparable to the top mine (mine type 1), due to the similarity in both the direction of the channel and the position on the leaf (Figure 7.9B).

Table 7.4. A summary of the different fossil traces from Seymour Island that are comparable with modern traces collected in Chile. References are given for the corresponding photographs within the chapter and are highlighted in bold. mGLC = marginal general leaf chewing. nGLC = non-marginal general leaf chewing. Continued on p191.

Trace Morphotype	**Trace type**	**Number of fossils in morphotype similar to modern traces**	**Number of fossils of this morphotype not matched to a modern trace**
S1.1	mGLC	3 **(Figure 7.7A, p191)**	0
S1.2	mGLC	1 **(Figure 7.7B,p191)**	0
S1.3	mGLC	2 **(Figure 7.7C, p191**	0
S1.4	mGLC	2	0
S1.5	mGLC	1	0
S1.6	mGLC	1 **(Figure 7.7D, p191)**	0
S2.1	nGLC	1 **(Figure 7.8A, p192)**	0
S2.2	nGLC	3	0
S2.3	nGLC	3	0
S2.4	nGLC	1 **(Figure 7.8B, p1192)**	0
S2.5	nGLC	1	3
S2.6	nGLC	1 **(Figure 7.8, p192)**	0
S2.8	nGLC	1	0
S2.9	nGLC	1	1
S2.10	nGLC	1	0

Trace Morphotype	Trace type	Number of fossils in morphotype similar to modern traces	Number of fossils of this morphotype not matched to a modern trace
S2.11	nGLC	1	0
S3.1	Leaf gall	**1 (Figure 7.9A, p193)**	0
S3.2	Leaf gall	0	1
S4.1	Leaf mine	1 **(Figure 7.9B, p193)**	0
Total trace fossils		**24**	**4**

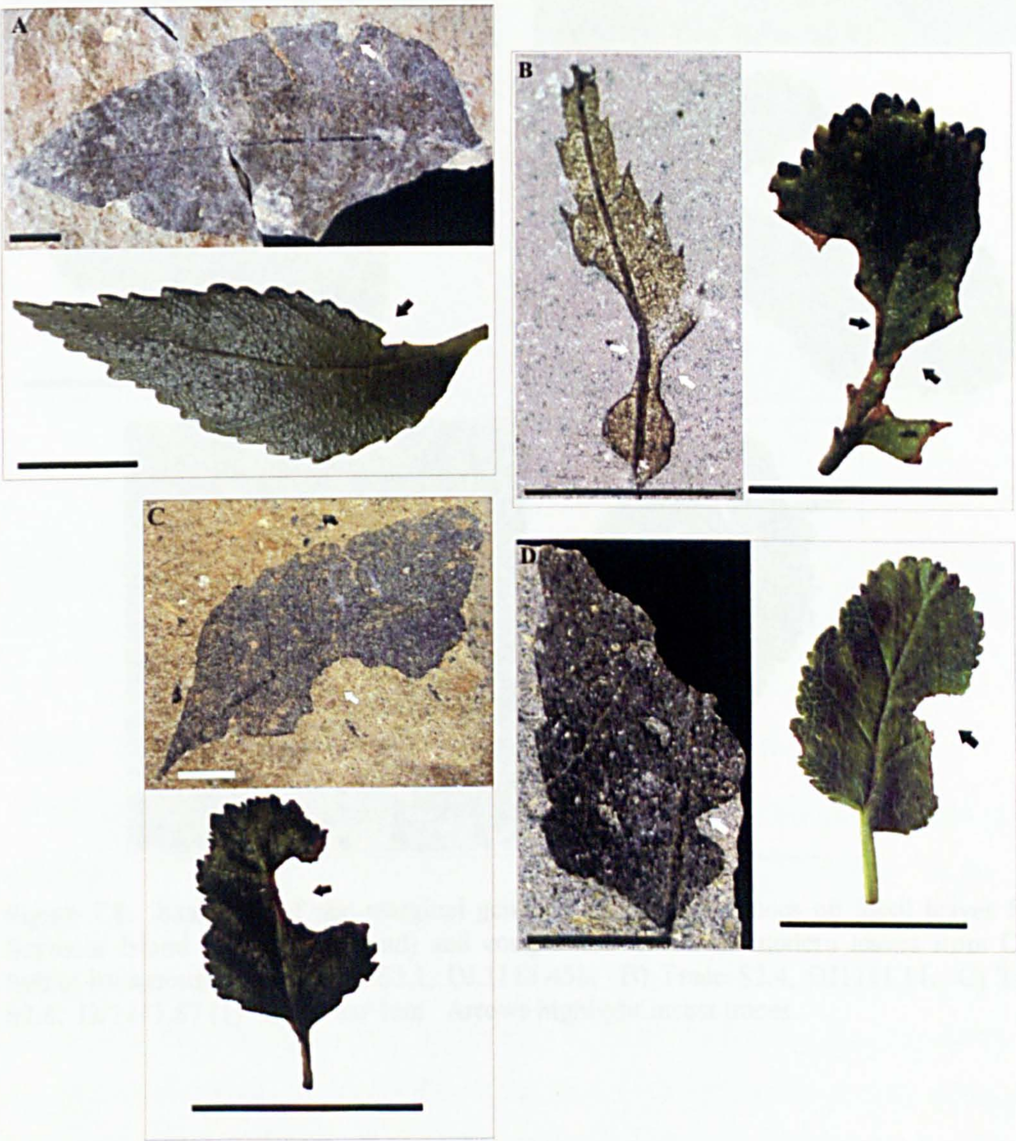


Figure 7.7. The range of marginal general leaf chewing traces on fossil leaves from Seymour Island (dark background) and comparable traces on modern leaves from Chile (white background). A) Trace S1.1: DJ.11105.88. B) Trace S1.2: DJ.1113.117a/b. C) Trace S1.3: DJ.1105.13 (1). D) Trace S1.6: DJ.1113.218. Scale bar 1cm. Arrows highlight insect traces.

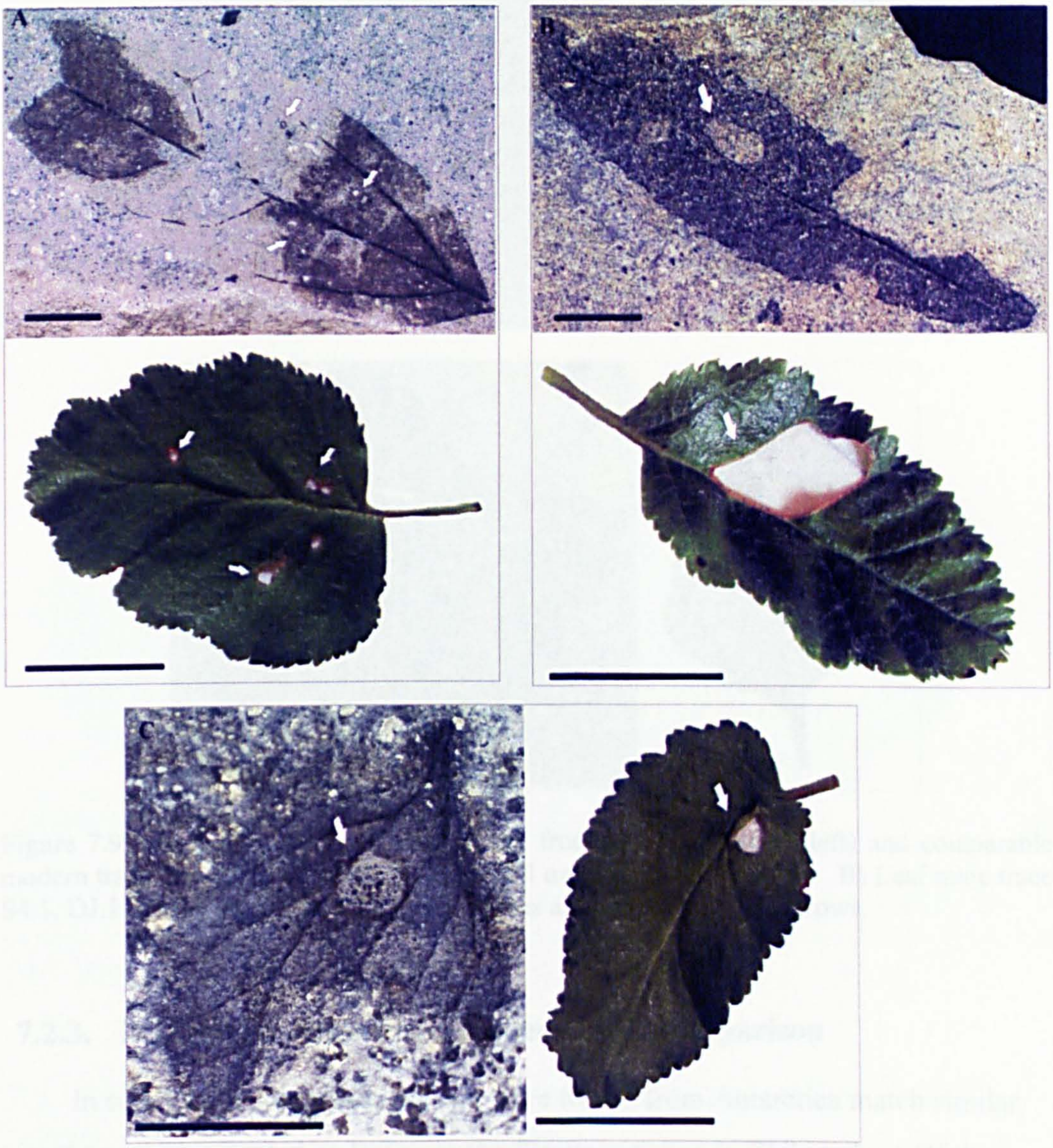


Figure 7.8. Examples of non-marginal general leaf chewing traces on fossil leaves from Seymour Island (dark background) and comparable traces on modern leaves from Chile (white background). A) Trace S2.1, DJ.1113.45b. B) Trace S2.4, DJ1111.11. C) Trace S2.6. DJ1111.87 (1). Scale bar 1cm. Arrows highlight insect traces.

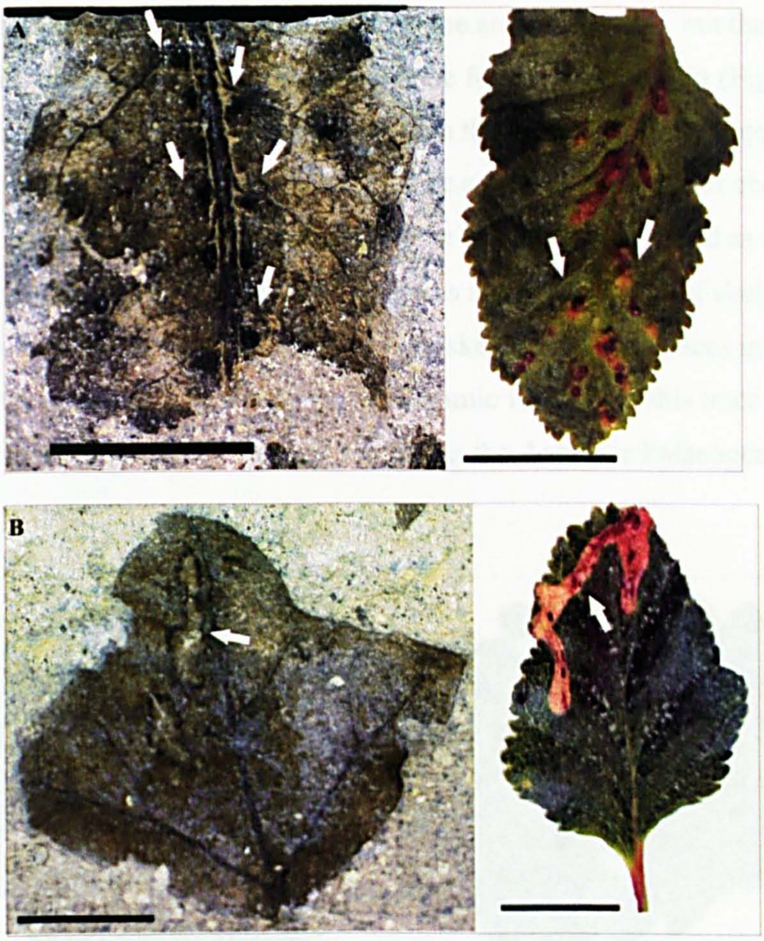


Figure 7.9. Examples of insect trace fossils from Seymour Island (left) and comparable modern traces from Chile (right). A) Leaf gall trace S3.1, DJ.1113.161. B) Leaf mine trace S4.1, DJ.1113.86. Scale bar 1cm. Insect traces are highlighted with arrows.

7.2.3. Discussion of fossil and modern trace comparison

In conclusion, many of the insect trace fossils from Antarctica match similar insect traces represented on leaves in the forests growing in Chile today. Of the fossil traces not found in modern Chile, such as the multiple, very small non-marginal marks, one possible explanation could be that the fossil traces were preserved during an earlier stage of chewing or earlier in the season and therefore the traces were small. However, the leaves from the Chilean forests were collected at different times within the season and randomly within the tree; hence all stages of leaf chewing would be sampled. Also, since there are multiple examples of this trace type, another explanation could be that the traces are actually a type of skeleton feeding. The very small traces are the same size as a range of skeleton feeding traces that were found to be concentrated beside the primary and secondary vein areas. A distinct ridge was present around the area of damage, but the inner part of the marks was quite fragile and many were found partially split (Figure 7.10). Therefore, during preservation the fragile veins in the centre of the damage could have become detached, making the trace look like a type of general leaf chewing. The evidence for skeleton feeding could therefore be under represented as it resembles general leaf chewing marks. There was a rarity of traces of skeleton feeding on the fossil leaves, but an abundance of skeleton feeding traces in the Chilean forests. This could be due to the taphonomic filtering of this trace type, creating an under estimation of skeleton feeding in the Antarctic Palaeogene forests.

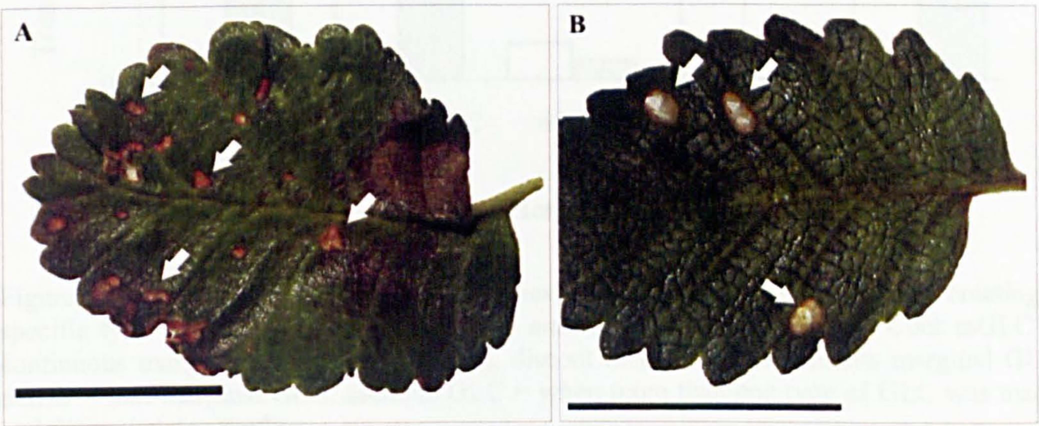


Figure 7.10. A) *N. pumilio* leaf with old multiple patches of skeleton feeding. B) *N. pumilio* leaf with multiple patches of skeleton feeding with tears in the tissue. Scale bar 1cm. White arrows highlight insect traces.

7.3. Quantitative comparisons of fossil and modern herbivory traces

7.3.1. Herbivorous insect species

To estimate the possible diversity of insect species which could have made the fossil insect traces during the Palaeogene in Antarctica, the different species that were observed making a particular trace (general leaf chewing or skeleton feeding) on the modern leaves was examined. In general, there were no trace types on either *N. pumilio* or *N. antarctica* that were created by only one species of insect (Figure 7.11). It is only when the trace types are further divided that links between types of damage and specific insect species can be made. For example, multiple non-marginal areas of leaf chewing were created by only one species (Coleoptera:

Curculionidae: Leptopinae) during the feeding observations. Multiple types of trace were also made by the same insect on the one leaf, such as skeleton feeding and continuous marginal chewing (Lepidoptera: larva species 3) (Appendix V).

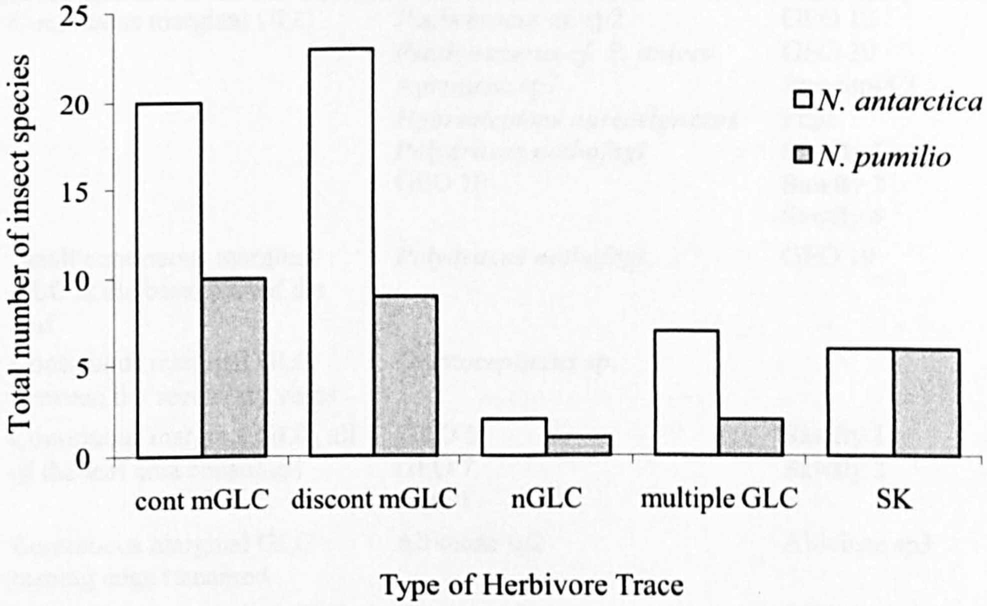


Figure 7.11. The total number of insect species (reported in Chapter 5) observed creating a specific type of trace on *N. pumilio* and *N. antarctica* leaves from Chile. Cont mGLC = continuous marginal general leaf chewing, discont mGLC = discontinuous marginal GLC nGLC = non-marginal GLC, multiple GLC = when more than one type of GLC was made and SK = skeleton feeding.

Several species of insect were, however, observed making all the other trace types. The highest number of insect species was associated with marginal leaf chewing (65 species, 76.5% of all species), in particular, discontinuous marginal feeding (30 species, 46.2% of all species making marginal traces). The numerous insect species making each trace type (Table 7.5, 7.6 and Appendix V) suggests that many species could also have created each of the fossil traces. The trace types collected in Chile were very similar to the insect trace fossils, therefore, it is highly likely that each fossil trace type in the general leaf chewing categories does not represent just one insect species, but actually several insect species. Hence, as there were 37 trace types from King George Island and 16 trace types from Seymour Island, there could have been at least 37 species and 16 species of insect respectively making such traces. There were probably considerably more herbivorous insect species .

Table 7.5. The different trace types created during feeding observations by different species of insect on *N. antarctica* leaves. Insect species that were not identified were separated into morphospecies. Insect species that created more than one type of trace are in bold. GLC = general leaf chewing. GEO = Geometridae, COL = Coleoptera, and LEP = Lepidoptera.

Trace description	Insect species observed creating the trace	
Continuous marginal GLC	*Pachybracus* cf. sp2	GEO 18
	Psathyrocerus cf. P. unicor	GEO 20
	Apioninae sp7	Parasitoid 2
	Hybreoleptops aureosignatus	Pupa 1
	Polydrusus nothofagi	**Sawfly 1**
	GEO 10	**Sawfly 2**
		Sawfly 4
Small continuous marginal GLC at the basal part of the leaf	***Polydrusus nothofagi***	GEO 19
Continuous marginal GLC between the secondary veins	***Cryptocephatus* sp.**	
Continuous marginal GLC, all of the leaf area consumed	**GEO 5**	**Sawfly 1**
	GEO 7	**Sawfly 2**
	Pupa 1	
Continuous marginal GLC rasping edge remained	Alticinae sp2	Alticinae sp3
Discontinuous marginal GLC	***Cryptocephatus* sp.**	GEO 1
	Eumolpinae sp2	GEO 2
	Eumolpinae sp3	GEO 3
	Eumolpinae sp4	GEO 4
	Eumolpinae sp6	**GEO 5**
	Psathyrocerus cf. P. unicor	GEO 8
	COL 1	GEO 13
	Anthribidae sp2	*Perilopsis flava*
	Apioninae sp2	**Sawfly 2**
	Dasydema hirtella	Sawfly 4
	Polydrusus nothofagi	***Hybreoleptops aureosignatus***
Discontinuous marginal GLC with rough edges created	**Eumolpinae sp3**	***Polydrusus nothofagi***
Single non-marginal GLC	***Hybreoleptops aureosignatus***	
Multiple non-marginal GLC	Leptopinae sp1	
Continuous marginal GLC & skeleton feeding	LEP 3	
Discontinuous marginal GLC & skeleton feeding	***Cryptocephatus* sp.**	
Discontinuous marginal & non-marginal GLC	GEO 19	
Skeleton feeding	cf. *Varicoxa*	Apioninae sp6
	COL 3	LEP 1
	Smurf sp.	Sawfly 5

Table 7.6. The different trace types created during feeding observations by different species of insect on *N. pumilio* leaves. Insect species that were not identified were separated into morphospecies. Insect species that created more than one type of trace are in bold. GLC = general leaf chewing. GEO = Geometridae, COL = Coleoptera and LEP = Lepidoptera.

Trace Description	**Insect species observed creating the trace**	
Continuous marginal GLC	*Aegorhinus vitulus vitulus* ***Hybreoleptops aureosignatus*** GEO 10	GEO 15 LEP 1 **Sawfly 2** Sawfly 4
Small continuous marginal GLC at the basal part of the leaf	Apioninae sp6	
Continuous marginal GLC between the secondary veins	**Sawfly 1**	
Continuous marginal GLC, all of the leaf area consumed	GEO 5	
Discontinuous marginal GLC	*Psathyrocerus cf. P. unicor* COL 1 ***Hybreoleptops aureosignatus*** ***Polydrusus nothofagi***	*Polydrusus sp.* GEO 9 **Sawfly 1** **Sawfly 2** *Plagiotelum irinum solier*
Single non-marginal GLC	Sawfly 4	
Continuous & discontinuous marginal GLC	***Polydrusus nothofagi***	
Skeleton feeding	cf. *Varicoxa* Apioninae sp4 Apioninae sp5	*Eucalus thoracicus* *Parergus axillaris* micromoth 1

7.3.2. Occurrence of leaf damage

The proportion of herbivory on fossil leaves in the fossil assemblages was calculated as 9.75% of leaves for King George Island and 2.64% of leaves for Seymour Island (Chapter 3). In comparison to the modern study sites (Chapter 4), the extent of herbivore damage was lower in the fossil floras, as damage to the leaves in Chile ranged from 9.05% to 48.25% for *N. antarctica* and 9.61% to 60.08% for *N. pumilio* (Chapter 6, Figures 6.2 and 6.3). The variation in the extent of insect damage for each site in Chile is large and, despite no significant latitudinal trend (Chapter 6), the most southern sites, RNMag and Isla Nav, have the lowest median of insect-damaged leaves. This is very similar to the proportion of insect-damaged leaves amongst the fossils of King George Island.

7.3.3. Intensity of Skeleton Feeding

The level of damage from skeleton feeding on the fossil leaves was also lower than that on leaves from modern sites (only one example found from the fossil leaves from King George Island and none from Seymour Island). The intensity of damage from skeleton feeding varies for each modern site, but it was present at all sites (Figure 7.12 and Figure 7.13). For both tree species there was statistically no significant difference in skeleton feeding levels between sites. Although there was site variation in the level of skeleton feeding, the extent of damage by skeleton feeding was generally quite low compared to the level of general leaf chewing. The level of skeleton feeding in Chile, however, was still higher than the level in the fossil floras. For *N. antarctica*, the median levels of skeleton feeding for all sites were very low; the largest proportion of skeleton feeding was found at Isla Nav and the smallest at Tolh. Similar patterns were observed for *N. pumilio*, with very low proportions of skeleton feeding at all sites, but Nah had the largest proportion of skeleton feeding on the leaves and Torres had the smallest (Figure 7.13).

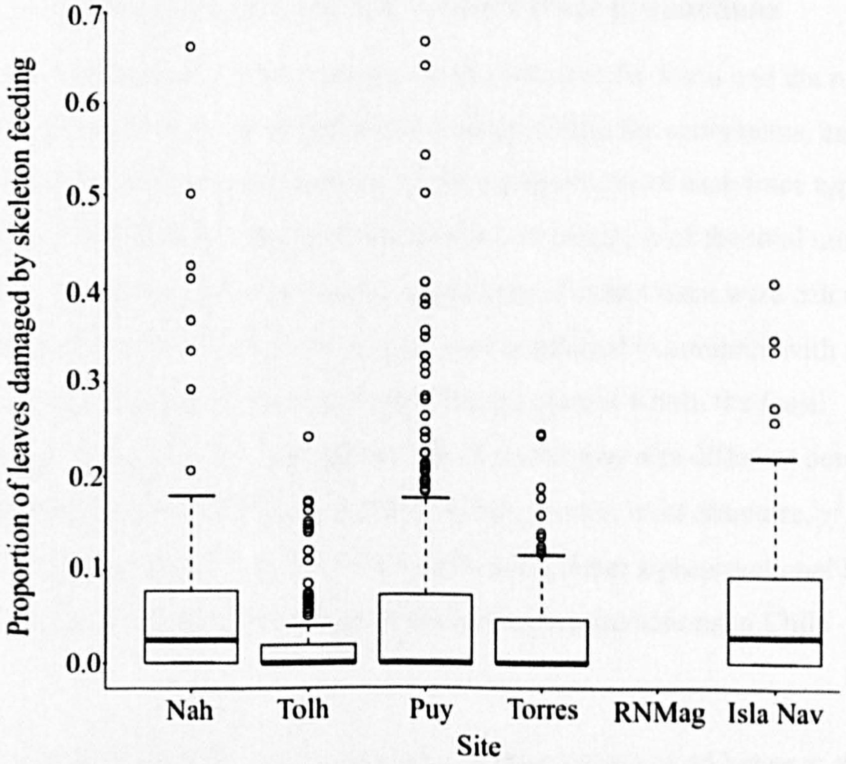


Figure 7.12. The proportion of *N. antarctica* leaves damaged by skeleton feeding at each sampling site (quantitative method).

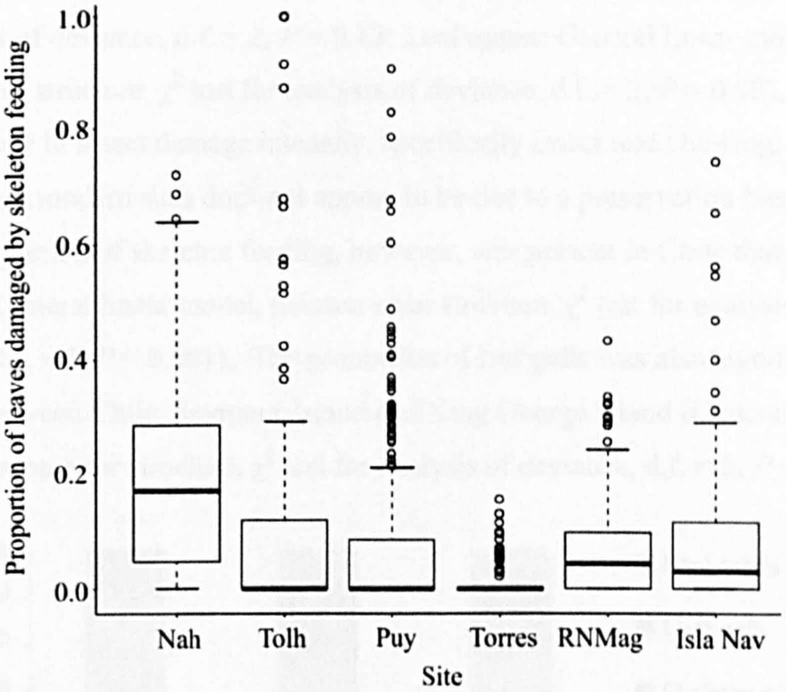


Figure 7.13. The proportion of *N. pumilio* leaves damaged by skeleton feeding at each sampling site (quantitative method).

7.3.4. Comparisons of fossil and modern trace proportions

If the differences in insect damage levels between the fossil and the modern sites is representative of the actual insect damage within the ecosystems, and is not due to a preservation artefact, then the relative proportions of each trace type would be the same for the fossil sites as in the modern, irrespective of the total number collected. Therefore, the proportions of each type of insect trace were calculated. The data for *N. pumilio* and *N. antarctica* were combined to compare with the fossil systems more closely as several plant species are present within the fossil assemblages (Figure 7.14). The proportion of trace types was different between the fossil localities and Chile (General linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 6, $P < 0.001$) indicating either a preservational bias or a possible difference due to evolution of further insect associations in Chile

To discover however the assemblages of trace types varied between the sites, each trace type was then analysed separately. For both general leaf chewing and leaf mines there were no significant differences between the proportions at each locality (General leaf chewing: General linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 2, $P = 0.12$; Leaf mines: General linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 2, $P = 0.98$). Hence, the difference in insect damage intensity, specifically insect leaf chewing, between the fossil and modern sites does not appear to be due to a preservation bias. A greater proportion of skeleton feeding, however, was present in Chile than the fossil localities (General linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 2, $P < 0.001$). The proportion of leaf galls was also significantly different between Chile, Seymour Island and King George Island (General linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 2, $P < 0.001$).

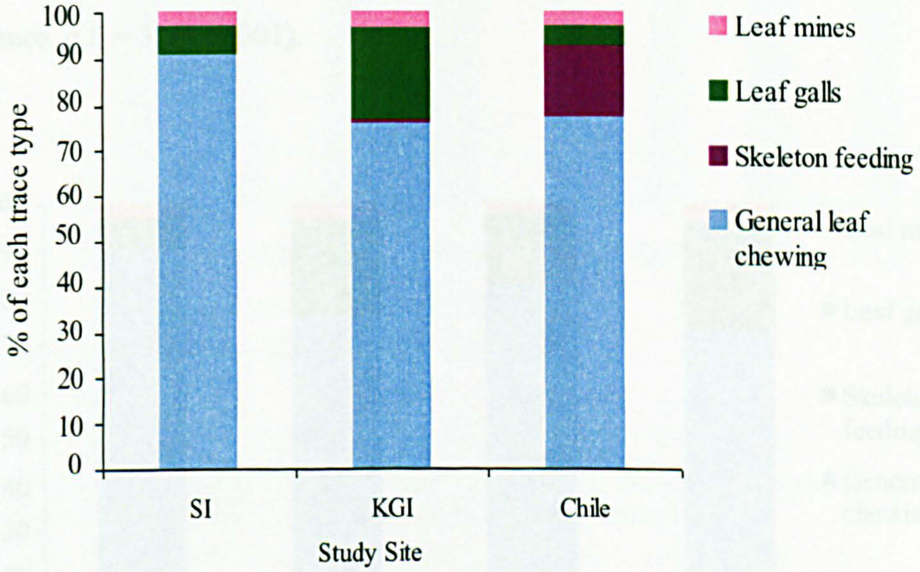


Figure 7.14. The proportion of different herbivore trace types collected for the two fossil sites, Seymour Island and King George Island and the modern forests in Chile.

The proportions of each trace type from the fossil localities were also compared with the proportions found on the two modern tree species (Figure 7.15). This was to examine the effect that tree species may have had on the proportions of each trace type, because one tree species may have had more insect species associated with it and as a consequence a greater number of specific trace types. There was a significant difference in the proportions of each trace type between the fossil sites and the data for each living tree species (Generalized linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 9, $P < 0.001$). The proportion of leaf galls on King George Island was greater than the proportion of galls on both Seymour Island and on the modern *N. pumilio* and *N. antarctica* leaves (Generalized linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 3, P <0.001). As mentioned in Chapter 3, this was attributed to a high proportion of galls on fossil leaves from the Dufayel Island locality in the King George Island assemblage (60% of total number of leaves with galls). The galls were on 5 plant morphotypes, but the majority of galls were found on leaves belonging to the Myricaceae and Lauraceae. Only one gall, from the Dufayel Island locality, was on Nothofagaceae leaves. This may explain the greater proportion of leaf galls in the King George Island assemblage than from the other sites. The proportion of skeleton feeding on the modern leaves was also greater than in the fossil sites (Generalized linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 3, P <0.001).

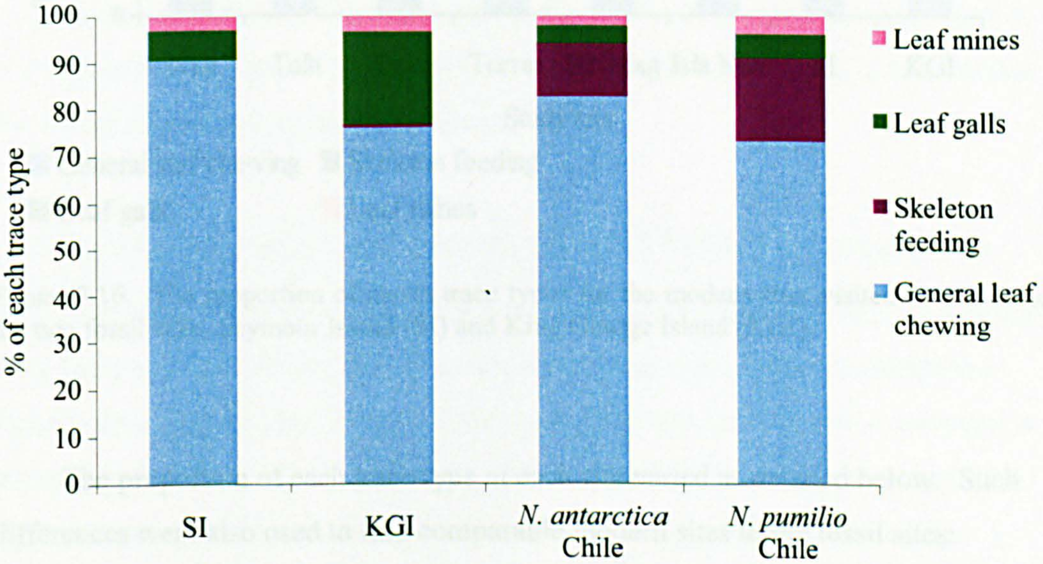


Figure 7.15. The relative proportions of each herbivore trace category at each site, including the two fossil localities, King George Island (KGI) and Seymour Island (SI) and the main tree species examined in Chile, *N. antarctica* and *N. pumilio*.

The proportion of each herbivore trace type at each site was also examined (Figure 7.16). There are distinct variations between not only the fossil and modern sites, but also within the modern sites. For all sites, the largest proportion of leaf damage was due to general leaf chewing, even though RNMag and Isla Nav, the more southerly sites, had a lower overall proportion of this type of trace. The next most abundant trace type at the modern sites was skeleton feeding on the leaves. The next most abundant trace type became dependent on the site. For the northern sites and Torres, leaf galls were the next most common and then leaf mines. For the fossil sites, leaf galls were the most abundant trace type after general leaf chewing and then leaf mines.

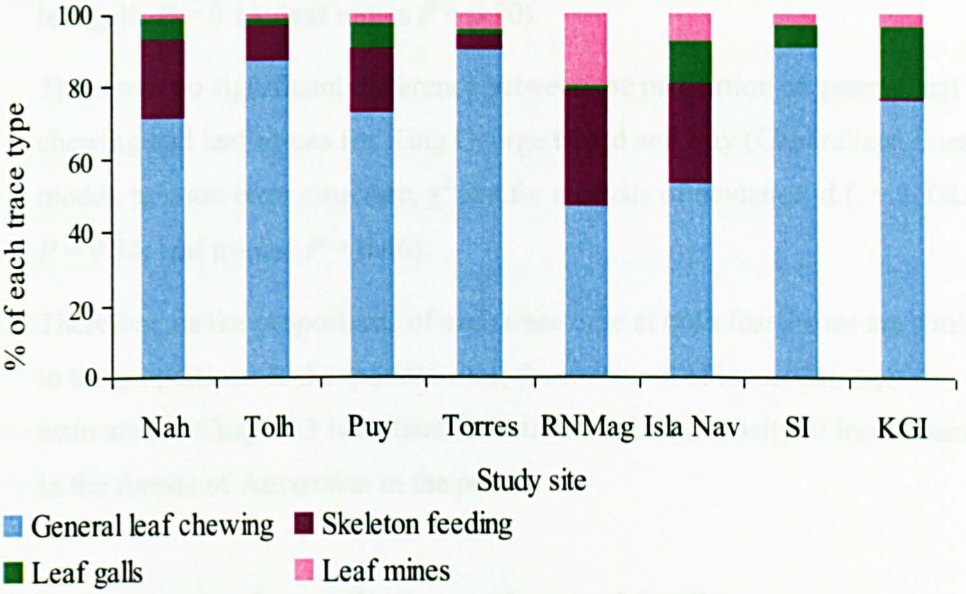


Figure 7.16. The proportion of insect trace types for the modern sites visited in Chile and the two fossil sites, Seymour Island (SI) and King George Island (KGI).

The proportion of each trace type at each site varied as detailed below. Such differences were also used to find comparable modern sites to the fossil sites:

- The proportion of traces that were leaf mines at the southern sites was different from both the modern and fossil sites. RNMag had a significantly greater proportion of mines than the other sites (northern sites consist of Nah, Tolh, Puy: Generalized linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 3, $P < 0.001$; southern sites consist of Torres, Isla Nav, King George Island and Seymour Island: Generalized linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 4, $P < 0.001$).

- Both RNMag and Isla Nav had a greater proportion of skeleton feeding traces than the other modern sites or the fossil sites. The most notable difference between the modern and fossil sites is the significantly lower number of skeleton feeding traces recorded from the fossil localities.
- The proportion of insect trace types for Seymour Island was similar to the proportion at Torres (Generalized linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 1; GLC $P = 0.99$, Skeleton feeding $P = 0.12$, leaf galls $P = 0.13$, leaf mines $P = 0.80$).
- There was no significant difference between the proportion of general leaf chewing and leaf mines for King George Island and Puy (Generalized linear model, poisson error structure, χ^2 test for analysis of deviance, d.f. = 1, GLC: $P = 0.34$, leaf mines: $P = 0.46$).
- Therefore, as the proportions of each trace type at both fossil sites are similar to the proportions at the modern sites, the low level of insect damage estimated in Chapter 3 is a plausible estimate of the intensity of insect damage in the forests of Antarctica in the past.

7.3.5. Summary of quantitative modern and fossil trace comparison

In general, the range of fossil trace types appears comparable with the modern trace types collected from Chile. There were no trace types that were exclusively found only on modern leaves or on fossil leaves; the same trace types were found in the modern and fossil leaf assemblages. If the level of insect damage on the leaves for both general leaf chewing and skeleton feeding are considered, the fossil localities are more similar to the southern sites in Chile than the northern sites (Figure 7.12 & 7.13). The fossil sites are, however, more similar to the northern sites and Torres when the proportions of trace types at each locality are considered. There are also similar trace types to the fossil traces that are found only in the northern sites. The difference in the extent of damaged leaves, however, is greater for the northern and fossil sites than for the southern and fossil sites.

7.3.6. Comparison of herbivory traces from the fossil localities with those in Chile.

The previous results indicated similarities in the proportions of general leaf chewing and leaf mines in the King George Island flora and the Puy forests. Seymour Island and Torres also had similar proportions of each trace type. This relationship was tested further using cluster analysis (method explained in Section 3.4.1) to determine the similarity within the modern and fossil sites. The similarities in the herbivory traces at each fossil and modern site may also indicate similar climates as the insects that created the traces could only be present within certain temperature or precipitation ranges (Chapter 8). The characters used to perform the test were: proportion of insect-damaged leaves, proportion of damaged leaves with skeleton feeding, proportion of the damaged leaves with general leaf chewing, proportion of damaged leaves with leaf mines and the proportion of damaged leaves with leaf galls. Although the characters do not need to be given codes, Gower's method for distance was used as it is proportional data and Ward's method was used as the cluster algorithm. Bootstrapping was also carried out to give p-values to test the reliability of the groupings.

The dendrogram (Figure 7.17) suggests that the trace fossils in the fossil sites form a distinct cluster, but are more similar to those from the northern sites of Nah, Tolh and Puy, and the southern site of Torres. The northern sites are clustered together with Torres as a significant grouping, with Isla Nav and RNMag forming a separate group. This is in accordance with previous results that indicate that King George Island and Seymour Island have similar trace proportions to Torres and Puy (Section 7.3.4). The separation of the fossil sites from the northern sites and Torres may be caused by the difference in leaf damage level between the modern and fossil sites. The characters used in the analysis such as trace proportion was similar for the fossil localities, the northern sites and Torres, but the proportion of insect-damaged leaves was different (Chapter 6). The proportion of insect-damaged leaves at the fossil sites was very low in comparison to the proportion at the modern sites, in particular Torres, Nah and Tolh.

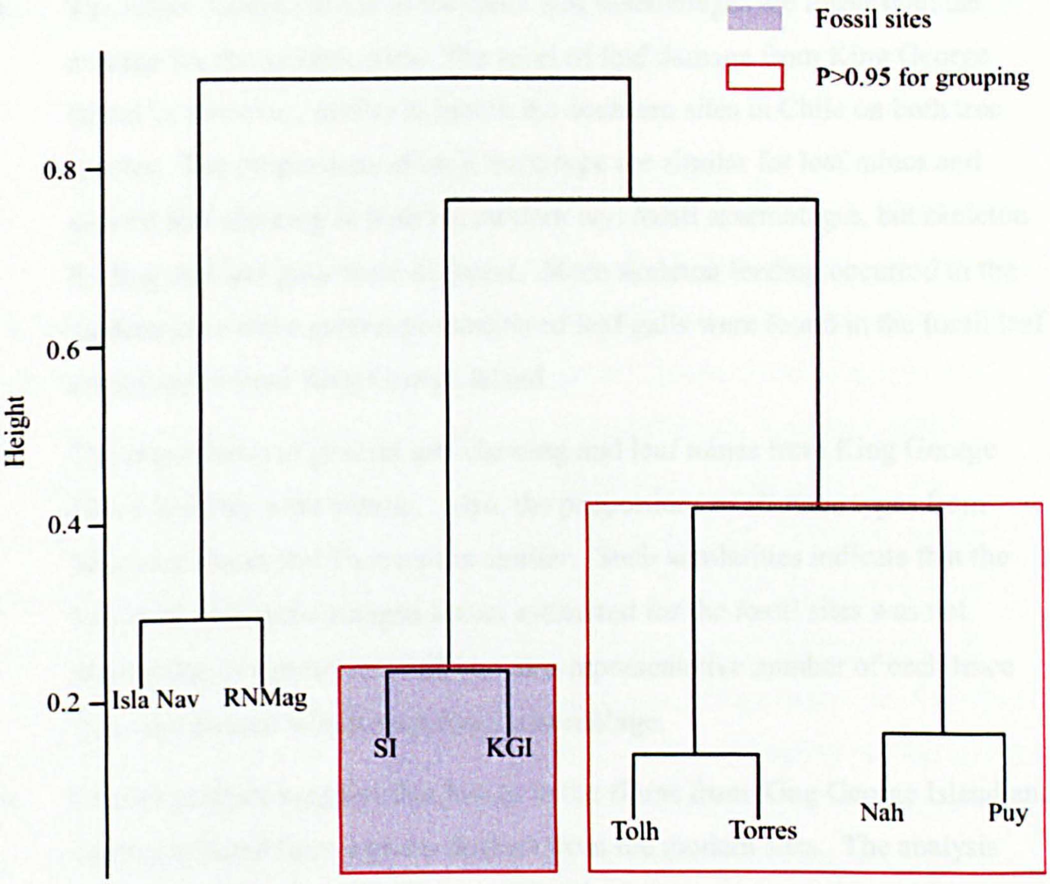


Figure 7.17. Dendrogram showing the results of the cluster analysis to determine similarities between insect trace proportions in the modern sites in Chile and the fossil sites in Antarctica. The clusters with red rectangles indicate the reliable clusters with 95% confidence. The y-axis is a measure of distance between each separation, which allows the tree to be cut at various points in the analysis.

7.4. Summary

- Traces from all insect herbivory categories (general leaf chewing, skeleton feeding, leaf mines and leaf galls) that were found on the fossil leaves from Antarctica were represented within the modern sites in Chile.
- There were no herbivory traces that were exclusive to either the fossil or modern assemblages.
- Several insect species were observed making similar general leaf chewing traces. In addition, the same insect species created several different chewing traces. Therefore, the presence of one fossil insect trace could represent several different insect species.

- The insect damage levels in the fossil leaf assemblages are lower than the average for the modern sites. The level of leaf damage from King George Island is, however, similar to that in the southern sites in Chile on both tree species. The proportions of each trace type are similar for leaf mines and general leaf chewing in both the modern and fossil assemblages, but skeleton feeding and leaf galls were different. More skeleton feeding occurred in the modern sites and a greater proportion of leaf galls were found in the fossil leaf assemblages from King George Island.
- The proportions of general leaf chewing and leaf mines from King George Island and Puy were similar. Also, the proportions of all trace types from Seymour Island and Torres were similar. Such similarities indicate that the low level of insect-damaged leaves estimated for the fossil sites was not mainly due to a preservational bias as a representative number of each trace type was present within each fossil assemblage.
- Cluster analysis suggests that leaves in the floras from King George Island and Seymour Island form a group distinct from the modern sites. The analysis shows that they are, however, more similar to the herbivory traces from the northern sites and Torres in Chile than the southernmost sites of Isla Nav and RNMag.
- The similarities between the fossil leaf assemblages and the specific modern sites enables the trace fossils to be used to provide other environmental estimates, such as temperature and precipitation, for the forests in Antarctica in the past. The differences between the fossil and modern sites mentioned above, were attributed to either a preservation bias or a greater number of galls at a locality that was not *Nothofagus* dominant.

Chapter 8. The Climatic Significance of Fossil and Modern Trace comparisons

8.1. Climatic range of herbivorous insects in Chile

The climate data for the study areas in Chile were obtained from the Dirección Meteorológica de Chile (www.meteochile.cl) for both the study period (Dec 2006 to March 2007) and the previous year, which includes January 2006 to March 2007. The average monthly maximum and minimum temperatures, the total monthly precipitation and the total monthly hours of daylight were obtained from the meteorological stations nearest to the study sites (Figure 8.1). The most northerly station, Aerodrome Maquehue at Temuco (38°45'S, 72°38'W), is situated at an altitude of 114m and is the closest station to the Nahuelbuta (140km away) and Tolhuaca (70km away) study sites. The station closest to Puyehue is the Aerodrome Cañal Bajo at Osorno (40°36'S, 73°04'W) and it is at 65m elevation (45 km away). The most southerly station is the airport at President Carlos Ibáñez del Campo at Punta Arenas (53°00'S, 70°58'W) situated at an altitude of 37m above sea level. This meteorological station is closest to the sites at Torres (290km away), RNMag (25km away) and Isla Nav (300km away). For the remainder of this section the stations will be named, in latitudinal order, Temuco, Osorno and Punta Arenas. All stations are located at lower elevations than the elevations at which the trees were sampled, and therefore the measurements used are likely to represent the top end of the range for temperature and at the lower end of the range for precipitation for the sites visited.

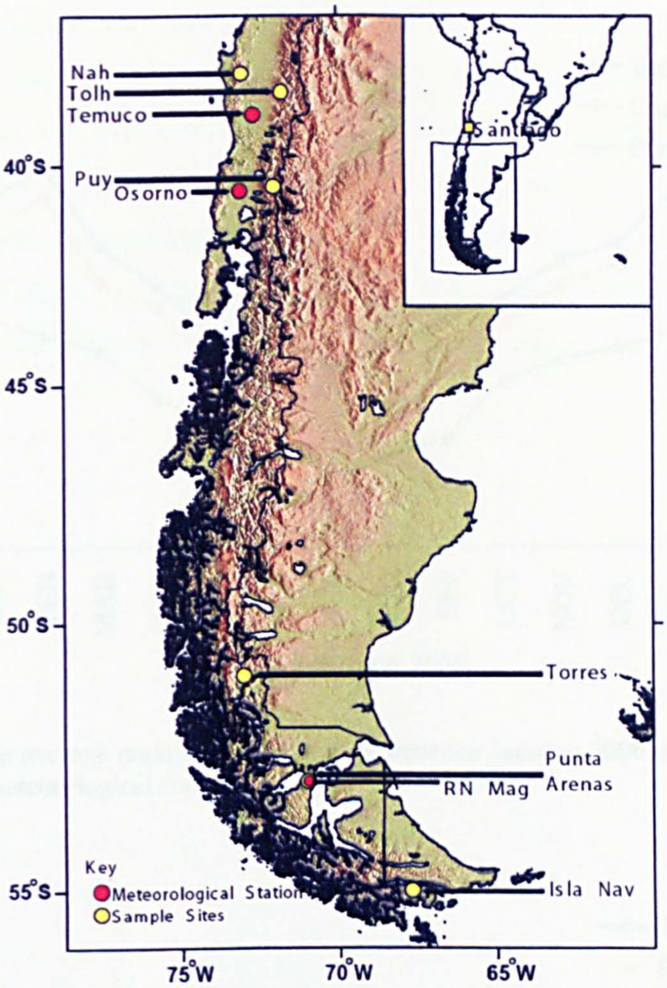


Figure 8.1. Map of Chile showing the location of the meteorological stations used to obtain climate data for the sampling sites.

The climate data for all sites are presented in Figures 8.2 – 8.4. The average maximum and minimum temperatures vary as expected between the three stations, with both maximum and minimum monthly temperatures having a negative relationship with latitude (Figure 8.2 and 8.3). The most northerly station, Temuco, has the highest maximum monthly temperature, and the most southerly station has the lowest maximum monthly temperature (Figure 8.2). The minimum monthly temperature has a more complex relationship with latitude. The minimum monthly temperature at Temuco is generally lower than at Osorno and only during the winter months (May to August) is the minimum monthly temperature at Temuco higher than Osorno (Figure 8.3). The most southerly site, however, still has the lowest minimum monthly temperature throughout the year.

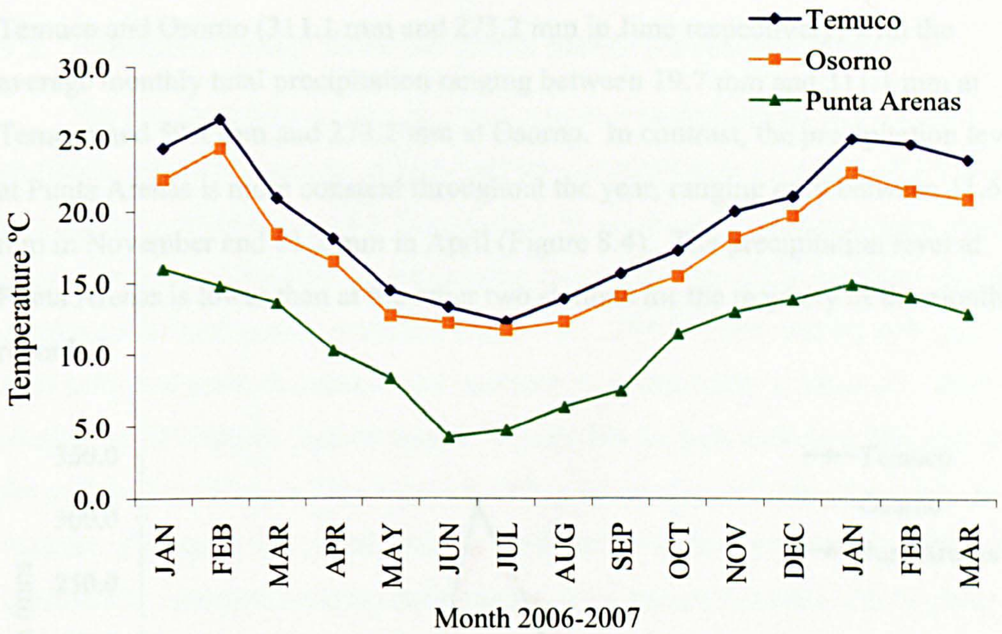


Figure 8.2. The average maximum temperature between January 2006 and March 2007 in Chile at three meteorological stations.

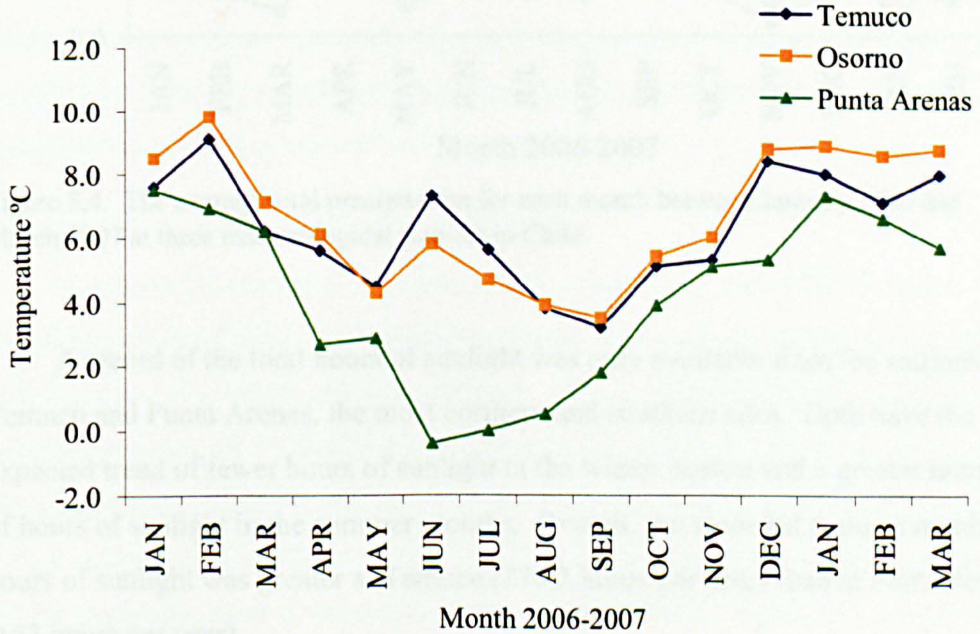


Figure 8.3. The average minimum monthly temperature between January 2006 and March 2007 in Chile at three meteorological stations.

The average monthly total precipitation peaks in the winter season at both Temuco and Osorno (311.1 mm and 273.2 mm in June respectively) with the average monthly total precipitation ranging between 19.7 mm and 311.1 mm at Temuco and 59.1 mm and 273.2 mm at Osorno. In contrast, the precipitation level at Punta Arenas is more constant throughout the year, ranging only between 11.6 mm in November and 81.2 mm in April (Figure 8.4). The precipitation level at Punta Arenas is lower than at the other two stations for the majority of the months recorded.

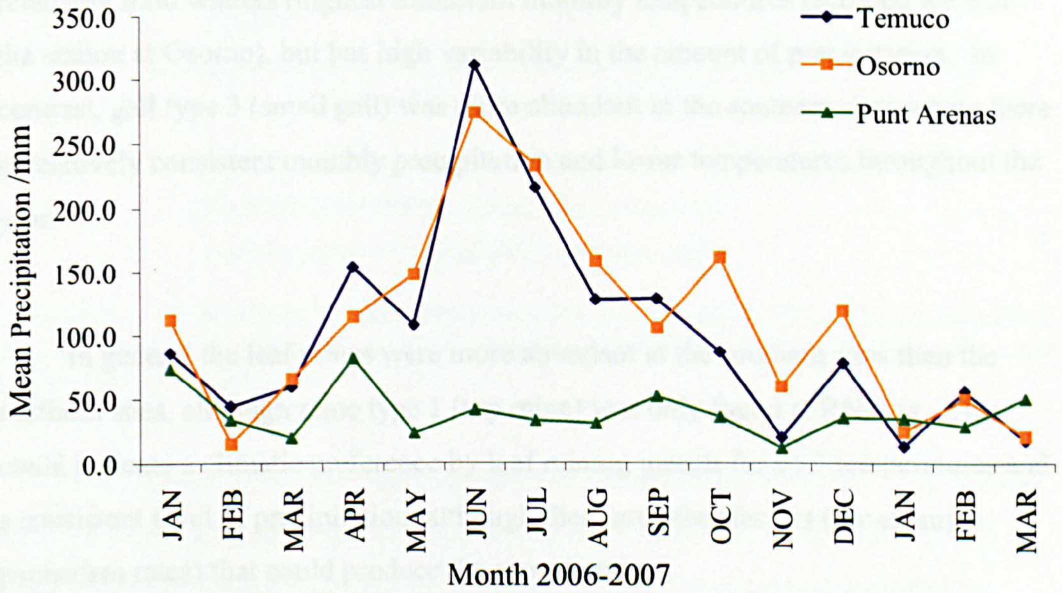


Figure 8.4. The average total precipitation for each month between January 2006 and March 2007 at three meteorological stations in Chile.

A record of the total hours of sunlight was only available from the stations at Temuco and Punta Arenas, the most northern and southern sites. Both have the expected trend of fewer hours of sunlight in the winter season and a greater number of hours of sunlight in the summer months. Overall, the recorded average number of hours of sunlight was greater at Temuco (170.2 hours per year) than at Punta Arenas (153 hours per year).

8.2. Climatic range deduced from insect traces in Chile

The site specificity of certain insect species was highlighted in Chapter 4, in particular for the leaf miners and leaf gallers. In addition to the range of the plant host species and other isolating factors, such as geographical features and habitat fragmentation, one possible factor limiting the distribution of the herbivorous insect species could be climatic conditions (Bale *et al.*, 2002). For example, gall type 1 (ball gall) was more abundant in the northern sites, especially at Puyehue, which has relatively mild winters (highest minimum monthly temperatures recorded were at the station at Osorno), but has high variability in the amount of precipitation. In contrast, gall type 3 (small gall) was more abundant in the southern sites where there is relatively consistent monthly precipitation and lower temperatures throughout the year.

In general the leaf mines were more abundant at the southern sites than the northern sties, although mine type 1 (top mine) was only found at RNMag. This could indicate a climatic preference by leaf mining insects for cool temperatures and a consistent level of precipitation, although there are other factors (for example, parasitism rates) that could produce the same patterns.

A quantitative method, Coexistence Approach (CA), was used to obtain temperature estimates for the insect trace fossils. CA uses the nearest living relative of a fossil taxon to obtain climatic extremes. Where several fossil taxa exist then the climate estimates for all fossil taxa are obtained (Yang *et al.*, 2007). A region of overlap, or coexistence, is obtained where the climatic tolerances of all taxa are within the boundaries (Figure 8.5). This is used to give the palaeoclimate estimates for the study site, such as mean annual temperature (Mosbrugger & Utescher, 1997).

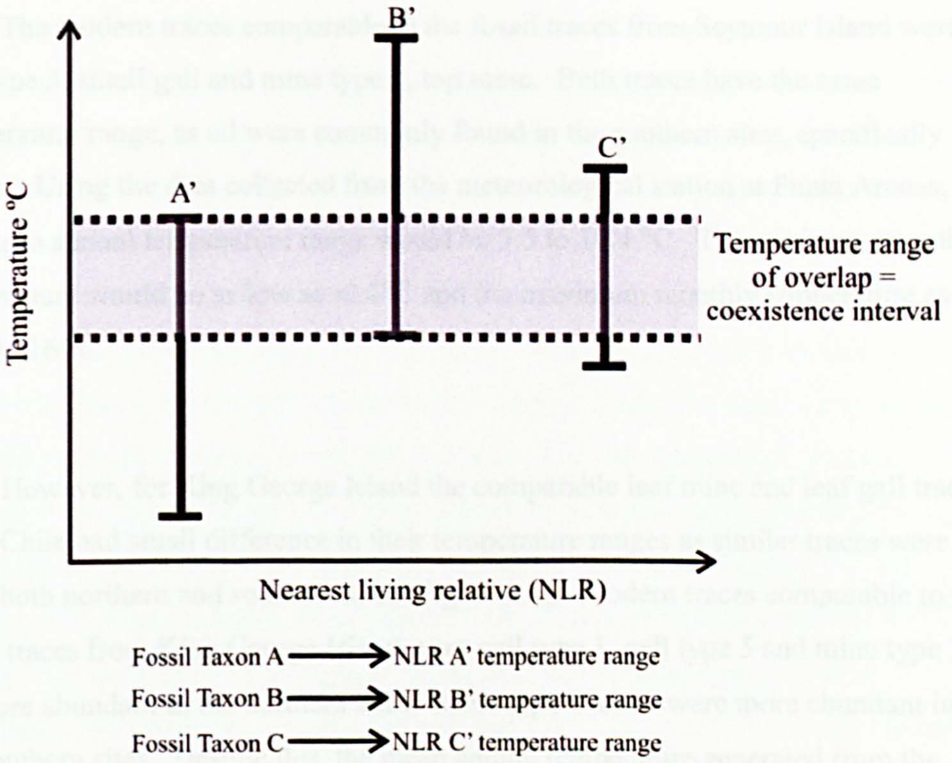


Figure 8.5. The principles of coexistence analysis (CA) used to obtain climate estimates, such as temperature, for fossil taxa by comparison with the climatic tolerances of nearest living relatives (NLRs).

The Coexistence Approach was modified to enable insect trace fossils, instead of insect fossil taxa, to be used to generate climate estimates. The nearest living relative used in the analysis was a comparable modern insect trace instead of the modern insect taxon. The modern insect traces from Chile that were comparable with the insect trace fossils from each fossil site were described in Chapter 7. Only comparable leaf gall and leaf mine traces were included due to such trace types being species specific. The presence of each comparable trace in the modern Chilean sites was used to give a temperature tolerance range for each trace. The temperatures were obtained from the meteorological stations as described in Section 8.1. In Chapter 7, the ratio of trace types at each modern site was compared to the ratio of trace types at the fossil sites. This indicated that the King George Island fossil traces were most similar to those at the modern Puyehue site and the Seymour Island fossil traces were most similar to those at the modern Torres site. The temperature range for the most similar modern site was included in Figure 8.6 to obtain the region of overlap for the fossil assemblage.

The modern traces comparable to the fossil traces from Seymour Island were gall type 3, small gall and mine type 1, top mine. Both traces have the same temperature range, as all were commonly found in the southern sites, specifically Torres. Using the data collected from the meteorological station at Punta Arenas, the mean annual temperature range would be 3.5 to 10.4 °C. The minimum monthly temperature would be as low as -0.4°C and the maximum monthly temperature as high as 16 °C.

However, for King George Island the comparable leaf mine and leaf gall traces from Chile had small difference in their temperature ranges as similar traces were from both northern and southern sites (Figure 8.6). Modern traces comparable to the fossil traces from King George Island were gall type 1, gall type 5 and mine type 11; all more abundant in the northern sites. Mine type 3 and 7 were more abundant in the southern sites. Despite this, the mean annual temperature generated from the coexistence interval is also 3.5 to 10.4°C, but the minimum and maximum monthly temperature could have been as low as 3.5°C and as high as 24.3°C.

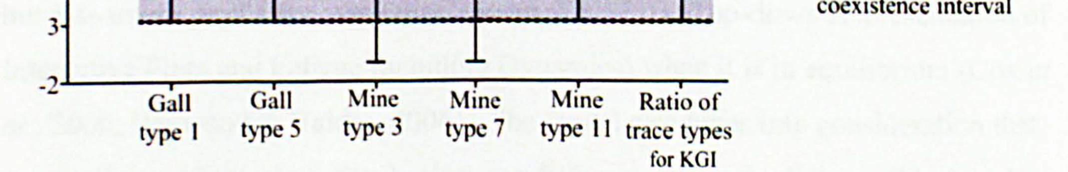


Figure 8.6. The temperature ranges of modern leaf mines and leaf galls that are comparable to the fossil traces from King George Island. The coexistence overlap interval is shown, with the mean annual temperature range for this interval. The ratio of trace types for King George Island, comparable to Puyehue (Chapter 7) was also used to give a temperature range.

In summary, the site specific traces collected in Chile are comparable to the insect trace fossils from Antarctica, thus giving an indication of the climate range during this time. The fossil traces that have modern equivalents were more abundant in the southern sites, suggesting that during the Palaeogene both King George Island and Seymour Island had cool temperatures and relatively consistent precipitation levels, similar to the climate at the southern study sites in Chile today. This is also supported by the level of herbivore damage at the fossil localities, which was lower than at the majority of the modern sites in Chile, but more comparable with the southern sites at Isla Nav and RNMag. The low intensity of skeleton feeding at the fossil sites was also more similar to the lower levels found at the southern site at Torres. The results of the coexistence analysis suggest that the climate of Seymour Island (maximum and minimum monthly temperature -0.4°C and 16 °C) would have been slightly cooler than that of King George Island (maximum and minimum monthly temperature 3.5°C and 24.3°C), despite the same mean annual temperature range (3.5 to 10.4°C).

8.3. Comparison of palaeoclimate estimates from the fossil data with computer climate model outputs.

8.3.1. The Palaeoclimate Model

Palaeoclimate data were obtained from simulations for the Eocene generated from BRIDGE, Bristol Research Initiative for the Dynamic Global Environment. The model is based on the HADCM3L coupled predictive model (Cox *et al.*, 2000), but also uses a predictive vegetation model, TRIFFID (Top-down Representation of Interactive Flora and Foliage Including Dynamics) when it is in equilibrium (Cox *et al.*, 2000; Haywood & Valdes, 2006). The model also takes into consideration that the continental land mass distribution was different during the Eocene (Markwick *et al.*, 2000). Also, the solar constant was reduced by 0.4% in the Eocene and the CO_2 levels were estimated to be six times greater than pre-industrial levels. However, as studies on proxy measurements have given a large range in the CO_2 concentrations for the Eocene (100 – 3500 ppmv) (Pearson & Palmer, 2000; Beerling & Royer, 2002; Lowenstein & Demicco, 2006), several models were used with 1 times, 2 times, 4 times and 6 times the pre-industrial levels of CO_2 in the atmosphere in order to determine the variation in the output this would create.

The climate data were obtained for King George Island and Seymour Island from the climate output produced from the models (Figure 8.7). The climate estimates were obtained from the output by reading the pixels on which each fossil site was located. Due to the model resolution (150km per square grid) each fossil site was located on two or more pixels with different values. Thus, an average of the estimates for all pixels was then used to obtain the final value. The climate data during the Eocene for the sites in Chile were also obtained and compared with the climate data for today. The data for 6x CO_2 and 4x CO_2 concentrations are shown in Appendix 7 to highlight the increase in warm month mean annual temperature and cold month mean annual temperature from the Eocene to today. Several climate models, which had different concentration of atmospheric CO_2, were used to generate the climate variables all for the Ypresian age of the Eocene (55.8 Ma to 48.6 Ma).

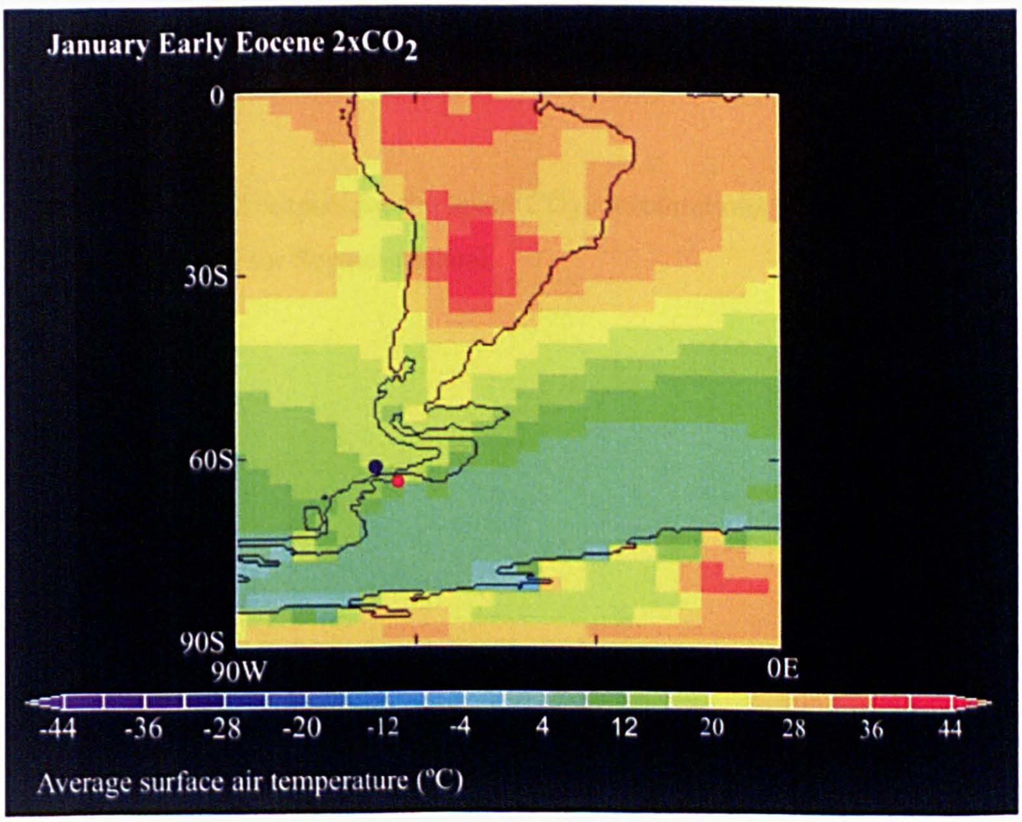


Figure 8.7. Climate model output for the average surface air temperature for the Antarctic Peninsula and South America during the Ypresian. The blue circle is King George Island and the red circle is the location of Seymour Island.

8.3.2. Temperature estimates from palaeoclimate model outputs

The surface air temperature estimates from the climate models were compared to the modern observations of temperature in Chile. The data are presented in Figures 8.8 and 8.9. The estimates from the models indicate that:

- The fossil localities had similar warm month and cold month mean temperatures as the data obtained from both Osorno and Temuco.
- The average warm month temperature for Temuco was slightly higher than the average warm month temperature from all model outputs (Figure 8.8).
- A higher than average warm month temperature was estimated from models with $4xCO_2$ and $6xCO_2$ concentrations.
- The cold month mean temperatures obtained from the models are similar to the estimates for the modern and the fossil sites.
- The mean temperatures at the $4xCO_2$ and $6xCO_2$ concentrations are similar to the mean temperatures for Osorno and Temuco, however, the temperatures estimated for $2xCO_2$ are more similar to modern temperatures at Punta Arenas (Figure 8.9).
- Only the model outputs for the lower CO_2 concentrations predict temperatures below freezing for Seymour Island.

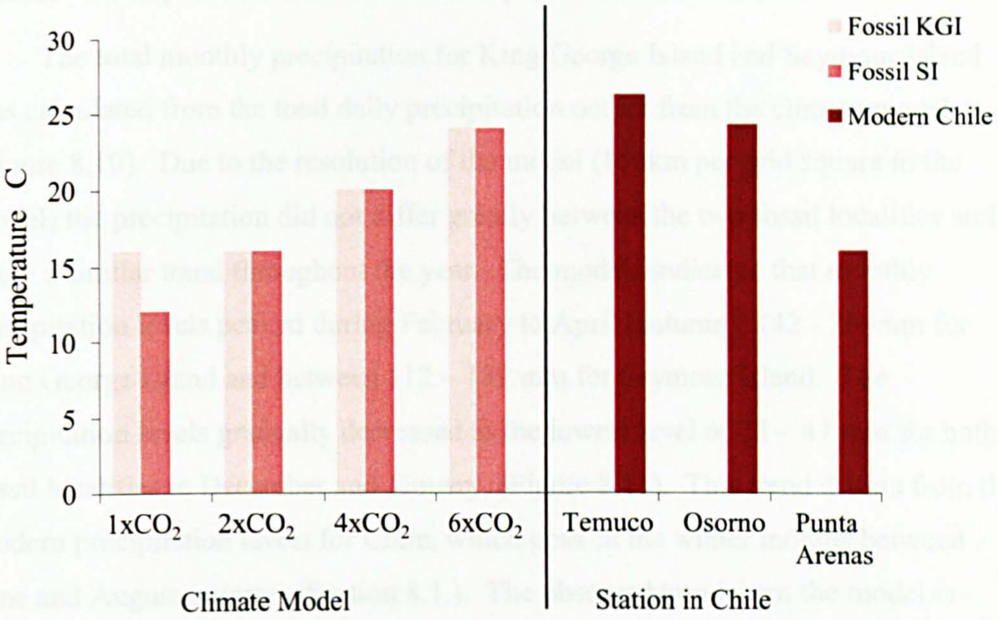


Figure 8.8. The average temperature for the warmest month from the model outputs at different CO2 concentrations for the two fossil localities, King George Island (KGI) and Seymour Island (SI) compared with modern climate data from three meteorological stations in Chile (January 2006-March 2007).

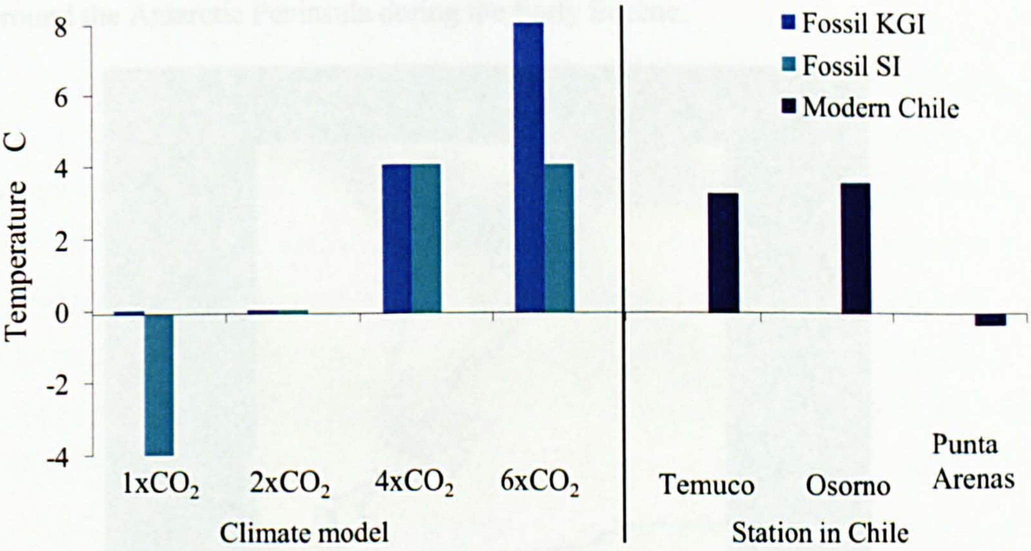


Figure 8.9. The average coldest month temperature estimates from the palaeoclimate models for two fossil localities King George Island (KGI) and Seymour Island (SI) at different CO_2 concentrations compared with the temperatures recorded from three meteorological stations in Chile (January 2006-March 2007).

8.3.3. Precipitation estimates from palaeoclimate models

The total monthly precipitation for King George Island and Seymour Island was calculated from the total daily precipitation output from the climate model (Figure 8.10). Due to the resolution of the model (150km per grid square in the model) the precipitation did not differ greatly between the two fossil localities and show a similar trend throughout the year. The models indicated that monthly precipitation levels peaked during February to April (autumn); 142 - 204mm for King George Island and between 112 – 189 mm for Seymour Island. The precipitation levels gradually decreased to the lowest level of 23 – 47 mm for both fossil localities in December and January, (Figure 8.11). This trend differs from the modern precipitation levels for Chile, which peak in the winter months between June and August (winter) (Section 8.1.). The observed trend from the model is attributable to the surface wind strength, in particular the strength of the westerly prevailing winds across the Pacific Ocean. During the Early Eocene, the westerlies were located more towards the south pole in the period of February to April and have a greater strength than today, according to climate models. This causes more evaporation and hence more precipitation when the westerlies reach the land surface around the Antarctic Peninsula during the Early Eocene.

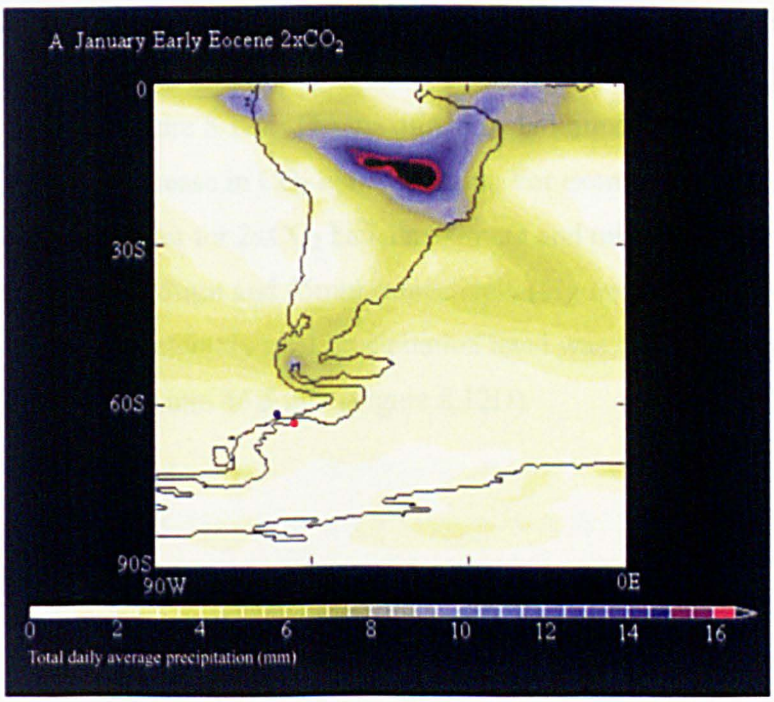


Figure 8.10. Climate model output for the total daily average precipitation level for the Antarctic Peninsula and South America during the Ypresian. The blue circle is King George Island and the red circle is the location of Seymour Island.

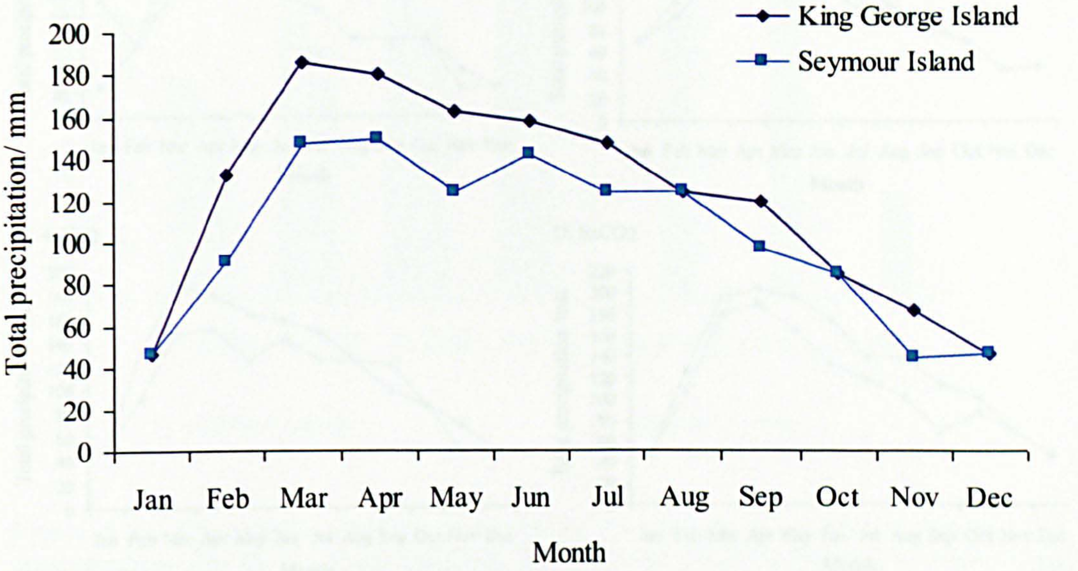


Figure 8.11. Total monthly precipitation estimates for King George Island and Seymour Island from climate model outputs with $4xCO_2$ concentration for the Ypresian age of the Eocene.

The precipitation peak in March is present in the model outputs for all of the CO_2 concentrations used, with the exception of $2xCO_2$ where the precipitation peak occurred in May (Figure 8.12). The maximum and minimum precipitation values increased with an increase in CO_2 concentration. For example, for King George Island the model output for $2xCO_2$ had a maximum and minimum monthly total precipitation of 162.75mm and 45mm respectively (Figure 8.12B). With $6xCO_2$ levels the maximum monthly total precipitation level was, however, predicted to be 204mm and the minimum 46.5 mm (Figure 8.12D).

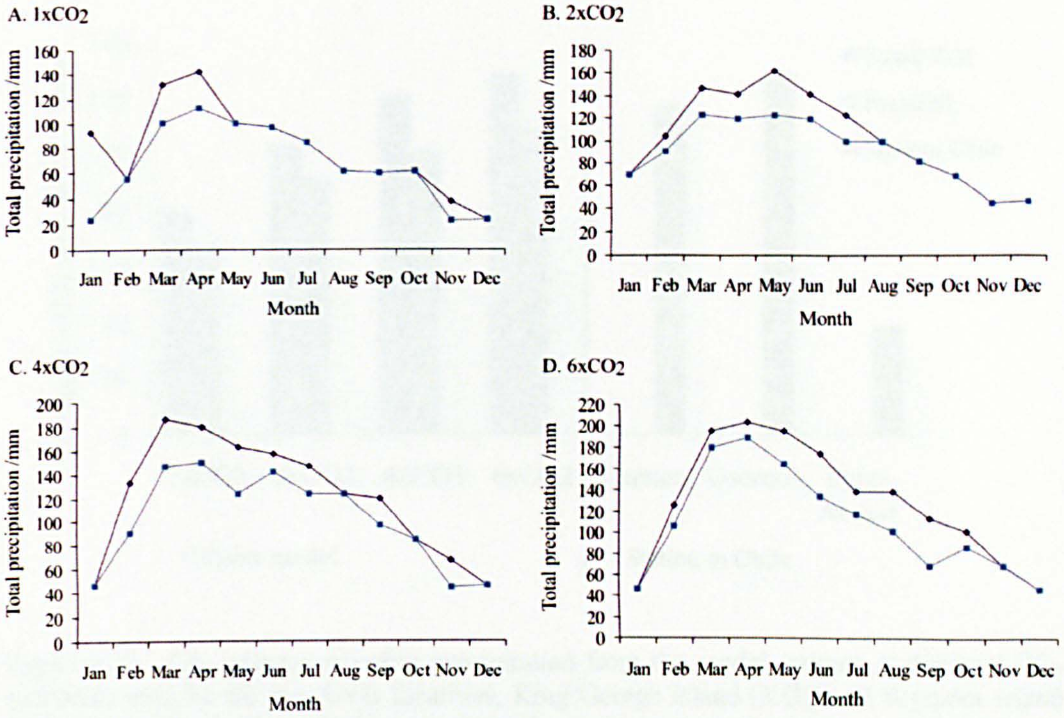


Figure 8.12. Total monthly precipitation estimates for King George Island (dark blue) and Seymour Island (light blue) from climate models for the Ypresian age of the Eocene. A) $1xCO_2$ concentration. B) $2xCO_2$ concentration. C) $4xCO_2$ concentration. D) $6xCO_2$ concentration.

In contrast, the modern precipitation levels for southern Chile are lower than the climate model predictions at all CO_2 concentrations (Figure 8.13). For example, Punta Arenas today has an average monthly precipitation of 39.05 mm, whereas the lowest level produced by the models was 67.15 mm (Seymour Island under $1xCO_2$ concentration). The average monthly precipitation levels for both Temuco and Osorno are very similar to the predictions from the models for King George Island and Seymour Island, particularly the levels predicted from the $4xCO_2$ and $6xCO_2$ concentrations for King George Island. This supports the conclusion that the climate of King George Island was most similar to the climate in the northerly sites in Chile (Chapter 7), but it also contradicts the estimates from the trace fossils that suggest a larger difference in monthly precipitation at the two fossil sites.

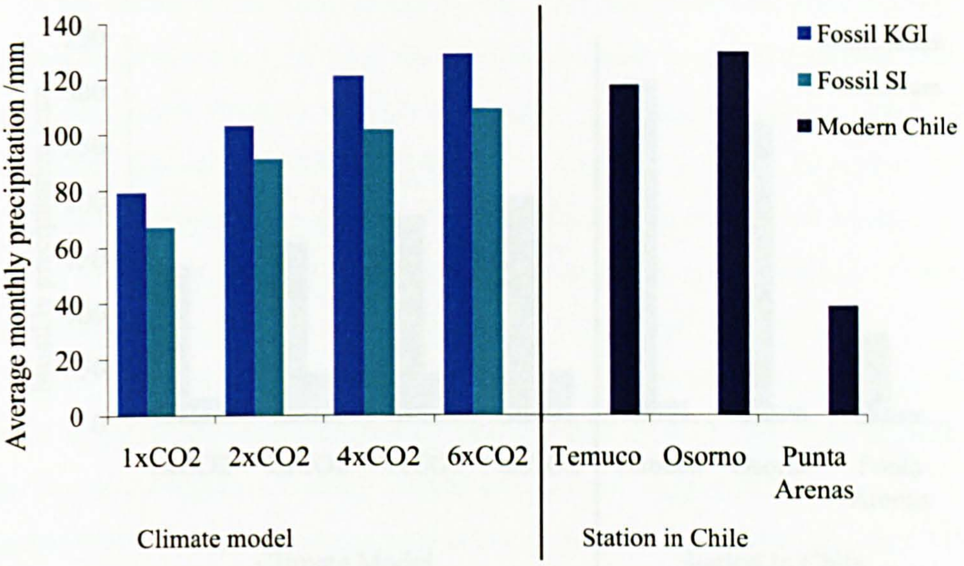


Figure 8.13. The average monthly precipitation from the model outputs at different CO_2 concentrations for the two fossil localities, King George Island (KGI) and Seymour Island (SI) compared to modern climate data from three meteorological stations in Chile (January 2006-March 2007).

The maximum monthly total precipitation values for both Temuco and Osorno are greater than the predicted values from all palaeoclimate models; these are the sites that are more similar to King George Island than Seymour Island. The minimum monthly total precipitation from the same stations is lower than that from the palaeoclimate models (Figure 8.14). Therefore, the model predictions for precipitation levels for both localities are less variable than for the precipitation levels at the Chilean sites between January 2006 and January 2007. This could be due to the precipitation level in the sampling period being in a particularly variable year or that the model cannot detect the small scale variation for the fossil localities due to the resolution of the model grid (150km per square grid).

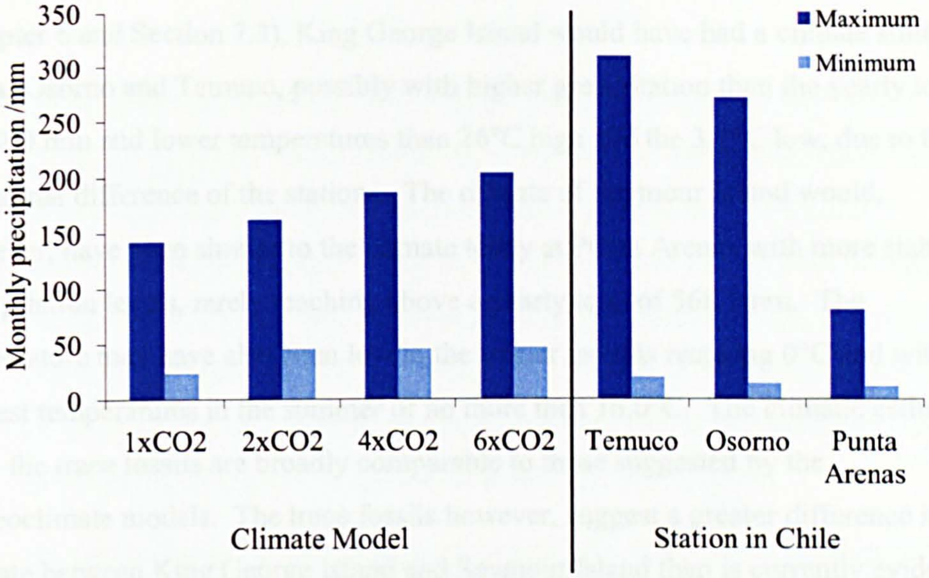


Figure 8.14. The maximum and minimum monthly precipitation estimates from the model outputs at different CO_2 concentrations for King George Island compared to modern climate data from three meteorological stations in Chile (January 2006-March 2007).

8.3.4. Comparison between palaeoclimate model and trace fossil climate estimates

Generally, the palaeoclimate model predictions for Seymour Island and King George Island with CO_2 levels 4 to 6 times greater than pre-industrial levels, are similar to the estimates obtained for central Chile today. With lower CO_2 concentrations in the models, the palaeoclimate estimates are more similar to the climate of modern southern Chile, which is not surprising as the CO_2 concentration from Middle to Late Eocene has been estimated from carbon isotope values from deep sea cores as between 1000 and 1500 parts per million per volume (Pagani *et al.*, 2005). This is approximately equal to the 4 and 6 times pre-industrial levels of CO_2 used in the model. Hence, the estimates from the models with high levels of CO_2 represent the climate of King George Island and Seymour Island during the Eocene. Overall, both fossil localities are predicted to have been relatively warm and wet, with temperatures rarely reaching below freezing, but, do the climate estimates obtained from the interpretation of the insect trace fossils from Antarctica agree with such palaeoclimate predictions?

From the fossil and modern insect trace evidence previously documented (Chapter 6 and Section 7.3), King George Island would have had a climate similar to that at Osorno and Temuco, possibly with higher precipitation than the yearly total of 1500 mm and lower temperatures than 26°C high and the 3.2°C low, due to the altitudinal difference of the stations. The climate of Seymour Island would, however, have been similar to the climate today at Punta Arenas with more stable precipitation levels, rarely reaching above a yearly total of 568.3mm. The temperature may have also been low in the winter months reaching 0°C and with highest temperatures in the summer of no more than 16.0°C. The climatic estimates from the trace fossils are broadly comparable to those suggested by the palaeoclimate models. The trace fossils however, suggest a greater difference in the climate between King George Island and Seymour Island than is currently evident from the models at all CO_2 concentrations.

8.4. Summary

- The climate data obtained from three meteorological stations in central and southern Chile show that the northern and southern sites had different climatic regimes. The northern sites had relatively mild winters (highest minimum monthly temperatures recorded were at the station at Osorno), but with high variability in the amount of precipitation (Temuco ranged between 19.7 mm and 311.1 mm total monthly precipitation). The southern sites had relatively consistent monthly precipitation (ranging only between 11.6 mm in November and 81.2 mm in April) and lower temperatures than the northern sites throughout the year.
- Coexistence analysis of the modern insect traces that were comparable to the insect trace fossils was used to determine temperature ranges at the fossil sites. This indicated that Seymour Island would have had a mean annual temperature range of 10.4 to 3.5°C and a maximum and minimum monthly temperature of 16 to -0.4°C. King George Island would also have had a mean annual temperature range also of 10.4 to 3.5°C, but the maximum and minimum monthly temperature would have been 24.3°C and 3.5°C respectively. The

temperature estimates for both fossil localities are most similar to southern Chile today.

- Palaeoclimate models used to predict the palaeoclimates of King George Island and Seymour Island during the Early Eocene indicate similar climates for both localities. At $4xCO_2$ concentrations (most similar to the Early Eocene) the mean monthly precipitation would have been 121mm on King George Island and 102mm on Seymour Island. The mean warm and mean cold month temperatures would have been 20°C and 8°C for both localities. An increase in CO_2 concentration caused an increase in the warm and cold month mean temperatures and the total monthly precipitation.
- The palaeoclimate model outputs agree with the climate predictions made from the climate analysis of the fossil herbivory traces. The climates deduced from the insect traces do, however, indicate a difference in the climates of King George Island and Seymour Island, not predicted by the models. The climate estimates from the fossil insect traces also suggest lower mean annual temperature ranges than the palaeoclimate models, but the maximum and minimum monthly temperatures are similar.

Chapter 9. Discussion

9.1. Introduction

The diversity of insect traces and the intensity of insect-damaged leaves from Antarctica have enabled a comparison with the forests of southern Chile, the modern-day analogue of the Palaeogene forests of Antarctica. In addition, the study of the insect faunas of modern trees in Chile has examined the effect of environmental factors on the diversity of insect traces and has helped to determine the factors which could have affected the insect activity in the Antarctic fossil forests. The comparison between modern and fossil insect traces supports previous studies which concluded that the forests of the Palaeogene of the Antarctic Peninsula were similar to the Valdivian and Magellanic forests of modern Chile, in both vegetation and climate. This study has shown that the insect herbivory in Palaeogene Antarctic forests was also similar to that in modern Chilean forests. This chapter discusses the results of this project and the implications in more detail.

9.2. The diversity of fossil insect traces on Palaeogene fossil leaves from Antarctica

9.2.1. Introduction

Four main functional feeding types were recognised on the fossil leaves from King George Island and Seymour Island: general leaf chewing, skeleton feeding, leaf mines and leaf galls. Due to the variation in the characteristics of the trace fossils, each main functional feeding type was further sub-divided. The trace types varied in abundance, with general leaf chewing being the most common trace at both localities and leaf mines the least common. Only one fossil specimen had evidence of skeleton feeding and that specimen was from King George Island.

The range of different trace types on the fossil leaves may have been dependent on the plant type. The abundance of each trace category on the most common fossil plant type, *Nothofagus*, was, however, not significantly different from the abundance of traces on fossil plant types considered together. As expected, there was a significant correlation between the number of traces observed and the abundance of the fossil plant type. For example, of the 62 *Nothofagus* fossil leaves examined from King George Island 19 had traces on them. The next most abundant plant family, Cunoniaceae, had 7 individual insect traces on 17 fossil leaves. This implies that the more abundant a particular fossil plant type, either due to a preservation bias or the dominance of that plant in the Palaeogene forest, the more common the insect traces from that plant type. The cluster analysis for general leaf chewing traces (Chapter 3) also supports this interpretation as there were specific trace character combinations that were unique to leaf morphotypes from specific sites, in particular the *Nothofagus* specimens from King George Island. For example, multiple, small, circular-shaped areas of non-marginal damage on the leaf, with the damage throughout the surface, was only found on *Nothofagus* leaves from King George Island. Thus, the abundance of a trace type and the diversity of trace types seen in the fossil floras may only represent a minimum level due to the preservation bias on leaves of specific plant types.

The proportion of general leaf chewing on the fossil leaves was 9.83% for King George Island and 2.64% for Seymour Island. Previously published studies of fossil herbivory in other floras report much higher levels of damage (Table 9.1). The variation in proportion of leaves damaged by herbivory amongst the floras in Table 8.1 could be due to changes in the number of plant-insect associations with time, differences in forest type, such as tropical to temperate environments, as well as preservation biases. For example, it is known that in modern environments the level of insect damage is significantly greater in the tropics than in temperate systems (Coley & Barone, 1996), therefore, such differences in fossil systems are also to be expected. Both the diversity of vegetation present within the modern environment and the range of plant species that the traces are found on could also dramatically influence the overall density of insect traces observed, even when only angiosperms are considered. Hence, comparisons between different fossil herbivory systems would only be valid if plant species diversity is also taken into account and the type of plant (angiosperm or conifer) considered.

Table 9.1. A summary of data from other fossil herbivory studies, adapted from Smith & Nufio (2004). Abbreviations for the forest types: Trop = tropical, DTr - Temp = transitional dry tropical to temperate, Temp = temperate. NA = where data is unavailable, nGLC = non-marginal general leaf chewing. The data in bold are from the two Antarctic localities, King George Island (KGI) and Seymour Island (SI).

	Age of Fossil Flora Studied							
	Palaeocene[1] (North Dakota USA)	Palaeocene[2] (Wyoming USA)	Early Eocene[2] (Wyoming USA)	Middle Eocene[3] (Utah, USA)	Middle Eocene[5] (Colorado USA)	Late Eocene[5] (Colorado USA)	**Late Palaeocene/ Eocene (KGI)**	**Late Palaeocene - mid Eocene (SI)**
Forest Types	Trop	Trop	DTr-Temp	DTr-Temp	DTr-Temp	Temp	**Temp**	**Temp**
Leaf area damaged (%)	NA	NA	NA	NA	2.5	1.4	**1.7**	**3.6**
Leaves damaged (%)	4	28	35	19.4	34	23	**9.8**	**2.6**
Dominant Feeding Guild	NA	NA	NA	NA	nGLC	nGLC	**nGLC**	**nGLC**

[1]Labandeira *et al.* 2002, [2]Wilf & Labandeira 1999, [3]Wilf *et al.* 2001, [5]Smith 2000.

In comparison with other studies (Table 9.1), a greater number of distinct trace types were identified for King George Island (54 different trace types) even though the sample size was smaller. For Seymour Island the number of distinct trace types is lower than other studies, with 19 trace types identified. A study of the Eocene Patagonian flora of Argentina found 52 discrete damage types after examination of 3,599 angiosperm leaves. This was found to be significantly greater than that in North American floras, whether for all collections or individual species of plant (Wilf *et al.*, 2005). This suggests that at higher latitudes during the Eocene the diversity of insect-plant associations was greater than at middle latitudes with similar climates. Caution has, however, to be taken not to over-interpret the results, as only two fossil localities in southern latitudes have been studied and the number of specialist plant-insect associations will be dependent on the range of plant species preserved, the specific fossil specimens collected and the extent to which types of feeding damage are sub-divided.

9.2.2. The effect of preservation (taphonomy) on the insect trace fossils record from Antarctica

The diversity of fossil herbivory traces is largely dependent on the preservation quality of the specimens and the range of fossil plant species that are actually preserved. Both are influenced by taphonomic filtering of the leaves, which is controlled by a range of factors including the species of plant, whether it is evergreen or deciduous, the forest structure, the forest type, the extent of previous damage on leaves, the distance the leaves have travelled from the original source and the sediment in which the leaves are preserved (Ferguson *et al.*, 1985). For example, tall trees generally have a large crown that covers a large area and thus more leaves are exposed to high winds, plus the leaves on the crown tend to be smaller-shaped 'sun' leaves, rather than the larger 'shade' leaves. Therefore, the 'sun' leaves are more likely to be transported further from the source than leaves from smaller trees or shrubs in the understorey. In addition, leaves from evergreen trees weigh more, on average, than deciduous leaves and they are unlikely to be transported as far (Ferguson *et al.*, 1985). This is particularly important in a closed forest as, in order for the leaves to be preserved, they must be in contact with water and be transported to a nearby lake or river. The leaves must then sink to the bottom of the lake or river to be covered with sediment. The time taken for a leaf to sink is species dependent, due to the water absorbency of the leaves. Leaves with thin cuticle and large numbers of stomata will sink faster than leaves with a thick waxy cuticle (Spicer, 1990). Hence, such factors would affect the relative number of leaves, and thus the trace types that are preserved as fossils.

If leaves are transported over long distances then they are more likely to be damaged and fragmented than if they fall directly into a body of still water. If the water is turbulent then this will also increase the probability of fragmentation of the leaf. The sediments in which the leaves are preserved may also affect the preservation quality (Ferguson *et al.*, 1985). Clays are a more suitable matrix for leaf preservation than sands or gravels due to the limited interstitial space in the clay, leading to anaerobic conditions in which the micro-organisms that breakdown the leaves cannot survive. At both fossil localities the leaves are preserved in siltstones and sandstones yet the preservation quality is better on King George Island than Seymour Island. Therefore, other environmental effects may have controlled the preservation potential of the leaves on Seymour Island, such as transport over a longer distance or in a higher energy environment. The depositional environments on King George Island includes shallow and deep water lacustrine settings and low energy fluvial deposits. In contrast, the leaves on Seymour Island would have been washed along the shore and deposited in a high energy shallow marine environment. This probably contributed to the lower number of insect traces observed from the Seymour Island fossil leaf assemblage than from the King George Island assemblage. Studies have also shown that non-marginal general leaf chewing allows water to penetrate the leaf lamina easily and actually causes the leaf to sink in water more rapidly, thus increasing the probability that the leaf is preserved (Ferguson *et al.*, 1985; Spicer, 1989). The same may be true for other insect trace types which change the leaf cuticle properties, making the leaf absorb water more easily.

In studies of fossil leaf herbivory levels from other regions, the general leaf chewing trace type is the most abundant, in particular the non-marginal type (Labandeira *et al.*, 1994; Wilf & Labandeira, 1999; Wilf *et al.*, 2001)(Table 9.1). Leaf piercing and phloem-feeding traces are commonly found in other fossil assemblages, but were not found on fossil leaves from Antarctica. The preservation quality of the fossil leaves from both Antarctic sites would not have impeded observation of this trace type if it was present. Leaf piercing and phloem-feeding in modern environments is mainly performed by insects belonging to the order Hemiptera (Homoptera) such as the aphids, cicadas and leaf hoppers. The mouthparts are adapted to form a long proboscis which can pierce the leaf surface to access the fluid (Borror *et al.*, 1989). There are several possible explanations for the absence of this trace type in the Antarctic floras: leaves with this trace type were not preserved, the trace type is difficult to distinguish due to the small size of the piercing in the surface or perhaps there were relatively few leaf-piercing traces because hemipterans were rare or absent in the fossil forests of Antarctica. The hemipterans, especially the aphids, have many known species-specific host associations (Strong *et al.*, 1984; Blackman *et al.*, 2003). In particular, *Nothofagus* species are known to have several host-species-specific homopteran associates (Schlinger, 1974; McQuillan, 1993; Wardhaugh *et al.*, 2006). Therefore, it could be assumed that hemipterans may have been associated with *Nothofagus* species in the forests of the past. This is also supported by the finding of fossil homopteran wings in Jurassic and Permian deposits in Antarctica (Carpenter, 1969; Tasch & Riek, 1969). Consequently, taphonomic filtering or misidentification of the trace type are plausible explanations for the rarity of piercing and sucking trace types in the fossil record from Antarctica. In addition, leaf piercing and phloem-feeding traces are difficult to observe on modern leaves due to the small size. Therefore, such traces would be extremely difficult to observe on impression or compression fossil leaves that are fragmented.

9.2.3. Other potential trace makers

The trace fossils found on the Palaeogene leaves have all been attributed to invertebrate damage, specifically insects, which is especially valid for the leaf mines because only insects have evolved this habit (Hering, 1951). There is, however, evidence of other herbivorous organisms at this time on Seymour Island. Eocene fossils of various tree dwelling mammals have been identified, such as "opossum-like" Antarctic marsupials (Goin *et al.*, 1999), the small Antarctic sloth (Vizcaino *et al.*, 1998) and an extinct South American ungulate group (Reguero *et al.*, 2002)(Figure 9.1). Their main source of food would have been plant material such as fruit, leaves and stems (Vizcaino *et al.*, 1997; Reguero *et al.*, 2002). That said, herbivorous mammals are commonly known to consume the entire leaf and, in general, the chew marks that they leave behind would be larger than those seen on the fossil leaves. Also, the Antarctic marsupials were probably fruit and insect feeders (Goin *et al.*, 1999).

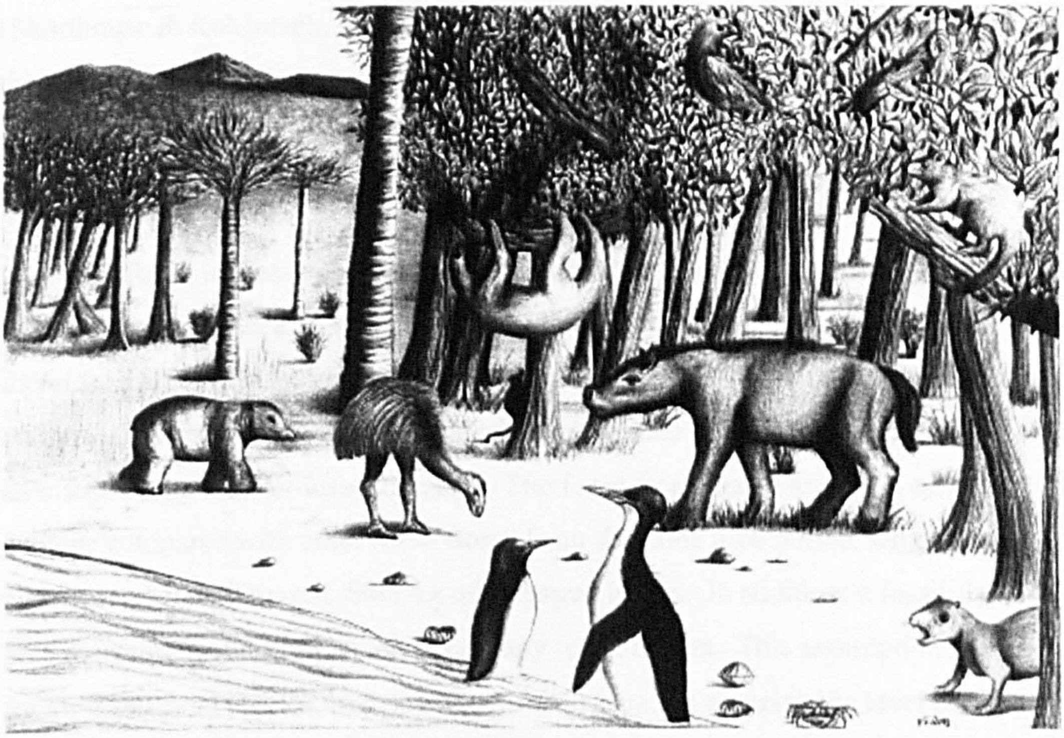


Figure 9.1. Reconstruction of forests on Seymour Island during the Middle - Late Eocene based on palaeontological evidence of mammals, birds, plants and molluscs from La Meseta Formation. From Reguero *et al.*(2002).

In modern ecosystems, terrestrial molluscs such as snails and slugs (Gastropoda: Pulmonata) also consume a large proportion of leaf material, but due to the difficulty of preservation of soft body tissue, evidence of fossil slugs has not been reported and there is no fossil evidence of terrestrial snails from either locality on Antarctica. Both terrestrial snails and slugs feed using a radula, a specialised mouthpart which can scrape, tear or cut the plant tissue (Hickman Jr. *et al.*, 2001). Molluscs can still create a range of traces, from marginal and non-marginal general leaf chewing to skeleton feeding (Cates & Orians, 1975). Slug damage to plants characteristically has long narrow holes within the leaf tissue, which is sometimes only on one lamina. This damage type is not seen on the Antarctic fossil leaves and due to an absence of terrestrial snail fossils being preserved, it can be assumed that species of terrestrial snails and slugs did not create the fossil traces studied.

The leaf gall traces could have been caused by species of insects, such as the Cecidomyiidae (Diptera) and the Cynipidae (Hymenoptera), but also by species of Tylenchida (Nematoda), Eriophyidae (Acari) and species of fungus and bacteria

(Shorthouse & Rohfritsch, 1992; Williams, 1994). Therefore, it is concluded that the general leaf chewing traces and leaf mines were created by insects, but the leaf galls may have been created by other invertebrates as well as insects.

The fossil leaf assemblages examined could represent an accumulation of leaves over several seasons or years and possibly from a range of altitudes and therefore, different vegetation types (Hunt & Poole, 2003). The taphonomic effect on the leaves and trace types preserved was mentioned previously, but this also has an effect on the level of insect damage. The fossil floras had a low level of insect damage compared with other fossil floras from the same time period, which may partly represet taphonomic filtering of damaged leaves. In addition, a fossil flora may represent leaf accumulation over many tens of years. This assumption is made for all fossil flora studies, irrespective of forest type. In contrast, the level of insect damage in modern forest systems generally represents only one season (Coley & Barone, 1996), especially if collected from the trees.

9.3. Specific traces made by insects in Chile

9.3.1. The range of insect traces on modern leaves

Examination of the range of herbivory traces and the insects that created them in Chile can aid identification of the species of insects which may have created the traces on leaves in the Palaeogene forests of Antarctica. This section documents the main species-specific traces that were observed in Chile and the interpretations that can be made from them. The insect species and the traces they create plus the leaf miners and leaf gallers are compared with previous studies where possible. The herbivory trace types on the two deciduous *Nothofagus* species were documented in Chapter 5.

Both the adult and larval insects collected made a variety of general leaf chewing marks on the leaves. The majority of traces were made by several species, but one trace was both species- and site-specific (made by an Alticinae species). *H. aureosignatus* (Coleoptera: Curculionoidea) was site-specific and only found in Nah and one species was specific to the more northerly sites of Nah and Tolh (*cf. Psathyrocerus unicor* (Eumolpinae)). The most common insect observed skeleton feeding *cf. Varicoxa* sp. (Coleoptera: Chrysomelidae: Alticinae) was collected only from Puyehue.

It is difficult to compare the number of insect species, leaf mines and leaf galls collected in Chile with other studies because the same methodology was not applied (for example, different collection techniques and different tree species studied). Overall trends and the proportion of different feeding types can, however, aid interpretation of the insects associated with *Nothofagus* forests. Kushcel (1960) reported that the insect fauna of Magellanic forests represented only a subset of that from the more diverse Valdivian forests further north. Another study found that certain species of *Nothofagus* had a higher diversity of associated insects than others, notably *N. obliqua* had a more diverse insect fauna than *N. antarctica* and *N. pumilio*, and the diversity of insects changed with increasing altitude and latitude (Welch, 1988). Conversely, Russell *et al.* (2000) found that *N. obliqua*, *N. pumilio* and *N. antarctica* had the largest insect faunas in a study of all Chilean *Nothofagus* species. As this study examined the insects collected and the traces they created on two plant species with the largest insect fauna (*N. pumilio* and *N. antarctica*), the greatest diversity of possible traces for comparison were collected, hence, improving the range of modern traces collected that could be compared with the fossil traces from Antarctica.

Four types of leaf gall were frequently present in Chile and each had a specific distribution pattern across the sites (Chapter 5). For example, gall type 1 (ball gall) was more commonly found on *N. pumilio* and was more abundant in the northern sites. The complexity in phytophage community structure was also revealed because species of Eriophyidae (Acari) and Tychlenida (Nematoda) were the gall inducers and not an insect. There was also evidence of complex interactions, such as the leaf galler-parasitoid-inquiline interaction seen in gall type 1 (Chapter 5). Therefore, the presence of leaf galls does not represent a two level trophic interaction, but actually a multitrophic interaction.

Eight types of leaf mine were commonly found, each at particular sites. In general, there was higher leaf mine diversity in the southern sites than in the northern sites. The leaf-mining insect fauna included species of Lepidoptera, Hymenoptera and Coleoptera. Their associated parasitoid fauna were also present, highlighting the multi-species interactions that are present. Some species of leaf miner have the ability to move to other leaves half way through larval development. For example, when a mine is created on a small leaf the larva may not complete development inside that one leaf, but will move onto another leaf to create a new mine. When recording the presence of leaf mines, the two mines would, however, be counted as two separate occurrences of leaf mining insects. Leaf mining insects in the genus *Heterobathmia* (Lepidoptera: Heterobathmiidae), associated with *Nothofagus*, commonly create several mines before development is complete (Grimaldi & Engel, 2005). This problem was overcome in this project by using the number of leaves rather than number of mines in the quantitative analyses, and the assumption had to be made that larvae did not travel between leaves.

9.3.2. Factors influencing diversity of insect species in Chile

Identification of the phytophagous insects, both adult and larvae, was not easy due to the limited number of relevant taxonomic papers or books for identification. Also, identification of specific leaf mining and leaf galling species was difficult due to limited success with rearing of larvae. The small number of insects reared could be attributed to the non-optimal conditions for insect development during the field work. In addition, some leaf mining insects do not complete their development within the same season, but overwinter as a cocoon in the soil and emerge as adults the following spring. For example, larvae of *Heterobathmia* are known to spend the summer, autumn and a large part of the winter as a cocoon in the ground and pupate during late winter, emerging as an adult in the early spring (Kristensen & Nielsen, 1983). Therefore, if the development of the insect during the leaf mining stage occurs over a long interval, the adults would never have been successfully reared within the sampling period.

The species richness of phytophagous insects found in this project, including the leaf mines and leaf galls, is similar to reports from other studies. Previous work on *N. pumilio* found that dipteran species (including all feeding guilds) were the most abundant insects in Magellanic forests (Lanfranco, 1991; Spagarino *et al.*, 2001). As the adults of dipteran leaf mining species are, however, not leaf chewers and are fast flying, they are highly unlikely to be collected using a beating technique; rather a malaise trap or flight-interception trap would be more suitable (Leather, 2005). Many adult dipteran species were observed at the sites during sampling in this project but no larvae were collected. Also, no dipteran leaf miners were collected but this is in accordance with other studies on *Nothofagus* (Spencer, 1990). In the Northern Hemisphere, it is reported that Argromyzidae leaf miners are commonly found on *Quercus* species but relatively few on *Fagus* species (a genus closely related to *Nothofagus*) (Spencer, 1990). Therefore, it is not surprising that they are not found on the Southern Hemisphere genus *Nothofagus*. Orthopterans were also absent from this study, although there are several studies reporting high damage levels due to orthopteran nymphs on *Nothofagus*, in particular producing non-marginal general leaf chewing (Rentz & Gurney, 1985; Lanfranco & Dungey, 2001). Orthopterans were reported by Welch (1988) to be a less significant component of the leaf chewers in *Nothofagus* forests; instead lepidopteran larvae and both the larvae and adults of coleopterans were the most abundant phytophagous species (Welch, 1988).

Nothofagus forests support a wide range of epiphytes, including mosses, lichens, ferns, algae and fungi, which have a diverse and characteristic fauna of invertebrates associated with them. Many of the sampling sites contained trees with mistletoe species in the family Misodendraceae, which is endemic to southern South America. Also common throughout the sites were lichen species (*Usnea* sp.), growing on the branches and trunks. Such abundant epiphytes could act as important overwintering and sheltering sites for many herbivores on the *Nothofagus* trees, which could increase the potential diversity of insects found on any given tree (McQuillan, 1993). From observations during fieldwork, epiphytes were not significantly more abundant at any one site, apart from a mistletoe species at Torres which had infested large areas of the forest. However, such trees were not sampled and therefore the effect of epiphytes on the species richness of insects collected was limited.

An important factor to consider is that the insect species that are associated with one tree are not independent of each other. The abundance and distribution of one insect species will affect others within the same or different trophic level or feeding guild through interspecific competition (Denno *et al.*, 1995; van Veen *et al.*, 2006). Along with other factors that interact to affect the distribution or abundance of any particular insect species, competition between different species for access to resources, which in this case are the leaves, can have an effect on the numbers of each species. The insects may belong to different feeding guilds such as general leaf chewers, leaf miners and leaf gallers, hence the presence of a leaf miner could affect the presence of a general leaf chewer or leaf galler at any one time (Denno *et al.*, 1995). Interspecific resource competition may influence the proportion of trace types on both the modern and fossil leaves because several traces on the same leaves were documented for both, but the specific types that were present in multiples per leaf differed. On the fossil leaves, multiple types of general leaf chewing were found on one leaf but not with leaf mines. In the modern floras, several leaf mines and general leaf chewing were found together. The multiple mines were generally of the same type and were possibly made by a single larva. Therefore, inter-guild competition between leaf miners, leaf galls and general leaf chewing herbivores may influence the proportion collected. As phytophagous insects include a variety of feeding guilds that have restricted or non-overlapping host ranges, as well as having various specialisations, interspecific resource competition may have a limited effect on the herbivorous insects' distribution and the proportion of each feeding type found (Lawton & Strong, 1981; Strong *et al.*, 1984; van Veen *et al.*, 2006).

9.3.3. Insect traces from evergreen *N. betuloides*

The range of traces collected from the evergreen *Nothofagus* species, *N. betuloides*, helped highlight the differences between evergreen and deciduous species, specifically the different species of leaf miners and leaf gallers found. The traces on *N. betuloides* were distinguishable from the traces on deciduous species of

Nothofagus and therefore they may be useful for determining if a fossil leaf belonged to an evergreen or deciduous species. Skeleton feeding was also common on both tree types, but the plant defence reaction was more distinctive on the evergreen leaves. The number of different leaf mines and leaf galls on deciduous and evergreen *Nothofagus* was similar, although the evergreen *Nothofagus* had marginally fewer species.

It was expected that the associated insect fauna on an evergreen tree would be different from that of a deciduous tree, even within the same genus, due to different levels of chemical and physical defences (Russell *et al.*, 2000; Lavandero *et al.*, 2009). Both the insect traces and the insect species collected during this study were different on the evergreen and deciduous *Nothofagus* trees. It should also be noted that plant species within the same genus, and specifically ones that are closely related, may also have the same or closely related insect species associated with them (Lavandero *et al.*, 2009). This could explain the presence of the same insect, leaf miner and leaf galler species on the deciduous *N. pumilio* and *N. antarctica* and may not be related to leaf longevity.

9.4. Factors that affect the level of insect damage on modern leaves

9.4.1. Introduction

The effect of different abiotic factors on the presence of modern insect damaged leaves, intensity of damage on the leaves and the number of leaf mines and leaf galls is dependent on both the species of plant and the species of insect involved (Vehviläinen *et al.*, 2007). Other studies have also concluded that the impact of the abiotic factors on herbivorous insects is determined to some extent by the biotic factors of the species involved (Forkner *et al.*, 2008). In the modern sites in Chile, there was however, variation in the number of insect-damaged leaves with latitude, height and orientation within the tree (Table 9.2). This indicates that abiotic factors do have an effect on the phytophagous insect communities. In the following sections, the proportion of insect-damaged leaves on *Nothofagus* will be compared with other studies and then the interpretation of the effect of the abiotic will be discussed.

Table 9.2. Summary of the effect of different abiotic factors on the proportion of insect damaged leaves on *Nothofagus* trees in Chile. The effects are discussed fully in sections 9.4.1 to 9.4.6.

Factor	Tree Species	Effect
Latitude	*N. pumilio*	More damage on *N. pumilio* than *N. antarctica* in the southern sites
	N. antarctica	More damage on *N. antarctica* than *N. pumilio* in the northern sites
Height within the tree	*N. pumilio*	Lower height levels within the tree have the greatest proportion of insect-damaged leaves, but more pronounced in the southern sites
	N. antarctica	Lower height levels within the tree have the greatest proportion of insect-damaged leaves, but only in the northern sites
Orientation within tree	*N. pumilio*	More damage on the northern facing branches than the southern
	N. antarctica	More damage on the northern facing branches than the southern, but only in the northern sites
Season	*N. pumilio* *N. antarctica*	No significant difference in the proportion of damaged leaves throughout the same season
Canopy	*N. pumilio*	Lower canopy layers of the tree have the greatest proportion of damaged leaves
Altitude	*N. antarctica*	Trees at mid-altitudes have more damaged leaves than trees growing at lower or high altitudes

The proportion of insect damage on different species of *Nothofagus* has been investigated previously (Lanfranco *et al.*, 1999; Russell *et al.*, 2000). One study found that *N. antarctica* had a significantly higher proportion of insect-damaged leaves (20% of leaves damaged, 80% of leaves not damaged) than N. *pumilio* (3% of leaves damaged, 97% of leaves not damaged) (Russell *et al.*, 2000). The study concluded that the amount of damage was lower on *N. pumilio* because this species has a better chemical defence system to deter feeding insects. The damage levels recorded in this project contradict the report above because it was found that both *N. pumilio* and *N. antarctica* had similar levels of insect damaged leaves. The difference in results could be due to specific defoliators having a greater influence on a particular plant species at certain sampling times, as well as differences in the study sites. Russell *et al.* (2000) sampled *N. pumilio* from a more northerly site (Altos de Lircay National Reserve (35°32'S, 70°50'W) and *N. antarctica* only from Nahuelbuta (the proportion of insect damaged leaves on *N. antarctica* for that study was 38.4%) which could explain the difference in damage levels. In contrast, a study of *N. pumilio* further south in Region de Magallanes observed a leaf damage level of between 25-75% on the trees studied (Lanfranco, 1991). That amount is greater than the proportion of damage from the RNMag site in this study, in which 10.2% of *N. pumilio* leaves were damaged.

9.4.2. Effect of latitude on level of insect damage on modern leaves in Chile

There is no simple relationship to explain the variation in insect-damaged leaves with latitude in Chile (Section 6.3.1 and 6.3.2) (Table 9.2). However, the proportion of damaged leaves found on the two species of *Nothofagus* was different in all sites examined (apart from at Nahuelbuta). In the southern sites, the insect damage level on *N. pumilio* was greater than on *N. antarctica* (at Torres del Paine, R.N. Magallanes and Isla Navarino), but in the northern site, the level of insect-damaged leaves was greater on *N. antarctica* than on *N. pumilio* (at Tolhuaca and Puyehue). This contrast may be due to the altitude range of the tree species. *N. pumilio* was the dominant tree species, but it occurred across a broader range of altitudes in the southern sites. It is possible, that in the southern sites, the increase in abundance of *N. pumilio* affected the number of monophagous and polyphagous insect species associated with each tree. A study on mixed deciduous beech (*Fagus* sp.) and coniferous plantations found that the ratio of the tree species affected the relative abundance and dominance of associated monophagous and polyphagous insect species differently. Monophagous insects increased in abundance when its host plant was the dominant species, but the polyphagous insect species showed no difference with change in forest composition (Heiermann & Schütz, 2008). The results from this study in Chile are also consistent with the observation that the number of insect species feeding on a host plant increases with an increase in the plant's geographical range because the more widespread the plant the greater the variety of habitats the range encompasses and more habitats supports more species as species vary in their habitat requirements (Strong *et al.*, 1984; Leather, 1986).

Nothofagus antarctica occurred as a smaller tree in the more southerly sites, shown by a significantly smaller mean tree height in Torres and Isla Nav than the northern sites. The architectural complexity of a host plant, whether it is the size, the number and position of branches, growth form, persistence and the variety of above-ground parts of the plant that are present, is known to significantly affect the species richness of insects associated with that plant (Southwood *et al.*, 1979; Strong & Levin, 1979; Lawton, 1983). The phytophagous insect fauna decreases with a decrease in architectural complexity; for example, trees were found to support more insect species than shrubs, which themselves supported more insects than were supported by herbs (Leather, 1986). Even when considering the same plant species, the number of insect species was found to increase with architectural complexity (Moran, 1980). Hence, on smaller *N. antarctica* trees there may be a smaller array of branches or less preferential foliage for the insects to feed on, as well as providing fewer sites for oviposition, overwintering or escape from predators (Strong *et al.*, 1984; Riihimäki *et al.*, 2006).

9.4.3. Effect of height and orientation on the proportion and intensity of insect damage on modern leaves

Species specific effects can explain the different effects of factors such as height and orientation within the tree on proportion of insect-damaged leaves (Table 9.2). Leaf damage on *N. pumilio* in the cooler southern sites varied considerably at different heights in the tree and with location on the north-facing or south-facing sides of the tree. In contrast, the variation of damage on *N. antarctica* within the tree was only significant in the northern sites. In the cooler southern sites, the insects on *N. pumilio* may have to maximise their use of warmer parts of the tree, such as on the lower branches or at the branches on the north-facing sections. In the northern sites, the insects on *N. antarctica* may, however, obtain optimal feeding conditions through changing their distribution within the tree, by feeding on leaves on lower branches and at the south-facing sides of the tree.

The observed difference in the proportion of insect-damaged leaves could also be attributed to tree height and therefore the temperature or environmental gradient within the tree. *N. antarctica* may have been too small in the southern sites to produce such an effect. For both species overall, there was a greater proportion of insect-damaged leaves at the lower height levels, possibly due to a higher insect abundance at the more sheltered areas within the tree, as the higher sections are exposed to higher wind speeds than lower in the tree (McGeachie, 1989). The intensity of damage (area of leaf consumed on damaged leaves) on *Nothofagus* was, however, relatively unaffected by such factors, regardless of site or tree species. There was greater variation in the intensity of damage between the damage categories (0-25%, 26-50%, 51-75% and 76-100% of the leaf area damaged) than due to between-tree or between-site differences. Previous studies on damage levels on *Nothofagus* species have not focussed on the amount of leaf area removed from the tree by insect damage, but rather the number or proportion of damaged leaves present (Lanfranco *et al.*, 1999; Russell *et al.*, 2000). Therefore, it is difficult to determine if the intensity of damage on the leaves is characteristic of the Chilean sites or the observed trend is atypical and is dependent on the sampling period.

9.4.4. Seasonal effect on level of insect damage on modern leaves

There was no statistically significant difference in the number of leaves damaged by insects within the same season at one site (Table 9.2), but some variation did exist (may be due to a change in the diversity and abundance of insect species throughout the season). The difference in the number of damaged leaves is usually more defined between seasons (i.e. between summer and winter) rather than between periods within the same season (i.e. different times within summer). A study by Lanfranco *et al.*(1999) on *N. obliqua* found significant differences in damage levels between two years, but no difference between two sampling periods within the same season. For example, the conditions in one year may be beneficial for one species of herbivorous insect, which causes a population explosion and high levels of damage on the host tree. The next year the conditions change and the species returns to a relatively normal population density and hence the amount of insect damage is also reduced. Therefore, sampling at different times within the same season was justified for this study because no seasonal effect was observed.

9.4.5. Effect of canopy level on insect damage

There were also a significantly greater number of damaged leaves in lower levels of the tree canopy than in the middle and upper levels (Table 9.2). A study of the canopy insect fauna of *Nothofagus* by Arias *et al.* (2008) found that the beetle fauna of *N. dombeyi* and *N. obliqua* was very large (600+ species), with half of the species previously undescribed. That study used canopy fogging which is unselective in the species it will collect (Stork *et al.*, 1997) and therefore is not directly comparable with a study of insect damage alone. There are no other previous studies on the canopy insect fauna of *Nothofagus*.

There was no significant difference in the amount of damaged leaves sampled from the ground and from the canopy. Hence, sampling from the ground alone will not lead to an under- or over-estimation of the proportion of damaged leaves. The extent of this effect would vary depending on the tree species under study (only leaves in the canopy of *N. pumilio* trees were sampled in this study) and the height of the trees sampled, but the greatest amount of damage was always recorded from the lower branches of the tree. Thus, the lower levels of the tree provide the maximum estimate of insect damage. A study by Spagarino *et al.* (2001) examined the adult insect diversity of *N. pumilio* in Tierra del Fuego (54°06'S, 68°37'W) and found that the highest percentage of individuals were collected in the understorey level, while traps at the canopy level contained as little as 4% of the total insects collected. Although that study collected insects from various feeding guilds, it substantiates the results found in this project of decreasing insect damage levels with increase in height within the tree for *N. pumilio*. Therefore, extrapolation of the amount of damage at the lower levels to represent the total amount of damage in a tree may lead to an overestimation, due to different damage levels within a tree structure.

Insect diversity within the canopy layers of trees has been studied extensively, with work concentrated in tropical areas (Basset, 1992; Stork *et al.*, 1997; Winchester, 1997). The overall level of insect diversity generally is greater in tropical than in temperate areas (Coley & Barone, 1996). No consistent pattern of stratification within trees is evident because some insect species are more abundant in lower sections of the tree (Brown *et al.*, 1997) and others are more abundant at higher sections within the tree (Basset, 1992; Brehm, 2007). Although the height gradient in trees growing in temperate climates is not as large as in the tropics, there is still a microclimate effect within the tree that could affect the spatial distribution of the feeding insects (Yamasaki & Kikuzawa, 2003). In the tropics, forest structure is typically complex and the number of predators or parasites within the tree changes with height; for example, insect herbivores are more prone to bird predation in higher parts of the canopy, and so are less common than in the lower parts (Mazia *et al.*, 2004). If insect diversity differs with height within the tree canopy then this would affect the level of insect-damaged leaves, as was previously observed.

9.4.6. Altitudinal affect on the level of insect damage on modern leaves

Altitudinal differences affected the level of insect-damaged leaves in Chile (Chapter 5), but this was not a simple linear relationship (Table 9.2). There were a greater proportion of insect-damaged leaves on trees at middle altitudes than at low and high altitudes at the Puyehue site, although only minor differences in abundance of the tree species were recorded. The effect of altitude was, however, only observed in two tree species which are usually found at relatively higher altitudes or in cooler environments. Therefore, very low altitudes were not sampled. The altitudinal effect on herbivory could vary between tree species due to different populations of associated insects. These insect species may have a different range of altitudinal tolerances, which will influence the level of insect damage. In a study by Ashworth & Hoganson (1987) coleopteran diversity decreased with an increase in altitude at Puyehue, but the Coleoptera were sampled using a variety of collection techniques and in habitats with differing tree species that would affected the coleopteran species found and influenced the effect of altitude on the insect diversity. A peak in insect diversity at middle altitudes has been observed in Sitka spruce forests of the UK (Straw *et al.*, 2009), as well as in several tropical forests (Janzen, 1973; Olsen, 1994), which supports the increase in insect damaged leaves at mid-altitudes described here from Chile.

9.4.7. Effect of environmental factors on proportion of leaf mines and leaf galls

The effect of within-tree and between-tree factors on both the leaf miners and leaf gallers indicated that the effect was species dependent. Latitude, height and orientation had no effect on the frequency of leaf galls present in the Chile samples. There were, however, more leaf mines on leaves in the southern sites than in the northern sites on *N. pumilio*. This could indicate a higher diversity of leaf mining insects in the southern sites, but no distinct difference in the diversity of leaf mining insects was found. Hence, it is more likely that specific species preferred the environmental conditions in the southern sites, leading to a density increase (Section 9.3).

The variation in the proportion of leaf mines between tree species, sites, height levels in the tree and orientation within the tree suggest that the observed differences in both orientation and latitude may be related to the particular species of leaf mining insect. This could not be tested statistically due to the small numbers of each leaf mine type collected. As the environmental factors influencing the distribution of leaf-feeding insects seems to be dependent on the insect species in other studies (Brehm, 2007; Vehviläinen *et al.*, 2007), it can be assumed that in Chile the distribution of some of the leaf mining insects would have been affected by such factors. The environmental factors investigated in this study may not have the largest effect on the frequency of leaf mines. Rather, the spatial distribution pattern of the leaf mines reflects the selection for suitable oviposition sites by the adult leaf miner to limit parasitism or predation and increase the fitness of its offspring (Valladares & Lawton, 1991).

Therefore, the factors examined that affect the level of insect damage in the trees in Chile include latitude, height and orientation within the tree, altitude of the tree and age of the leaf on which the damage occurs. The effect of each factor is dependent on the tree species, the site in which the tree grows and the insect species that creates the damage. The relevance of this to the study of insect trace fossils will be explained in the next section.

9.4.8. Interpretation of the factors that may have affected herbivory in the Antarctic fossil forests

The study of insect damage and factors influencing its occurrence in modern forests in Chile can enable a more valid interpretation of the insect traces on the fossil floras. Comparison between fossil and modern herbivory should be carried out with a degree of caution, as not only are they separated by many millions of years of evolution, but also because of the sampling differences. The original position of the tree in the fossil forests is unknown because leaves from all altitudes, all heights in the tree and also all orientations are mixed together within the river or lake area in which they are preserved.

The taphonomic effect, previously mentioned, indicated that there could be a preservation bias of leaves of certain species and leaves from one particular section of the tree, usually the upper canopy. The proportion of insect damage is, however, significantly lower at higher levels of the canopy in *N. pumilio* in Chile. This suggest that if there are a greater proportion of higher canopy leaves preserved, the damage level recorded in the fossil floras may be an under-estimate of the true damage level for the forest.

The observed change in levels of insect damage with altitude also aids interpretation of the fossil herbivory. In a study of high latitude temperate forests in Siberia, a higher proportion of leaves were preserved from trees at middle and low altitudes than from trees at high altitudes because the leaves have to be transported further before they reach an area for preservation and are subject to mechanical damage during this transport (R.A. Spicer, pers. comm.). Therefore, it may be possible that the fossil leaves from Antarctica may also show a bias towards preservation of leaves from trees at middle to low altitudes. The greatest amount of insect damage on leaves in Chile were also found on trees at middle altitudes, suggesting that the proportion of insect-damaged leaves within the fossil forests is not an under-estimate. Although the effect of altitude may only be true for insect damage on *Nothofagus*, *Nothofagus* was the most common leaf type in the fossil floras studied and had the largest number of traces on the leaves. Thus, the level of insect damage preserved on the fossil leaves is considered to be a reliable estimate, despite the original position of the tree being unknown.

The fossil leaves, and hence the trace types found on them, could represent the range of leaves that accumulated on the forest floor during the period of leaf fall, which typically occurs in the autumn season. The leaves may have also become detached forcefully by the wind or rain storms. This suggests that a comparison of the fossil traces with the traces on modern leaves in the leaf litter may be more valid than a comparison with leaves collected directly from the tree. The leaves of plant species found on the forest floor may, however, not actually represent the range of plant species that have their leaves preserved because once on the forest floor it may be difficult for the leaf to be transported to a water body where it would be fossilised (Spicer, 1989). Also, the decomposition rate on the forest floor is dependant on the vegetation type, soil structure, soil type, soil depth, and the forest structure (Ferguson *et al.*, 1985). The decay rates in the fossil forests in Antarctica are unknown and they may not have been similar to the sites in Chile. Hence, it seems comparison of the fossil traces with the modern leaf litter would not be valid. Also, identification of the range of species which made each trace would not have been possible through a study of the modern leaf litter alone.

9.5. The diversity of modern herbivory traces in Chile as a model for interpreting the insect fauna in Antarctic fossil forests

Comparisons of the modern and fossil herbivory traces from Chile and Antarctica can enable a more complete interpretation of the past ecosystems in Antarctica. Traces from all of the herbivory categories of general leaf chewing, skeleton feeding, leaf mines and leaf galls found on the fossil leaves were also found on modern leaves in Chile. There is a long time interval in evolutionary development between the fossil and modern herbivory systems, hence the specific insect species that made the traces in the modern ecosystems may not have been present in the past (Smith & Nufio, 2004). However, the insect fauna in the fossil forests probably included species of Coleoptera, Lepidoptera and Hymenoptera (Chapter 5). Distinct traces are made by specific insect species in modern forests in Chile, but there are also cases where several types of insects made the same general leaf chewing trace and some insects made several different general leaf chewing marks at different times. Therefore, it could also be assumed that during the Palaeogene several insect species could have made the same type of trace (even though the Palaeogene insects may not have been the same species of insects observed in the modern forests).

The insect damage levels in the fossil floras were found to be lower than the average in the modern Chilean forests. For both tree species the damage level in the fossil flora was, however, more similar to that in the southern Chilean sites than those in the northern sites. The difference in damage levels between the fossil and modern forests was previously attributed to possible environmental factors (Chapter 8). It could also be due to the difference in insect abundance or diversity. In modern environments insect diversity and abundance will change between seasons and years. Therefore, sampling within one season allows interpretation of a snapshot within a specific window of time, but extrapolation to the next season or year would not be valid. Fossil floras represent accumulations over several seasons and many years and therefore it is difficult to determine the seasonal rate of damage to the leaves or the diversity at any one point in time to compare with modern floras.

Previous studies that compared fossil and modern levels of insect-damaged leaves usually reported a significantly greater amount of damage in the modern floras (Labandeira, 2002; Smith & Nufio, 2004). This is not unexpected as insect-plant associations have probably increased in number over time and, with the evolution of more insect species, there would be a greater number of insect species feeding on the leaves in the modern forests. Only *Nothofagus* trees were examined in the modern sites in this study, but the fossil floras included a range of plant types. Other work has shown that because *Nothofagus* has a wide latitudinal distribution in Chile it also has a rich insect fauna compared to other tree types in the forests

(Lavandero *et al.*, 2009). Thus, the high number of associated insect species may explain the higher levels of damage on modern *Nothofagus* when compared to the level of insect damage on the fossil leaves.

The ratios of each herbivory trace type were used to investigate similarities in patterns of feeding between the modern and fossil forests. The ratios of leaf mines and general leaf chewing were similar, but ratios of skeleton feeding and leaf galls were different. The ratios varied between the modern sites, but the ratios for the two fossil sites were not significantly different, suggesting that the insect faunas may have been similar in both Palaeogene forests. A greater proportion of skeleton feeding was found in the modern sites. The evolutionary development of insects that produce skeletonised traces, a more specialized form of feeding as opposed to general leaf chewing, may explain the difference between the modern and fossil skeletonised leaf occurrence, as well as a possible taphonomic effect. A greater proportion of leaf galls was found at the King George Island fossil site. This was previously explained by the inclusion of the specimens from Dufayel Island which had a significantly greater proportion of leaf gall traces than any other locality on King George Island (Chapter 3). There may have been more galls preserved due to the depositional environments in which the leaves were preserved, which have been interpreted as fluvial or lacustrine sediments (Hunt & Poole, 2003) and therefore had greater preservation potential in quiet depositional conditions. In contrast, the sediments on Seymour Island were deposited by transport along the coast, combined with mass wasting of material and localized runoffs from the valley margins. Therefore, the leaves from Seymour Island probably suffered more bioturbation and abrasion, hence the fragmented nature of the fossil leaves from this area. This may have resulted in the loss of many insect-damaged leaves and could be the reason why the Seymour Island flora contains less insect traces.

There were also similarities between selected modern sites and fossil localities: Puy and King George Island have similar ratios of leaf damage (Figure 9.2), as do Torres and Seymour Island (Figure 9.3). It was originally expected that the fossil herbivory traces would be most similar to sites in southern Chile due to the closer proximity of the modern and fossil sites during the Palaeogene when there was a land bridge between the Antarctic Peninsula and Patagonia (Swenson *et al.*, 2001a). The trace fossil assemblage from King George Island is, however, more similar to that from Puy, which is a northern site. This could indicate that King George Island had a different climate to Seymour Island during the Palaeogene. The forest structure and plant species may also have been different. Cluster analysis (Chapter 7) of the ratios of trace types suggests that King George Island and Seymour Island are a separate group from the modern sites, indicating that the trace type ratios and levels of herbivory were not the same as in the modern sites. Both fossil sites clustered as part of a larger group consisting of the northern sites and Torres because the ratios of trace types were similar. Also, the northern sites and Torres had a similar proportion of damaged leaves. The southern-most sites of Isla Nav and RNMag formed a completely distinct group. This suggests that, although the fossil sites were more similar to each other than to the modern Chilean sites, they were more similar to the northern sites and Torres than to the other southern sites.

The range of trace types on the evergreen *N. betuloides* was notably different to the traces on the deciduous *Nothofagus* trees. Therefore, if a fossil leaf has a herbivory trace that is characteristic of ones found on evergreen trees today (specifically leaf mines or leaf galls), it may be possible to conclude that the original tree was also evergreen. There are few features on fossil leaves that allow the deciduous or evergreen nature of the tree to be determined, so this evidence from herbivory maybe key to determining leaf longevity in fossil leaf floras

Comparison of modern insect traces in Chile with insect trace fossils from Antarctica has provided evidence of a variety of insect-plant interactions in the past. Each fossil trace could represent one specific insect species or it could have been created by several species. Identification of the species-specific traces on the fossil leaves was determined by the comparison with modern traces from Chile.

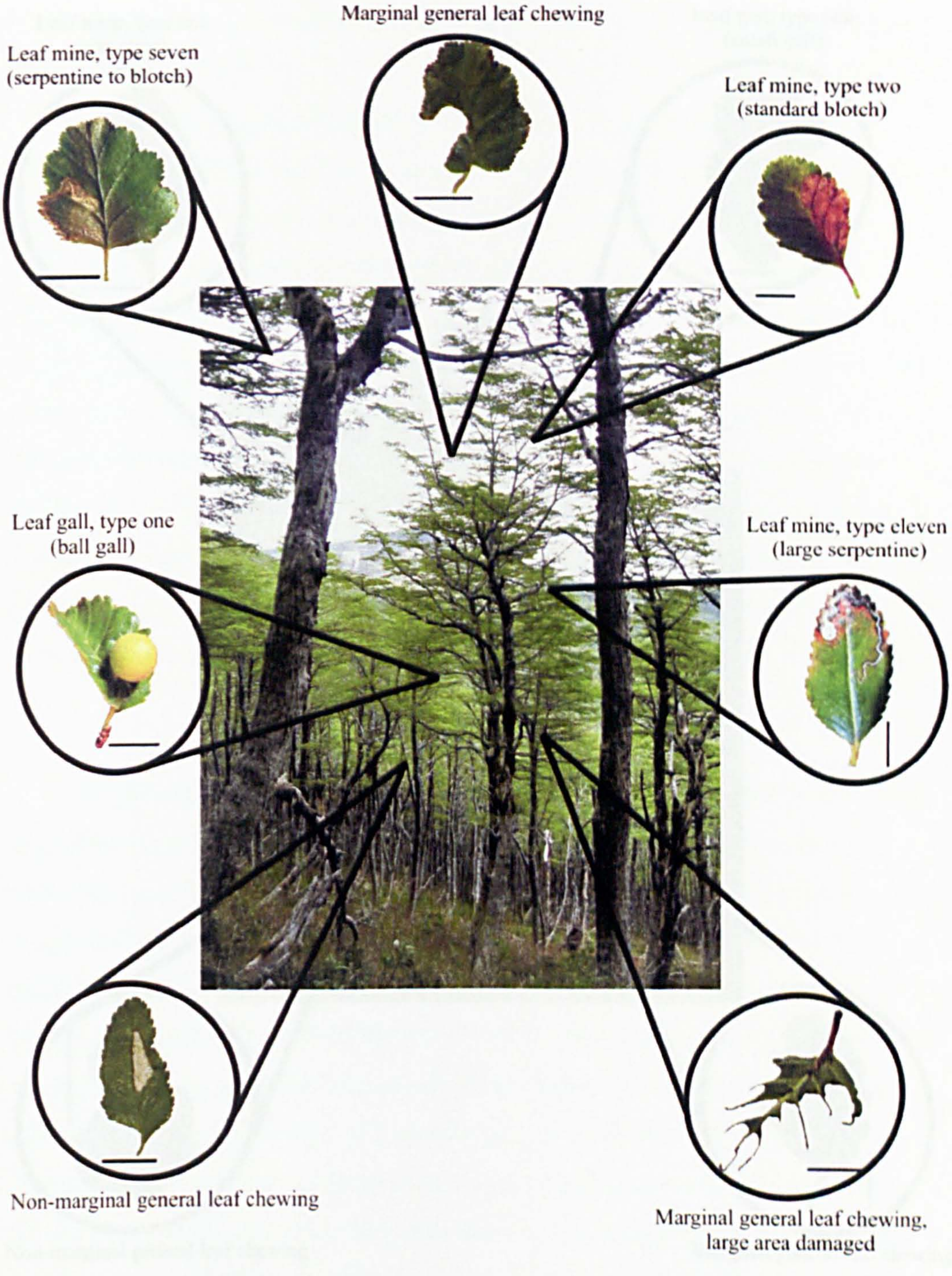


Figure 9.2. Reconstruction of the range of insect traces that may have been present on King George Island during the Palaeogene based on the comparison between modern herbivory traces from Chile and the fossil herbivory traces. The photograph is of a typical forest of *N. pumilio* at Puy, the modern site in Chile found to be most similar to King George Island. Scale bar is 1cm. The herbivory traces shown are on all three species of *Nothofagus* studied.

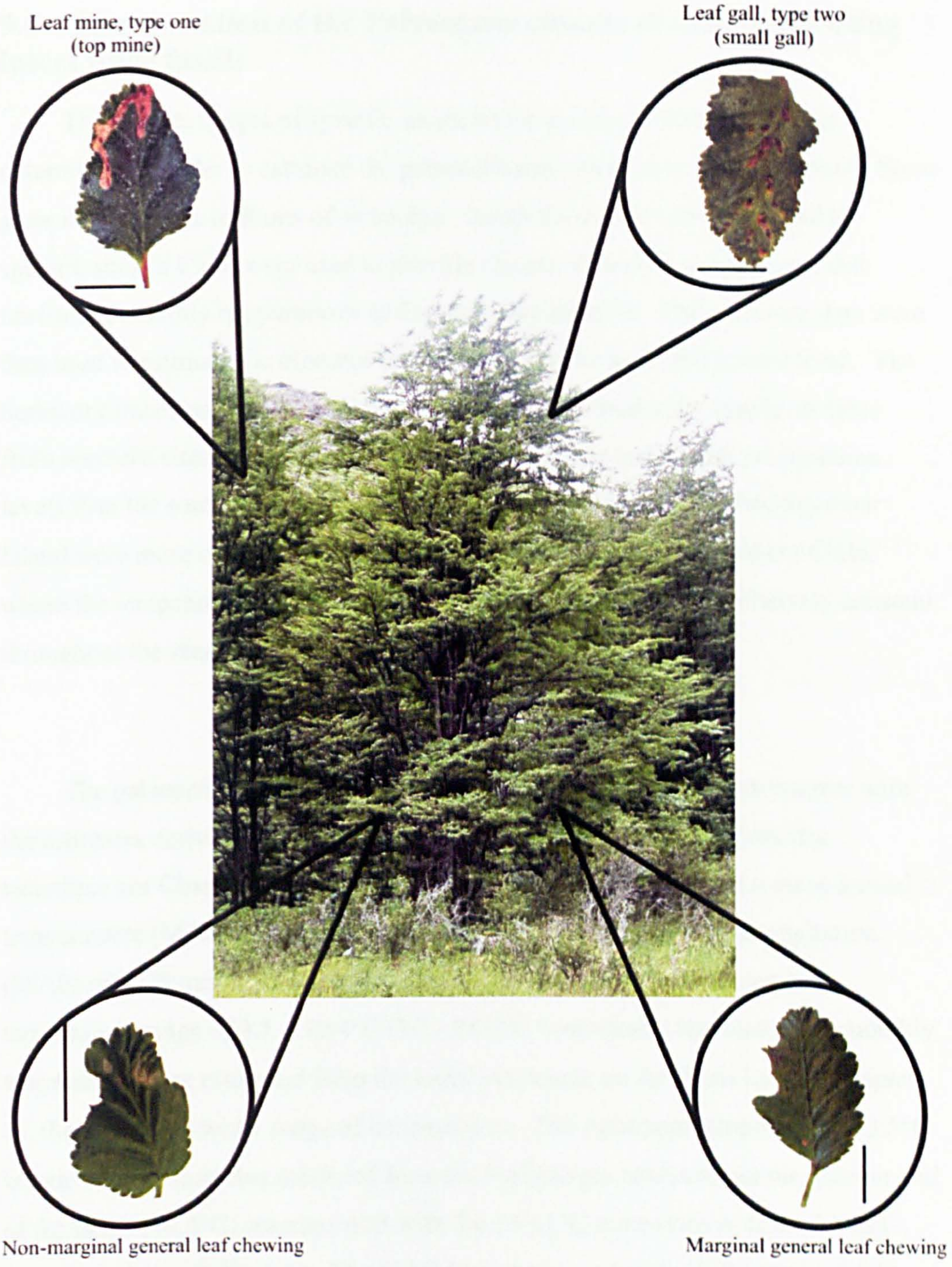


Figure 9.3. Reconstruction of the range of insect traces that may have been present on Seymour Island during the Palaeogene, based on the comparison between modern herbivory traces from Chile and fossil herbivory traces. The photograph is of a typical forest at Torres, the modern site in Chile found to have the most comparable herbivory traces to Seymour Island. Scale bar is 1cm. The herbivory traces shown are on the two deciduous *Nothofagus* species studied.

9.6. Interpretation of the Palaeogene climate of Antarctica using insect trace fossils

The climate ranges of specific insect traces on leaves from Chile were determined, in order to estimate the palaeoclimatic window in which the fossil floras grew in Antarctica millions of years ago. Insect traces that were restricted to specific sites in Chile were used to provide climate data such as minimum and maximum monthly temperatures and annual precipitation. These climate data were then used to estimate the climates in which the fossil leaves and insects lived. The herbivory traces on fossil leaves from King George Island were similar to those from northern sites in Chile, with warmer temperatures and higher precipitation levels than the southern sites. In contrast, the insect trace fossils from Seymour Island were more comparable with insect traces on leaves from southern Chile, where the temperatures are cooler and the precipitation levels are relatively constant throughout the seasons.

The palaeoclimate estimates based on analysis of the trace fossils agree with the estimates derived from palaeobotanical data. Leaf margin analysis (for technique see Chapter 1) of the King George Island flora generated a mean annual temperatures (MAT) of between 10°C and 11°C and mean annual precipitation (MAP) of 1039 mm (Hunt & Poole, 2003). In comparison, a mean annual temperature range of 3.5 – 10.4°C (3.5 - 24.3°C = minimum and maximum monthly temperature) was estimated from the herbivory traces on the fossil leaves (Chapter 8); this is a much wider range of temperatures. The minimum temperature of 3.5°C is much cooler than that predicted from the leaf margin analysis, but the warmer end of the range (10.4°C) matches well with the 10-11°C temperatures from the leaf margin analysis (Table 9.3). The MAP from the trace fossils (1500 mm) is higher than that estimated from the palaeobotanical data (1039 mm).

Table 9.3. Comparison of climatic data estimated for the Palaeogene leaf floras from Antarctica using different methods. MAT = mean annual temperature, MAP = mean annual precipitation.

Analysis used to give estimate	King George Island	Seymour Island
This study, based on comparisons with modern herbivory traces, Chile	3.5-10.4 °C MAT 3.5 – 24.3°C min and max monthly temperature 1500 mm MAP	3.5-10.4 °C MAT -0.4 - 16°C min and max monthly temperature 568 mm MAP
Palaeoclimate model for the Early Eocene (55.8 Ma to 48.6 Ma) (Chapter 7)	4 - 18°C MAT 1456 mm MAP	4 - 18°C MAT 1223 mm MAP
Leaf Margin Analysis of fossil leaves (Hunt & Poole, 2003)	10-11°C MAT 1039 mm MAP	-
CLAMP analyses of fossil leaves (Francis *et al.*, 2003; Francis *et al.*, 2008)	-	13.5 ± 0.7°C MAT 2110 mm MAP (Late Palaeocene ~55 Ma) 10.8 ± 1.1 °C MAT 1534 mm MAP (Late Early Eocene ~50 Ma)

For Seymour Island, a MAT range of 3.5 -10.4°C and MAP of 468mm was derived from this study, using comparisons with the climatic range of similar herbivory traces in Chile today (Table 9.3). Climate analysis of the fossil leaves using the CLAMP method (see Chapter 1) provided estimates of 13.5 ± 0.7°C at 55 Ma falling to 10.8 ± 1.1°C at ~50 Ma (Francis *et al.*, 2003). These temperature estimates match those derived from this study, with a particularly good match with that derived from CLAMP for the ~50 Ma flora (in this study fossil leaves from floras of 55 and 50 Ma were studied together to make a dataset of meaningful size). The estimate of MAP from this study of 468 mm is considerably lower than those of 2110 and 1534 mm from CLAMP (Francis *et al.*, 2003) (but MAP estimates from CLAMP are considered less reliable than MATs).

The estimates from the palaeoclimate models (Chapter 8) also agree with the estimates based on the modern and fossil trace comparison (Table 9.3). For both fossil localities the palaeoclimate model estimated the maximum and minimum mean annual temperature range to be 4 - 18°C. This is a wider temperature range than the MAT estimated using leaf margin analysis for King George Island. The range is also higher than the MAT estimated from CLAMP for both 55 Ma and ~50 Ma time periods. The palaeoclimate model gave MAP estimates of 1456 mm for King George Island and 1223 mm for Seymour Island; these are very similar to the estimates from the herbivory trace fossils (Table 9.3).

The climate data from Chile used to estimate palaeoclimate were obtained from Chilean stations at low altitudes. The modern insect traces on leaves were, however, actually collected at altitudes between 10m and 1420m above sea level. At higher altitudes the climate was cooler and wetter. This may explain the difference in precipitation estimates between the modern trace data and those from the CLAMP method, known to be unreliable (Jordan, 1997; Yang *et al.*, 2007). The actual MAT from the herbivory traces would be lower at high altitudes (using a lapse rate of 6.49°C for every 1000m if the standard lapse rate is applied (Jacobson, 2005).

The trace types used for the palaeoclimate analysis were mainly the leaf mines and leaf galls. The response of leaf mining insects and leaf galling organisms to changes in climate varies according to the species studied. The proportion of leaf mines in an assemblage was not correlated with either rainfall or latitude in a study by Sinclair & Hughes (2008). Other studies have found that the distribution of concealed feeders, such as the leaf-miners, is affected more by environmental factors than the affect of such factors on the distribution of surface active feeders (Novotny *et al.*, 2003). It has also been shown that leaf miners are more tolerant of desiccation than surface feeders (Connor & Taverner, 1997), with one study on *N. pumilio* in northern Patagonia forests of Argentina finding an increase in the damage caused by leaf miner activity at more xeric sites (Mazia *et al.*, 2004). Leaf galling insects have also been found to have increased survival rates in xeric sites by Fernandez & Price (1992), but other studies have found either greater numbers of galling insects in more mesic sites or no relationship between temperature and leaf galling insect diversity (Blanche, 2000; Blanche & Ludwig, 2001). Such differences in responses to external factors by leaf mining and leaf galling insects affect the use of their traces for climate estimates. In this study, the proportion of leaf mines did change with both height and orientation within trees, suggesting that in Chile the distribution of leaf-mining insects is to some extent influenced by factors, such as temperature and precipitation. The distribution of leaf mine and leaf gall traces does appear to be influenced to some extent by climate and therefore are considered here to be useful for comparison with fossil insect traces and subsequently obtain climate estimates for the past.

9.7. Reconstruction of Palaeogene fossil forests in Antarctica from fossil insect herbivory

The early Tertiary forests that once grew in Antarctica were composed of mixed deciduous and evergreen tree species dominated by the Nothofagaceae. Along with the Nothofagaceae, the Laurales, Cunoniaceae and Proteaceae were also important components of the angiosperm floras (Askin, 1992; Francis *et al.*, 2003; Hunt & Poole, 2003; Tosolini *et al.*, 2009). The coniferous vegetation included species of Cupressaceae, Araucariaceae and Podocarpaceae (Zastawniak *et al.*, 1985; Hunt, 2001; Poole *et al.*, 2001). Similar families are found growing today in the cool temperate forests of southern South America, New Zealand, Australia and Tasmania, all regions which were previously part of the supercontinent of Gondwana. These forests support a large diversity of insects which represent a variety of feeding guilds from sap-feeders, leaf miners, leaf gallers, leaf chewers, as well as insect predators and parasites (McQuillan, 1993; Reid, 2002; Austin *et al.*, 2004; Berry, 2007; Lavandero *et al.*, 2009)

Of the various Gondwanan plant groups the conifer genus *Araucaria* and the angiosperm *Nothofagus* have the best known insect herbivore faunas (Sequeira & Farrell, 2001). McQuillan (1993) stated that the invertebrate faunas of *Nothofagus* species throughout South America, Australia, New Zealand, Tasmania and New Caledonia show similar elements of a Gondwanan influence. Within this distribution, Tasmanian *Nothofagus* forests have a very low diversity of invertebrates compared to the other areas in which *Nothofagus* grow. Where *Nothofagus* is the dominant tree genus (New Zealand and Chile), its insect fauna is abundant (Welch, 1988); however, where the genus is surrounded by other tree species as in Australia and New Caledonia, the insect fauna is relatively restricted. Detailed comparisons of the insect fauna of *Nothofagus* from Chile with those from *Nothofagus* growing in other areas of former Gondwana would enable the evolution of insect-plant interactions on *Nothofagus* to be investigated, and is suggested as an extension of this project.

Other similarities are seen in both plants and insects with a Gondwanan distribution pattern. For example, *Araucaria* has an associated insect fauna rich in Coleoptera, five groups of which share comparable distributions with their *Araucaria* hosts in southern South America and Australia (Morrone, 1997; Farrell, 1998). Many insect groups found in Australia show characteristic Gondwanan distribution patterns throughout the southern continents including Thysanoptera, Hemiptera, Coleoptera, Lepidoptera, Diptera and Hymenoptera (Austin *et al.*, 2004).

Assuming that the modern insect fauna of the Gondwanan plant groups is representative of the insect fauna that lived in Antarctica during the Palaeogene, the majority of insect orders living today would have been present. The results of this project will help to define the possible insect diversity in Antarctic Palaeogene forests and enable further understanding of plant-insect interactions of the past. The study of both fossil and modern insect traces has highlighted that comparable traces from Chile are also present in the fossil floras in Antarctica. Insects that once lived in Antarctica would have been dependent on the plants as a source of food, shelter, reproduction and transport. This is evident through the types of trace fossils preserved, such as general leaf chewing, leaf mines and leaf galls. The characteristics of modern insect traces on leaves in Chile, comparable to those found on Antarctic fossil leaves, indicate the possible trace-makers that would have been present in the Palaeogene. For example, insects that created general leaf chewing traces on modern leaves include adults and larvae of Coleoptera (Curculionidae, Chrysomelidae), larvae of Lepidoptera (Geometridae) and larvae of Hymenoptera (Symphyta). It is therefore probable that ancestors of these insect families were present in Antarctica in the past and created similar traces on leaves in the Palaeogene forests.

In Chile, species of Coleoptera and Lepidoptera were collected; these created leaf mines. Insects such as species of Hymenoptera (Cynipidae) and Diptera (Cecidomyiidae), which created leaf galls, were also found. From the leaf mines and galls parasitoid wasps (Chalcidoidea and Ichneumonoidea) were reared. All of these species may have been present in Antarctica in the Palaeogene, creating galls and mines on the fossil leaves. Since parasitoid wasps evolved in the Early Triassic, it is possible that they were also living in the Early Tertiary forests of Antarctica. It is possible that other invertebrates may have created the leaf gall traces found on the fossil leaves, such as mites (Acari: Eriophyidae) and leaf galling nematodes (Tylenchida: Anguinidae), just as they do today in the Valdivian and Magellanic forests of Chile.

There have been numerous reports of insect trace fossils of Carboniferous and Permian age (Scott *et al.*, 1992; Beck & Labandeira, 1998; Labandeira, 2002), but insects diversified markedly in the Cretaceous with evolution of the angiosperms (Labandeira & Sepkoski Jr., 1993; Labandeira *et al.*, 1994; Grimaldi & Engel, 2005). With the exception of a study in Patagonia by Wilf *et al.* (2005) and the documentation of five leaf mines in Eocene deposits of Australia (Rozefelds, 1988), the main reports of fossil insect herbivory have originated in the Northern Hemisphere. Therefore, this work contributes to the greater understanding of the evolutionary history and past distribution of plant-insect interactions, particularly for the Southern Hemisphere.

General leaf chewing marks on modern leaves in Chile, created by Chrysomelidae and Curculionidea, are similar to general leaf chewing traces on the fossil leaves. Therefore, it is plausible that chrysomelid and curculionid beetles were present during the Palaeogene. General leaf chewing insects evolved as long ago as the Late Jurassic, since body fossils of Chrysomelidae and Curculionidea, phytophagous coleopterans, have been found in Jurassic rocks (Grimaldi & Engel, 2005). Some Coleoptera (Cerambycidae) appeared only in the Cenozoic (Grimaldi & Engel, 2005) and the majority of chrysomelid body fossils are of Cenozoic age.

Thus the record of the Coleoptera from Antarctica adds new records to the history of this group.

Definitive Eocene fossil galls on leaves from King George Island are very similar to the modern cynipid galls collected in Chile. Although identification of the fossil gall from Antarctica as a cynipid wasp gall cannot be verified without an insect body fossil, the comparison with the modern leaf galls in Chile implies the occurrence of cynipid galls in Antarctica in the past. The only definitive evidence of Cynipidae fossil leaf galls are of Cenozoic age from the Northern Hemisphere deposits (Scott *et al.*, 1994; Waggoner & Poteet, 1996; Waggoner, 1999). The presence of fossil leaf galls from Antarctica that are similar to modern galls formed by mites (Acari) and leaf galling nematodes (Tylenchida) provides evidence of invertebrate-plant interactions. The exact origin of the leaf galling ability in nematodes is unknown due to the lack of preservation of soft bodied organisms. Using phylogenetic relationships of modern species they are thought, however, to have originated in the Cretaceous, soon after the origin of grass- dominated systems (Siddiqi, 2000). Hence, the presence of leaf gall nematodes in the Palaeogene forests of Antarctica is possible.

The only fossil evidence of leaf mining lepidopteran from the Southern Hemisphere is in Late Eocene deposits from Victoria, Australia (Rozefelds, 1988) and from Early to Middle Eocene deposits in Patagonia, Argentina (Wilf *et al.*, 2005). However, this study found modern leaf mines in Chile that were created by lepidopteran larvae that are comparable to fossil leaf mines in Antarctica. This provides possible additional evidence of leaf mining Lepidoptera from the Palaeogene. The leaf mining habit is most extensively developed in the Chrysomelid beetles (Connor & Taverner, 1997), but most leaf mines found in Chile were created by Curculionoidea larvae. Coleopteran fossil leaf mines are reported from the Late Palaeozoic, but definitive evidence of Cretaceous and Cenozoic age rocks is rare (Krassilov, 2008).

Prior to this study, insect trace fossils and body fossils from Antarctica, and the Southern Hemisphere generally, were rare and the past insect fauna of Antarctica was unknown. This project has provided new, indirect evidence of several insect and invertebrate orders that would probably have lived in Antarctica during the Palaeogene. These are Coleoptera, Hymenoptera, Lepidoptera, Diptera, Nematoda and Acari.

Similarities between the fossil herbivory traces from Antarctica and modern Chilean insect traces provide evidence for past links between Antarctica and southern South America during the Palaeogene. During the Palaeogene Antarctica and southern South America were connected and insects could have dispersed easily throughout forests in Antarctica and South America. The lower sea level at the time implies that small islands could have existed between South America and Antarctic Peninsula and acted as 'stepping stones' between the two areas (Swenson *et al.*, 2001a; Swenson *et al.*, 2001b).

A more accurate reconstruction of the community of these southern forests of the past can now be made. The mixed deciduous and evergreen forests would have been dominated by *Nothofagus* similar to the cool temperate forests of southern Chile and Tasmania today. The forests probably contained coleopteran, lepidopteran and hymenopteran insects, leaf miners and leaf gallers, living in the forests during the Palaeogene. A reconstruction of the fauna of the forests is shown in Figure 9.4. This reconstruction shows the Eocene mammals of Seymour Island (as presented previously in Figure 9.1), but now the insect fauna, derived from this project, has been added. This has been made possible by examination of insect trace fossils that have provided unique evidence of insect life in Antarctica during the Palaeogene.

Figure 9.4. Reconstruction of forests on Seymour Island during the Eocene. Adapted from Reguero *et al.* (Reguero *et al.*). Evidence of an *Ibis*-like bird is from Jadwiszczak (2008). The Ibis species shown above is a buff-necked ibis (*Theristicus caudatus*), which can be found in South America today. In this reconstruction insects that may have lived at that time are added, determined from the herbivory traces studied in this project. Insects and ibis are not drawn to scale.

Chapter 10. Conclusions

10.1. Main Conclusions

1. The presence of phytophagous insect activity on fossil floras of Palaeogene age from Antarctica has been recorded for the first time. Insect body fossils are rare, but the herbivory traces on fossil leaves have provided a new reconstruction of insect life on Antarctica in the past.

2. Evidence of past insect life, preserved as traces of insect herbivory on fossil leaves, was found in Palaeogene fossil floras from two localities off the Antarctic Peninsula: King George Island and Seymour Island. The trace fossils found on the leaves indicate that a range of feeding guilds were present, including general leaf chewing, skeleton feeding, leaf mining and leaf galling. For both localities general leaf chewing was the most common trace type and leaf mines the least common type.

3. There were 130 fossil leaves from King George Island with traces preserved, with traces on *Nothofagus* leaves dominating the collection. The Seymour Island floras yielded 26 fossil leaves with traces preserved. On plant morphotypes that were different to those in the King George Island flora. The proportion of leaves with insect damage in these Antarctic forests was relatively small compared to other fossil studies of Eocene temperate forests around the world. However, the number of different trace types was higher than the range of trace types reported from fossil forests in North America and similar to the number of trace types found in Eocene forests of Patagonia.

4. In the Antarctic fossil forests the range of traces does not directly represent the diversity of insect species which could have been present, but rather the minimum number of insect species. This is because the range of traces found on the leaves is greatly influenced by the taphonomic bias on both the species of plant and the trace type that is finally preserved. Therefore, the traces

documented represent the minimum range that was likely to have existed in the past.

5. The Valdivian and Magellanic forests of Chile represent the closest modern analogue to the forests growing in Antarctica during the Palaeogene. Analysis of the range of modern insect traces preserved on leaves in the forests of southern Chile enabled a comparison with the fossil traces. The range of traces created by different species of insect, the diversity of leaf mining insects and the diversity of leaf galling insects on two species of deciduous *Nothofagus*, *N. pumilio* and *N. antarctica*, were investigated in several sites in Chile.

6. It is difficult to predict what effect external factors are likely to have on both the extent of insect damage to leaves and on the occurrence of leaf mines and leaf galls. In Chile, the occurrence of insect-damaged leaves between sites at different latitudes, at different height levels, and on branches with different orientations within trees was dependent on the tree species studied and the insect species involved. *N. pumilio* had a greater number of damaged leaves than *N. antarctica* in the southern sites in Chile, but *N. antarctica* had a greater number of damaged leaves in the northern sites.

7. When height within the tree was a major influence on the occurrence of insect damage, a greater number of damaged leaves were found in the lower levels of the tree. The relationship with orientation was more complex; on *N. pumilio*, the northerly facing sections of the tree had the greatest number of insect-damaged leaves, but on *N. antarctica*, the southerly facing sections had the greatest number. The northern sections are generally the warmest part of the tree compared to the southern side as they are exposed to the sunlight for a longer period, therefore, insect activity is likely to be greater on this side of the tree. The reaction of different species of insect to external factors such as orientation with the tree can explain why damage on *N. antarctica* was different. External factors did not affect the intensity of damage on either tree species, although the majority of leaves had a small area of the leaf damaged (<25% of the total leaf damaged).

8. The influence of several factors was used to justify between-site and between-tree comparisons and to confirm that the level of insect-damaged leaves in modern forests at all sites was a reliable estimate. The proportion of insect-damaged leaves was found to be constant throughout the season. The number of damaged leaves was found to be higher at middle altitudes than at high or low altitudes on *N. pumilio*. In addition, within the canopy of *N. pumilio* trees, the lower areas had a significantly higher proportion of insect-damaged leaves than middle or top areas of the canopy. Also, the proportion of damaged leaves collected using the canopy method was not significantly different from those collected using the ground based technique. Finally, there was a significantly greater proportion of insect damage on older leaves than on younger leaves.

9. The trace types on the evergreen tree species, *N. betuloides*, were distinct from those created on the deciduous species in that, although a similar range of general leaf chewing marks was present, the reaction rim around the damaged area on the evergreen leaves was thicker. The species of leaf mining and leaf galling insects on *N. betuloides* were different to those on the deciduous species and therefore created characteristically different mines and galls. Therefore, comparisons with trace fossils may enable the evergreen or deciduous nature of the tree types from which the fossil leaf was derived to be determined. This can often be difficult from examination of the fossil leaf alone.

10. The insect species that created the trace types on *N. antarctica* and *N. pumilio* in Chile highlight the community complexity within the forest system. The general leaf chewing and skeleton feeding insects included the adults and larvae of species of Coleoptera, larvae of hymenopteran species (sawflies) and larvae of species of Lepidoptera. Several insect species made the same type of damage marks and some species created several different types of marks. This indicates that the general leaf chewing traces on the fossil leaves could have been created by several different insect species, and so these trace types cannot be used to indicate the presence of a specific species or family of insects in the fossil forests of Antarctica.

11. The leaf mines on *N. antarctica* and *N. pumilio* were created by species of Coleoptera and Lepidoptera and the leaf galls were identified as being created by species of Diptera (Cecidomyiidae), Hymenoptera (Cynipidae), Acari (Eriophyidae) and Nematoda (Tylenchida). Both the leaf mining and leaf galling insects collected had a parasitoid wasp community associated with them, mainly species of Chalcidoidea and one species of Ichneumonoidea. Inquiline coleopterans (Curculionidae) were also reared from leaf galls. Parasitoids and inquilines highlight the complexity of trophic interactions even within two tree species. The leaf mines and leaf galls were comparable to fossil leaf mine and leaf gall traces, therefore, it is likely that trophic interactions, such as the leaf galler-parasitoid-inquiline interaction, were present in the forests of the past.

12. The insect damage levels and ratios of each trace type were used to examine the similarity between the fossil sites and the modern sites. Statistical analysis indicated that the insect damage on King George Island floras was more similar to that from Puyehue, and the herbivory traces on Seymour Island floras were more similar to those from Torres del Paine. Cluster analysis showed that the insect traces from both Antarctic localities are distinct from the modern sites, which may have been due to different forest structure in the past or evolution of plant-insect associations. Herbivory traces on both fossil floras more similar to traces from the northern sites and Torres del Paine than the more southerly sites in Chile, possibly related to climatic factors.

13. The climatic tolerances of selected feeding guilds were determined for several sites in Chile. These were then used in the method Coexistence Approach to determine the possible palaeoclimates that prevailed, under which insects lived in Antarctica in the past. King George Island was estimated to have had a minimum and maximum monthly temperature range of 3.5 - 24.3°C and Seymour Island had a minimum and maximum monthly temperature range of -0.4 -16°C. The mean annual temperature range was 3.5 - 10.4°C for both sites. Mean annual precipitation estimates were also calculated: King George Island was estimated to have an annual precipitation of at least 1500 mm and Seymour Island could have had an approximate annual precipitation of 570 mm.

14. The palaeoclimate estimates obtained for King George Island and Seymour Island from an Eocene global climate model and leaf margin analysis were similar to the estimates obtained from the modern insect traces. The precipitation estimates were higher than those obtained from the trace fossils, possibly due to the altitude at which the meteorological stations were situated. The temperature estimates were similar, however, indicating that if a correction for altitude of the meteorological stations is considered the temperature estimates from the herbivory traces could actually have been lower than the estimates the models predicted.

15. The differences in both trace type and abundance of each trace type at the two fossil sites indicates that each site should be analysed separately, otherwise the variation in herbivory trace types would be missed. The range of external factors, both biotic and abiotic, and their varying effects on the insect traces in modern systems also highlights the complexities in interpreting and predicting insect species richness and the traces they create. Hence, limitations exist when comparing fossil and modern systems, but as long as such limits are considered the method can be used to provide a greater understanding of the ecological interactions and climates of the past.

16. Overall, these studies of modern insect traces have provided a unique opportunity to investigate and reconstruct the past insect life of Antarctica during the Palaeogene. Investigation of past trophic interactions provides evidence of a faunal connection between Chile and the islands around the Antarctic Peninsula.

10.2. Possible further work

- The precise position of the fossil leaves within the stratigraphic column in the outcrop is unknown for many of the specimens of Seymour Island. However, if systematic sampling of the fossil specimens is possible then patterns of trace fossils at particular times may be evident or significant changes in the proportion of insect-damaged leaves at a finer scale within the Palaeogene may become apparent. This may help to understand if insect-plant interactions in the forest of Antarctica changed with changes in climate (specifically the Palaeocene Eocene Thermal Maximum and the Early Eocene Climatic Optimum).

- Examination of existing or new fossil leaf collections for insect traces for different time periods such as the Cretaceous and other times during the Tertiary or from a different area (West and East Antarctica) would enable a distribution pattern of specific insect traces, such as the leaf mines and leaf galls, to be obtained. This could be used to give a more precise range of climate estimates for specific times in the past and could highlight areas of high insect species diversity or endemism.

- Further identification of the insects collected in Chile could broaden existing species distribution data. New associations may also be documented, particularly the inquiline-leaf gall interaction and the parasitoid wasp community, for which plant and insect host species are unknown, and for which the parasitoid host species relationships have not been recently confirmed.

- This project would benefit from further sampling in the study sites in Chile to compare insect species richness and insect damage levels between years. This would provide insights into how such changes in insect damage over time could be related to the study of trace fossils, which themselves are assemblages of leaves that amassed over several seasons or years.

- Leaf collection from multiple tree species in Chile would indicate the variation in leaf damage and diversity due to different tree species compositions, or in forests with different structure and therefore, different levels of heterogeneity. This may provide a greater diversity of traces that could be comparable with the fossil traces, and as the fossil traces are found on many tree species, this may provide a more valid comparison than examination of one or two living tree species.

- Reports of Southern Hemisphere insect trace fossils are rare. It would be interesting to examine the distribution patterns of trace fossils on different land masses that were originally part of the supercontinent of Gondwana. The genus *Nothofagus* has been used as the key to understanding the biogeography of the Southern Hemisphere and the break-up of Gondwana, but there has been limited investigation of modern insect species on *Nothofagus* throughout Gondwanan continents to assess the distribution and the phylogenetic relationships of the insect families. Examination of insect trace fossils on fossil leaves, in particular *Nothofagus* leaves, in New Zealand, Chile, Australia, New Caledonia and Tasmania, may also provide evidence of a Gondwanan distribution of certain insect species. This would elucidate evolutionary developments throughout the Southern Hemisphere and indicate the extent of dispersal of past insect species.

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Appendix I. British Antarctic Survey Field Expeditions

Table I.1. British Antarctic Survey field collection details for King George Island and Seymour Island for all samples examined in the study.

Site	Formation	Site Codes	Collector	Date
King George Island	Dufayel Island	G.53	Bibby JS	31 Jan 1958
		G.312	Hobbs GJ	15 Jan 1959
	Potter Peninsula	P.232	Thomson MRA	25 Jan 1975
	Vaureal Peak	P.2799	Smellie JS	07 Mar 1996
	Fossil Hill	P.935	Davies RES	10 Dec 1975
		P.3030-P.3032, P.3034, P.3036	Hunt RJ	Feb-Mar 1999
	Rocky Cove	P.3029, P.3035	Hunt RJ	Feb-Mar 1999
	Collins Glacier	P.3025, P.3028	Hunt RJ	Feb-Mar 1999
	Dragon Glacier	G.9	H-Smith G	Dec 1948-Jan 1949
		P.236,	Thomson MRA	12 Feb 1975
		P.2810,	Smellie JS	22 Mar 1996
		P.3001-P.3007, P.3010, P.3011, P.3013	Hunt RJ	Jan 1999
	Smok Hill	P.1404	Davies RES	14 Jan 1976
	Platt Cliffs	G.50	Bibby JS	30 Jan 1958
		G.309, G.319	Hobbs GJ	14 Jan 1959
Seymour Island	La Meseta Formation	DJ1111, DJ1113	Tosolini, AMP Cantrill DJ Francis JE	Jan-Mar 2001
		DJ913	Cantrill DJ Francis JE	1998-99
		D512-523	Falkland Islands Dependencies Survey	1944

Appendix III. Double Rope Climbing Technique

III.I. Working Procedure for Canopy Access

1. The sampling area was assessed for possible hazards or hidden dangers and each tree was inspected to identify potential hazards within the individual working trees, such as dead branches and large splits in the trunk.

2. A work plan was formulated and agreed by both the climber and people on the ground. This included development of a communication system to enable rapid detection of any problems that may occur for the climber or the ground people. A maximum time limit was stated and clearly defined security arrangements were made for the site (gear storage, hazard tape).

3. A thorough inspection of all climbing equipment was performed before use including ropes, personal protective gear (harnesses and helmets) and sampling equipment.

4. A working line in the tree was established using a line installation technique with a Big Shot Catapult (Figure III.1A). Anchor points within the tree for the ropes were chosen that were strong and efficient for the work needed as was covered in training. Anchor points could include the main fork in the tree trunk at the top or in between two main large branches at the top of the tree (Figure III.1B).

5. The strength of the anchors was tested by two people hanging on the ropes to see if any branches would break within the tree canopy or that the ropes would change position. Two ropes were used throughout the sampling, a working line with two points of attachment and a safety line on which the climber was attached using a back-up device that locks with a sudden fall (Figure III.2A).

Figure III.1. A) Demonstration of using the Big Shot to place a throwline in a tree for sampling. B) An example of a safe anchor point, fork at the top of a tree, used to weigh the ropes on for climbing.

6. A ground based rescue system was set up which enabled the ground team to rescue the climber quickly and easily if required (Figure III.2B). This system involved both sets of climbing lines being tied to create two loops and fastened through descending devices to a suitable anchor point on the ground. The ropes were set up to ensure that enough length was available to lower the climber to the ground.

7. The climber sampled the tree by at first climbing to the very top of the rope, using a chest and hand ascender (Figure III.2C). The change was then made onto the stop (device for lowering to the ground) and the climber could then control the height they sampled at by stopping to collect the necessary samples (Figure III.2D). Samples are lowered to the ground immediately after collection for processing, using a butterfly knot in the safety line.

8. After climbing, all ropes were replaced with a thinner line to allow access to the same tree and anchor point next time if needed. This prevented the ropes from getting wet and therefore, potentially damaged.

9. All equipment was then logged and rechecked before storage and use at a later date.

Figure III.2. A) Working rope and safety rope set-up during a descent. B) Ground based rescue system with the two looped ropes secured to an anchor point (neighbouring tree trunk) using descending devices. C) Start of the ascent to the top of the tree. D) Sampling the tree during the descent phase.

Appendix VI. R Statistical Analyses

VI.1. Introduction to R Statistics

All analyses were carried out using the statistics programme R 2.6.1 (R: A Language and Environment for Statistical Computing, The R Development Core Team, 2008). An explanation of the statistical analyses performed and how they are used to analyse the data is given in below.

The statistical programme R provides the flexibility to analyse data sets which do not meet the assumptions of parametric tests, such as a normal distribution and equal variances. Traditional methods used to analyse categorical, proportional or count data that are not normally distributed, such as the Mann Whitney U test and the Kruskall Wallis method, normally need a large sample size to provide results with the same level of confidence as a parametric test. The modern insect data collected in Chile do not meet the assumptions of parametric tests (not normally distributed and the variances of the data are not equal) and therefore the programme R was used to perform all statistical analyses. The data are also nested, as several samples were taken from the same height, and also from several heights, within the same tree. The methodology in R enables analysis of such data sets.

The analyses in R are based on the creation of a model to describe the data, whether to examine the spread of a dataset from one area, to compare measurements from several different groups or to examine a relationship or correlation between two sets of measurements. The model is based on having a response variable (variable of interest) and one or more explanatory variables. For example, to find out if insect wing length differs between two populations, wing length would be the response variable and population would be the explanatory variable. Mathematically, the best model is one that has as many variables as there are data points, however, this is never possible in reality. Therefore, as a compromise, to explain the data with the limited variables, the first model includes all variables that may help to explain the observed range of data points (response variable) and is called the maximal model. The model is then adjusted in a step-wise fashion by removing any explanatory variables that do not significantly influence the data. Incremental deletion is tested by performing likelihood ratio tests to compare the explanatory power of two models to the data. If there is a significant difference when one variable is removed, that variable is retained in the final model.

An interaction term can also be incorporated into the final model, where two or more variables interacting together are used to explain the range in the observed data points. For example, a student wishes to look at the variability in the number of insect-damaged leaves at three height levels within a tree, as well as the difference in damaged leaves between two different areas of the forest (edge and centre). A model to test for the effect of height and area on damaged leaves would have the number of leaves as the response variable, with height and area as the explanatory variables. An interaction term between height and area could be included, because the number of damaged leaves may vary with height in one particular area of the forest. The number of damaged leaves could be highest at the top of the tree in the centre of the forest, but not change in relation to height at the edge of the forest. If the interaction between two variables is significant, but the variables alone are not, then they still need to be included in the final model. The interaction term can be removed from the model, but only in a step-wise fashion. Therefore, if the interaction between four variables is significant then this is included in the final model and no further simplification can be performed. The step-wise deletion of variables is carried out until the minimum number of variables needed to explain the response variable is reached, creating the minimal adequate model. The interpretation of the data is carried out using this model.

There are several different types of models which vary in both the assumptions and the constraints they apply (Figure V1.1). All models can be used to test for relationships between variables of interest, as well as examine differences between factor levels within each variable (Buckley *et al.*, 2003). The models examine how the variation in the response variable (the variable of interest, usually plotted on the y-axis) is affected by variation in the explanatory variable (usually plotted on the x-axis). A linear model has the same assumptions as parametric statistical tests. If explanatory variables are continuous a regression analysis is carried out, but if they are categorical an analysis of variance (ANOVA) is performed to test for a difference between the levels (different categories) of the explanatory variable. Many ecological data sets have a non-normal distribution or unequal variances (for example, proportional and binary response variables) so generalized linear models (GLMs) have to be applied (Venables & Ripley, 2002; Crawley, 2007).

VI.1.1. Generalized Linear Models (GLMs)

GLMs allow the flexibility for the error structure (distribution of the data) to be defined, such as poisson errors for count data and binomial errors for proportional data. Therefore, transformation of the response variable to give normally distributed data is not required. The model is related to the original data set using a linear predictor to calculate an estimated value for each individual data point of the response variable, but on a transformed scale. The predicted response estimate is obtained from the sum of the explanatory variables. The transformation of the response variable is determined by the link function which is, in turn, specified by the error structure used in the model. For example, to analyse the number of moths in a trap, a poisson error structure would be used because the counts do not have a normal distribution and the link function used would be the log of the counts to ensure that no negative values are generated. The error structure and link function used for each model is given throughout this chapter. Due to the transformation stage, the results of the model have to be back transformed for interpretation. For example, for binomial errors, the model estimates, x, have to be transformed back into proportions, p, to obtain the true means of the data using the following equation:

$$p = \frac{1}{1 + \frac{1}{\ell^{x}}} \qquad (1)$$

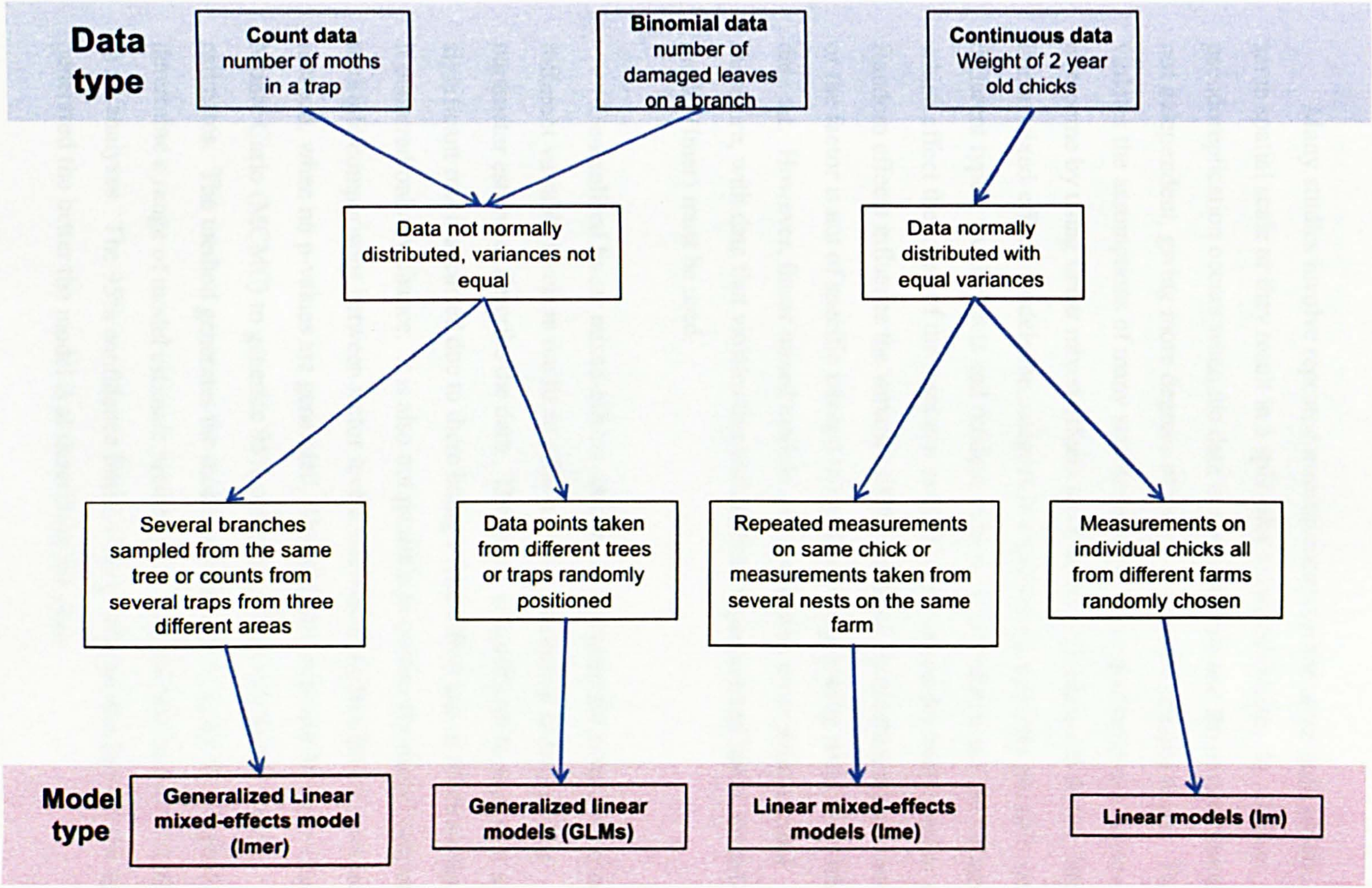

Figure VI.1. Flow chart to show the types of data that can be used for each of the models available in R and used for the statistical analyses in this chapter. An example of each type of data is given in the blue box at the tope of the chart and the models at the bottom in the red box.

VI.1.2. Generalized Linear Mixed-effects Models (lmer)

Many studies involve repeated measurements on the same individuals, on the same spatial scale or they result in a split-plot or nested design. In all cases, pseudoreplication occurs when the data is analysed because the measurements are not independent, giving more degrees of freedom than are actually there. This violates the assumptions of many statistical tests. Such problems can however, be overcome by using linear mixed-effects models (lme) (Pinheiro & Bates, 2000). In linear mixed-effects models the categorical explanatory variables are split into two different types, fixed effects and random effects. Fixed effects are the variables which affect the mean of the response variable and are usually controlled or set. Random effects influence the variance of the response by creating added variation, or the factor is not of specific interest but reflects the grouping structure within the data set. However, linear mixed models assume normal error structure and therefore, with data that violates this assumption, a generalized linear mixed-effects model (lmer) must be used.

Generalized linear mixed-effects models do not generate *p*-values for either the different variable levels in the fixed effects or for examining how good the parameter estimates describe the data. Therefore, it is difficult to state that a significant result occurred due to there being a true difference or relationship, or that it occurred only by chance. It is also not possible to correct the model output for multiple comparisons between factor levels, such as using Bonferoni's correction method, when no p-values are generated. One solution is to use Markov Chain Monte Carlo (MCMC) to generate 95% confidence intervals for the model estimates. The method generates the model several times, using the original data, to determine a range of model estimate predictions. The MCMC was run 10,000 times in all analyses. The 95% confidence limits were produced; the smaller the interval generated the better the model is at describing the data.

Such models were used in several analyses due to the pseudoreplication introduced when sampling several branches of leaves within the same trees. Models with different fixed effects were run using the maximum likelihood (ML) method to generate variance and covariance estimates. The models were then compared using Likelihood ratio tests, F-tests and Akaike's Information Criterion (AIC) depending on the type of model used (Crawley, 2002). The best model is the one with the lowest AIC number and the one with the minimum number of variables and factor levels. The minimal adequate model was then run using restricted maximal likelihood (REML) to obtain the variance estimates for the final model. The REML method is not biased as the errors of the fixed effects do not influence the estimates and so it enables the model to describe more accurately the different layers of variation associated with nested data. Minimal adequate models were also fitted to the data using the Laplace approximation method (for generalized linear mixed-effects models) to give variance estimates, which is more accurate than the penalised quasi-likelihood (PQL) estimation method for non-linear mixed-effects models (Crawley, 2007).

The datasets collected from Chile were analysed using the different models mentioned above to explain the differences between the numbers of insect-damaged leaves collected at the various levels of each factor. The model chosen was dependent on both the type of data and the combination of the response and explanatory variables. A model simplification process was carried out for all types used; summarised in Table VI.1. The minimal adequate model for each analysis is shown and interpreted in the remaining sections of the chapter to meet the objectives of Chapter 6 stated in section 2.1.4.

Table VI.1. Summary of the steps involved in model simplification when trying to find the best model to fit the data. After Crawley, 2007.

Step	Procedure	Explanation
1.	Create the maximal model	Include all variables, interactions and covariates of interest. Use the correct error structure relevant to the data.
2.	Start model simplification	Remove the least significant terms from the model first, usually those with the highest order interaction terms.
3.	If deletion caused a insignificant increase in deviance (F-test or Chi-squared tests)	Leave the term out of the model and look at the next least significant term.
4.	If deletion causes a significant increase in deviance	Put the term back in the model. This is the statistically significant term.
5.	Keep removing terms from the model	Repeat steps 3 or 4 until model contains nothing but significant terms. This is the minimal adequate model.

For all models described in this thesis, the first row of the model output shows the predicted parameter (known as the intercept), a mean value for the first level of a variable in alphabetical order, and the standard error of the mean. For example, if an explanatory variable called leaf area has three levels named apical, basal and central, row one would be the estimate of the apical area. The other estimates are not mean values, but are actual differences between the means of two variable levels and the standard error of difference between the two means. For example, a model output has an intercept (factor level apical) estimate of 9.90 units and a factor level basal estimate value of 1.60. This is interpreted as the mean for the factor level basal being 1.60 units greater than the factor level apical (11.50 units). The Student's *t* values are also given to test if the difference between the two means is statistically significant. If the *t* value is greater than 2 (ignoring the negative sign) there is a significant difference between the factor levels at the 95% confidence interval.

Appendix VIII. Palaeoclimate model results for Chilean Sites

VIII.1. Temperature estimates from palaeoclimate models

The warm month and cold month mean temperatures were estimated for the six sampling sites in Chile using the palaeoclimate model for the Early Eocene described in Chapter 8. Climate estimates from models with 4 and 6 times the pre-industrial CO_2 concentrations, the level estimated for the Early Eocene, were compared with the equivalent temperature estimates from meteorological stations near the sites today (Section 8.1). Figures VIII.1 and VIII.2 show the warm month and cold month mean temperatures for sites from the models and modern day data. They indicate that during the Eocene both the average warm month and cold month temperature were greater than the modern day levels. There was one exception as the estimates for the RNMag site during the Eocene were colder than the estimate of today for the cold month mean temperature.

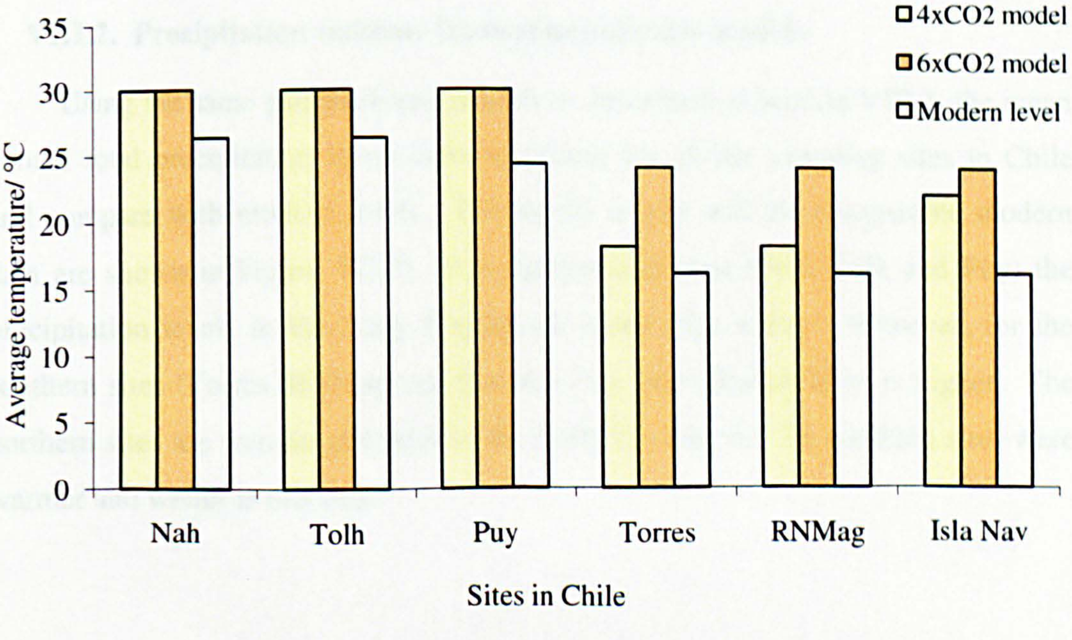

Figure VIII.1. The warm month mean temperature for the six sampling sites in Chile generated from a palaeoclimate model for the Early Eocene with 4 times and 6 times the pre-industrial CO_2 concentrations. Modern estimates were obtained from the three meteorological stations nearest to the sites. Data from one station were used for both Nah and Tolh and data from another were used for Torres, RNMag and Isla Nav.

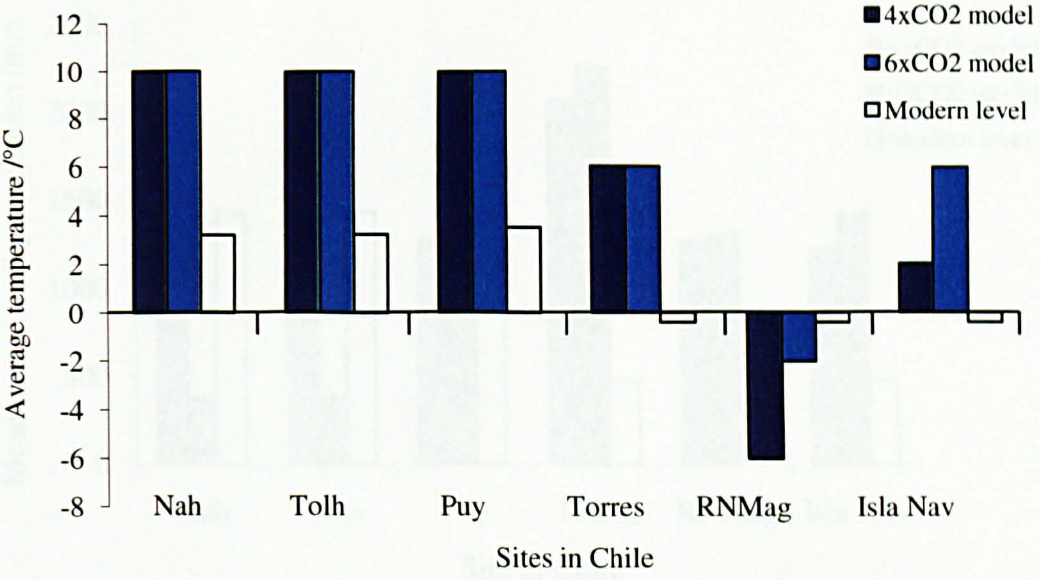

Figure VIII.2. The cold month mean temperature for the six sampling sites in Chile generated from a palaeoclimate model for the Early Eocene with 4 times and 6 times the pre-industrial CO_2 concentrations. Modern estimates were obtained from meteorological stations nearest to the sites. Data from one station were used for both Nah and Tolh and data from another were used for Torres, RNMag and Isla Nav.

VIII.2. Precipitation estimate from palaeoclimate models

Using the same palaeoclimate models as described in Section VIII.1, the mean annual total precipitation levels were measured for all the sampling sites in Chile and compare with modern levels. The model results and the comparable modern data are shown in Figure VIII.3. For the northern sites (Nah, Tolh and Puy) the precipitation levels in the Early Eocene are lower than today. However, for the southern sites (Torres, RNMag and Isla Nav) the precipitation level is higher. The northern sites are warmer and drier in the Early Eocene, but the southern sites were warmer and wetter at this time.

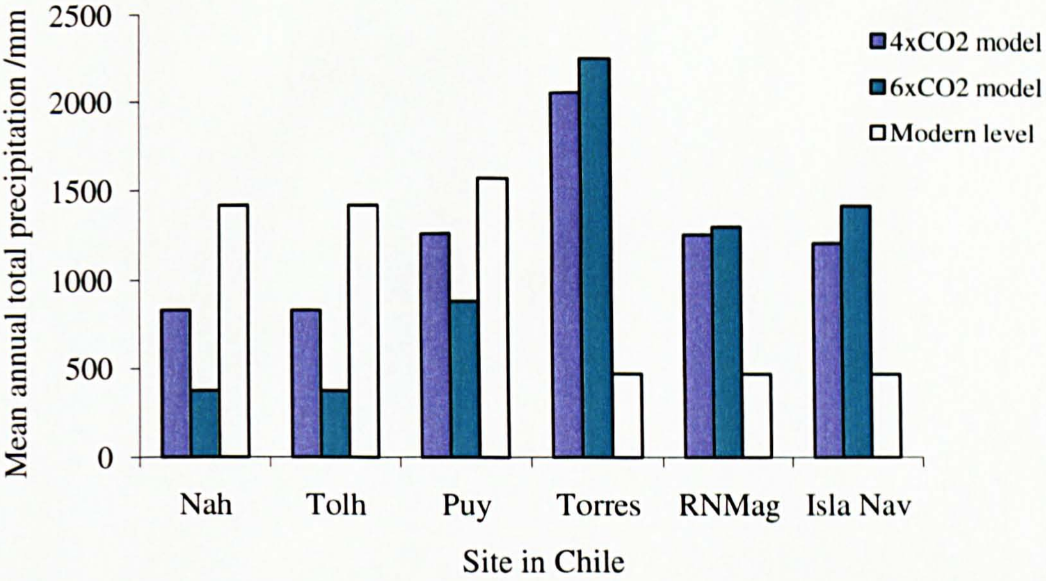

Figure VIII.3. The mean annual total precipitation levels for the sampling sites in Chile during the Early Eocene at 4 times and 6 times pre-industrial CO2 concentrations. Modern estimates were obtained meteorological stations nearest to the sites. Data from one station were used for both Nah and Tolh and data from another were used for Torres, RNMag and Isla Nav.