UNDERSTANDING THE SPATIAL PATTERN OF THE INVASIVE PLANT *CAKILE MARITIMA* IN AUSTRALIA

Submitted by

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DECLARATION OF ORIGINALITY

The work presented in this research project is to the best of the author's knowledge and behalf, original and her own work, except as acknowledged in the text, and the material has not been submitted, either in whole or part, for a degree at this or any other University.

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ABSTRACT

*Cakile maritima* (*Brassicaceae*) is a semi-succulent annual plant with a low spreading growth habit confined to sandy shores and stormy beaches. It originated from Western Europe and is believed to have been introduced accidentally throughout southern Australia. In the process of spreading it displaced another invasive *Cakile, C. edentula* in much of Southern Australia. The proliferation of *C. maritima* may be due to the amount of space not previously occupied by native species in these environments. Most studies have been done on species with a heterogenous habitat and *C. maritima* provided us with an opportunity to study spatial pattern in a relatively homogenous habitat. With so much unoccupied space along the beach, why did it replace the other species? What kind of spatial pattern does *C. maritima* exhibit?

This research compared the spatial pattern of *C. maritima* occurring in three habitats (sheltered, semi-open, and open beaches). In doing so, the following questions were addressed: (i) what is the species’ spatial pattern, (ii) what is the distribution of plant leaf morphotype within the pattern, and can leaf type be used as a marker to help us understand the distribution of progeny from source patches, (iii) do seeds collect under existing plants (forming patches), and are patches likely to persist over time?

Mapping of *C. maritima* plants was carried out using GPS along the three different sites (all on Phillip Island). Three different leaf shapes were identified and plants were classified according to these (entire (E), serrated (S) and dissected (D)). *C. maritima* exhibited an aggregation at less than one metre at all sites. The
correlation between aggregation and plants with similar leaf types was weak and inconsistent across sites.

Seeds were collected from parents of each leaf type (E, S, and D) and the progeny were grown until their leaf morphology could be reliably classified. Regardless of parent leaf shape, most of the progeny had serrated leaves. However, leaf shape assessment illustrated that the relative proportion of the parent’s leaf shape was higher amongst its progeny. For example, there was significantly more entire progeny from entire parents (30%), (8%) from dissected parents and insignificant (84%) from serrated parents. Principal component analysis (PCA) indicated that continuous variation in leaf shape exists within populations of *C. maritima*. The three categories used for classification in the field are therefore not justified and do not reflect a simple genetic system.

More viable seeds were found underneath existing plants and few in the soil between plants. Most seeds either fall directly into material under the parent or are blown until they are trapped around other plants, and thus patches are likely to be self-sustaining.

This thesis is the first study to report on the spatial pattern of *C. maritima* at different locations with different degrees of exposure. A very similar pattern was observed at all of the sites except for the distribution of plant leaf morphotype within the pattern, which varied from site to site.
The data collected in this thesis could be used as the basis for a genetic study to determine whether there was introgression between *C. maritima* and *C. edentula*. This will further explain the variation in leaf morphotype within a species.

**Key words:** *C. maritima, C. edentula*, spatial pattern, leaf morphotype and soil seed bank.
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CHAPTER 1

INTRODUCTION
1.1 **INTRODUCTION**

Non-indigenous invasive plants are an insidious, widespread and increasingly recognized threat to Australia's native plant communities and wildlife. To date, at least 2,700 species of non-native plants have naturalised in Australia. Many of these species have invaded native vegetation and replaced native plants (Parsons 1992). Almost every type of habitat in Australia has been invaded by non-indigenous species since European settlement. Two such invading species are *Cakile maritima* Scop. and *Cakile edentula* (Bigelow) Hook which have spread along the seashore around the coast of all southern States. The main objective of this research was to understand the spatial pattern of *C. maritima* within its habitat and investigate why it forms clumped spatial patterns given the large amount of unoccupied space on the beach. This Chapter will discuss the nature of this habitat, the attributes of species that are able to grow in this harsh environment and review the ecology of *Cakile maritima*.

1.2 **Coastal habitat systems**

The seashore is a dynamic place where the sea, the wind and the plants interact to shape the coastline. The coastal vegetation plays an important part in the stabilisation and maintenance of sand dunes, but inevitably the shores are continually changing. Aerial parts of plants intercept wind energy and cause sand and other material to be deposited around the vegetation. This sand deposition around plants results in increased height and width of the dunes, a process known as "plant-induced dune expansion" (Carolin and Clarke 1991). Coastal dunes form complex systems of habitats for plants due to the interacting steep environmental gradients related to the distance from the shoreline (Rippey and Rowland 1995).
The beach environment is very harsh for plant growth, with high salt concentrations in the water at high tide and dehydration at low tide, unstable saline sands, sudden erosion and dry heat in summer. The addition of fresh water from rain, streams or water seepage through the dunes from inland sources means that plants may experience a range of salinities from sea water to fresh water. The sand is deficient in nutrients and minerals due to leaching at high tide (Williams 1998). Because of a lack of vegetation and constant wind and water erosion there may be little organic matter in the substrate.

The result of the steep environmental gradient is a sharp zonation in vegetation. The swash area, where the waves break on the sand, is so physically stressful that few higher life forms survive there. The flowering plants of the sea are separated from those of the land by these few metres of turbulent bare sand. Landwards from the high-tide line, conditions become increasingly favourable for a series of plant communities to develop across the dunes. Pioneer strand plants survive the high spring tides on the beach and this zone is referred to as “an unstable strandline zone of colonising herbs” (Carolin and Clarke 1991).

On the fore dunes beside the beach, a wider variety of species forms the coastal heath. The dune heath consists of plants that are often less than one metre in height and which can tolerate winds, salinity and loose sand. This is also called a “semi-stable fore-dune zone of shrubs and ground plants” (Carolin and Clarke 1991). This gives way to the taller thicket of the stable hind-dunes (Figure 1.1). Zonation occurs because species respond differently to environmental conditions and because they interact (negatively and positively) with each other (Carolin and Clarke 1991; Rippey and Rowland 1995). The species most able to survive extreme conditions and that are most able to colonise after disturbances are found closest to the shore.
Figure 1.1: Zonation of plants along the open beach on Phillip Island. Strandline and fore dune is dominated by the introduced *Cakile maritima*, *Ammophila arenaria* and the native grass *Spinifex sericeus*.

1.3 Coastal weeds in Australia

Invasive plants are not only a problem for gardens and farming - they also invade natural ecosystems. About 11 per cent of Australian’s 18,000 species of wild plants have been introduced from other countries since European settlement. Many of the invaders resulted from intentional introductions for ornamental and culinary use, or erosion control (Parsons 1992). These species have expanded their abundance and range beyond their intended use to the extent that they impose a threat to human endeavors, or to native vegetation. There are few habitats in Australia that have not been invaded by at least some alien plants.

One of the harshest environments for plant growth is the strandline zone, but this has been invaded by a number of exotic plants. Weeds of southern Australian seashores include: *Ammophila arenaria*, *Salsola kali*, and *Cakile maritima*, all of which are native to the Mediterranean and Europe. *Ammophila*
arenaria (Marram grass), which has been used in many countries including
Australia for erosion control, has a history of replacing Spinifex sericeus in
Victoria and Tasmania (Carolin and Clarke 1991; Rippey and Rowland 1995).
However, other species may have been introduced accidentally, for example in
ballast from ships.

Strandline plants grow in a challenging environment and have developed
survival mechanisms that allow them to cope with the extreme coastal elements.
A prostrate growth form reduces sand blasting. A deep fibrous root system
allows them to gain access to fresh water at depth (Pakeman and Lee 1991a).
They also regulate the salt balance in the leaves through exudation of salts
through salt glands and hairs. Water storage in leaves is increased (succulence)
to keep the concentration of salts in tissues within the limits of physiological
tolerance (Larcher 1975). The succulent leaves enable the plants to survive
dehydration at low tide and excessive heat during summer (Carolin and Clarke
1991). Very few species can survive these harsh conditions and the vegetation
of such an environment is generally sparse and poor in species. This is a
characteristic of all sand dunes that are open to the ocean (Figure 1.5).

Cakile maritima is an ideal annual invasive species in which to study the
development of spatial pattern along a relatively homogenous and sparsely
populated habitat. Although its current range is from Western Australia to New
South Wales and Tasmania (Heyligers 1984), it does not appear to be a direct
threat to biodiversity because it occupies a previously un-occupied niche where
only a few natives could survive. It may, however, impact on ecosystem
function by causing the formation and stabilization of fore dunes. Since C.
maritima is a pioneer species, it creates favourable conditions for other beach-
loving species including Arctotheca populifolia, Salsola kali, Spinifex hirsutus
and Ammophila arenaria (Rippey and Rowland 1995).
1.4 Rationale of the study

Species growing in the strandline occupy an almost linear habitat. Such habitats are relatively rare in nature. Therefore, it is perhaps surprising that almost all theoretical models of population spread have explored the rate of population spread in one spatial dimension (Kot, Lewis et al. 1996). For example, (Fisher 1937) in his landmark paper on modelling the rate of population spread, states as his first assumption “consider a population distributed in a linear habitat such as a seashore...” This means that seashores are assumed to have linear habitat which might not be always the case. The reason for the focus on linear habitats is that the mathematics is much easier than in two dimensions.

When it comes to understanding the development of spatial pattern, however, theoreticians focus on two dimensional habitats (Lewis 1997). Field ecologists working on spatial pattern also concentrate on two-dimensional habitats, even when gathering transect (one dimensional) data. Many analytical packages for spatial pattern only work for a rectangular area. Hence, strandline species offer an opportunity to study spatial pattern in a one-dimensional habitat which will be directly relevant to most theoretical studies. In a relatively homogeneous habitat (in a direction parallel to the shore), the questions of interest are: do these species occur at random, or do they form distinctive patterns?

1.5 Ecological studies on Cakile species

1.5.1 Taxonomy and biology of Cakile species

The genus Cakile (Brassicaceae) in Australia comprises two non-native annuals C. maritima and C. edentula. Both species often occur near the high water mark. They are spreading, succulent annuals, up to about 70 cm tall, with entire to deeply lobed leaves. Different authors have described the leaf shape of C. maritima and C. edentula differently.
The Flora of Australia (George 1982) described *C. maritima* leaves as petiolate, deeply lobed to entire, ranging at a scale of 8 -10, Figure 3.2, (Cody and Cody 2004). *C. edentula* leaves has been described as ovate, obovate with upper leaves less lobed, and which were at a scale of 1 -7, Figure 3.2, (Cody and Cody 2004). The Flora Europaea (Tutin 1993) defined *C. maritima* as entire to deeply lobed and *C. edentula* as sub-entire to lobed.

However the leaves of *C. maritima* were classified as entire to deeply lobed or dissected leaves. Judging by the variation of the leaf types within one species, there is a possibility of introgression (Cody and Cody 2004) because the leaves match the description of both species (Figure 1.2 a).

*Figure 1.2 (a):* (i-ii) are *C. maritima* leaves from entire to deeply lobed, (Turner 2004). (iii-iv) are *C. edentula* leaves from entire to lobed respectively (Farmer 2002).
The petals of *C. edentula* are up to 8 mm while those of *C. maritima* are more than 8 mm long. The flowers of both species can be either purple or white (Figure 1.2 b). The species look similar at first glance but the fruits are the most distinguishing feature (Carolin and Clarke 1991).

![Figure 1.2 (b): (i) The flowers of *C. maritima* (Farmer 2002) and (ii) *C. edentula* (Saulys 2005).](image)

Both species produce succulent, green heteromorphic fruits which become brown when dry (Barbour 1972; Boyd 1988; Donohue 1997). The lower (proximal) part of the *C. maritima* fruit is as wide as the upper one with two blunt points at the top. In contrast, the upper (distal) part of the fruit of *C. edentula* is round and the lower part is narrower than the upper one (Figure 1.2 c). Each fruit separates into two sections when dry. Each segment of the fruit contains one, sometimes none or two, seeds (Heyligers 1984).
The top segment usually falls off and is dispersed further away from the parent plant, while the lower one remains attached to their pedicels and many are later buried with the plant (Barbour 1972; Keddy 1982; Boyd 1988; Donohue 1997). Distal fruit sections have thicker, aerenchymatous walls (Oliver 2001). The mode of dispersal is usually by water at high tide, wind and birds. The seeds can float for two weeks, and remain viable for a further three months while submerged (Barbour and Rodman 1970; Rippey and Rowland 1995; Cody and Cody 2004). Seedlings of *C. edentula* often grow in dense clusters around the remains of the previous year’s plants and seedling density declines with distance from the centre of the cluster (Keddy 1981). Initial observations in Victoria indicated that plant of *C. maritima* plants can be found either growing in clusters or as widely scattered individuals.

### 1.5.2 Local distribution within sites

Various studies on spatial pattern of *Cakile* species have been done along an environmental gradient perpendicular to the sea (Keddy 1981; Watkinson 1985; Cody and Cody 2004). Keddy (1981) in his paper on the demography of *C. edentula* discovered that its abundance at Martinique Beach, Nova Scotia was
greatest in the middle of the dunes and declined to both the landward and seaward ends (Figure 1.3). Survivorship and reproductive output varied along the gradient as a result of density-dependent and density-independent factors. These factors included seed production, fruit dispersal, and plant survival (Watkinson 1985).

![Figure 1.3: The sand dune cross-section of the barrier beach on Martinique Beach in Halifax County, Nova Scotia, Canada. Graph reproduced from (Keddy 1981).](image)

Both survival and fecundity were found to be density-dependent in the middle of the dunes where the plants were most abundant. Landward migration of seeds was responsible for the depletion of populations on the seaward end of the gradient and the maintenance of populations at the landward end (Keddy 1982; Watkinson 1985). Inputs at the seaward end of populations were balanced by losses due to seed movements, whereas losses at the landward end were result of seedling mortality. Barbour (1970b and 1972) observed no *C. maritima* seedlings behind the fore dune, although experimentally-sown seeds germinated there. He concluded that the landward limit of *C. maritima* in California was
due to (a) limited landward dispersal, (b) herbivore damage, (c) drought and (d) competition from other dune plants.

Cody and Cody (2004) found that at Pachena Beach, Vancouver Island in Canada, the two species appear to coexist but the zonation is different. *C. maritima* mostly occurred high on the beach while *C. edentula* occurred on the lower part of the beach. In high density plots, both species were in clusters at Pachena Beach and affected each other’s growth and reproduction. At Westlake Shore in South Australia, individual plants were unable to be identified as *C. edentula* or *C. maritima* based on the leaf morphology. The variation in spatial pattern between the two continents was assumed to be a result of different climatic conditions and different ecological preferences of the two *Cakile* species (Rodman 1986; Cody and Cody 2004).

Leaf morphology is often highly variable between species in the *Brassicaceae* family, as is the case between *C. maritima* and *C. edentula* (Heyligers 1984; Rodman 1986; Boyd and Barbour 1993; Cody and Cody 2004). Cody and Cody (2004) further analysed the differences in leaf morphotype within populations of *C. maritima* and *C. edentula* in Canada and Australia. They classified leaf morphotypes from 1-10 (from entire to deeply lobed leaves) to determine the variation in leaf shape between the two species (as will be discussed in detail in Chapter 3). All leaf morphotypes were widely distributed in relation to beach elevation in both continents. In Canada, most *C. maritima* leaves were scored at 9 and 10 and were more pinnatifid than *C. edentula* ones which generally scored in the range 2-5 (deeply crenate to sinuate-dentate). They suggested that the morphological variation in Australia could be attributed to introgression, but this has not been proven genetically.
1.5.3 Seed dispersal

Donohue (1998) suggested that seed dispersal patterns or migrations of *C. edentula* are determined by the maternal plant morphology, fruit morphology, and environmental conditions. She noted that taller, more sparsely branched plants with a greater proportion of primary fruit impeded dispersal and resulted in more dense seed distributions on beaches along the shores of the Great Lakes, Michigan. Environmental conditions also affected dispersal. Increased beach width, slope, and depth of burial were associated with shorter local dispersal distance and increased clumping. Plants on narrow beaches and on steep slopes also tend to have a greater percentage of dispersal to unsuitable sites (Donohue 1998). However, in this study environmental factors were not tested.

Keddy (1980) reported that the movement of *C. edentula* fruits in Nova Scotia was considerably greater than that reported for *C. maritima* on sand beaches in California (Barbour and Rodman 1970; Barbour 1972). Seedlings of *C. maritima* formed dense clusters around the remains of parent plants from previous years on protected ("sheltered") landward beaches, whereas on the seaward side plants were washed away by winter storms. However, the probability of survival near the parent is low due to high seedling density and increased probability of predation and pathogens. In his study on the population ecology of *C. edentula* on a gravel bar, (Keddy 1979) noted that seedling density declined with distance from the centre of seedling clusters.

Dispersal of fruits away from clusters usually produces seedlings with higher survivorship and/or reproductive output (Keddy 1980). Long distance dispersal in *C. edentula* in Michigan allowed plant adaptation at a larger spatial scale (Donohue 1997). Proximal seeds tended to remain close to the parent plant and had greater survival, whereas distal seeds had greater survival at farther
distances (Barbour and Rodman 1970; Keddy 1982; Heyligers 1989; Donohue 1997). It is uncertain whether the distal seeds belonged to those individual plants or came from neighbouring plants. This may lead to the argument that distal seeds found underneath plants are not necessarily from the same parent plant.

Various physical factors vary along the beach, including sand movement, exposure to wind and sun, salinity and soil nutrients (Keddy 1980; Heyligers 1984; Fenner 1985; Boyd 1988; Maun 1998; Cheplick and Demetri 1999). Sand movement and salt spray were the most important factors affecting the distribution of dune plants. It was demonstrated by Barbour (1970a;1970b;1972) and Boyd and Barbour (1993) that salt spray increased germination and growth of C. maritima seedlings in California. Shortage of nitrogen and competition from Ammophila breviligulata caused some variation in reproductive output of C. edentula in Canada (Keddy 1982). Both factors are likely to cause reduction in plant size, which is initially reflected in reduction of reproductive output. Further reduction in resources results in low plant density and mortality (Keddy 1981 and 1982).

1.6 Geographic distribution of Cakile in other countries

There are at least four species of Cakile which originated from Europe. These are C. maritima, which is found in the west and north-west of Europe, Mediterranean, Portugal, and Norway and rarely in the Baltic region. C. euxina is located around the Black Sea region, C. baltica is found along the Baltic Sea and south east of Norway, C. aegyptiaca occurs in the Mediterranean region and south Portugal (Tutin 1993). C. edentula originated from the Atlantic coast of North America (Oliver 2001) and its current distribution is San Diego, British Columbia, California, Mexico and Europe (Munz and Keck 1973). C. constricta
originated from south central Atlantic coast of Florida to eastern Texas and shores of the Gulf of Mexico. *C. lanceolata* is found in the Bahamas, Bermuda and Caribbean coast of Venezuela, Colombia, Florida and the Gulf of Mexico. *C. geniculata* is currently found in Mexico (Rodman 1974). Rodman (1974) also noted that *C. constricta* and *C. lanceolata* have naturally hybridised in Florida, while *C. constricta* and *C. geniculata* naturalised where their ranges overlap in the Gulf of Mexico and the Caribbean region.

The distribution of *C. edentula* and *C. maritima* in Australia is discussed in section 1.6.1. In North America, *C. edentula* was introduced from eastern coast to the San Francisco Bay area in 1880 and became widely spread in the next 50 years. *C. maritima* was first seen on the western coast in 1935, 24 km north of San Francisco where the two species grew together. By the late 1960s it had reached British Columbia and northern Mexico. Concurrently, *C. maritima* expanded its range and *C. edentula* disappeared from the southern beaches of Mexico (Rodman 1974, Heyligers 1989).

Several possible causes for the species replacement have been considered. The two species differ in a number of characters. These include: leaf form, petal size and colour (Rodman 1974), breeding system, spacing of flowers on the inflorescence, fruit morphology, flowering time and time of germination (Barbour and Rodman 1970). The most obvious factor is high seed production which might be sufficient to enable *C. maritima* to displace *C. edentula*, provided the beach has a limited carrying capacity for a plant population (Rodman 1974). Heyligers (1984) and Cody and Cody (2004) argued that *C. maritima* has a wider tolerance to growing conditions than *C. edentula* and it frequently produces a second generation, on the sand hummocks built by the first generation, while *C. edentula* prefers to start on virgin sand deposited by high tide.
Baker (1989) considered indirect effects of biotic factors on the decline of *C. edentula* in North America. He claimed that a possible factor is a "virus that might not produce obvious symptoms but effective enough to prevent reproduction in *C. edentula*". On the contrary Keddy (1980) found no evidence of diseases on plants grown in growth chambers. Boyd (1988) studied the distribution of the two species based on the preference of three *Cakile* herbivores using a growth chamber and artificial field tests at Point Reyes, Californian. One herbivore, *Platyprepia guttata* (Boisduval) preferred *C. maritima*, but another, deer mouse, *Peromyscus maniculatus* (Wagner), preferred *C. edentula*. He concluded, therefore, that *P. maniculatus* may have accelerated the replacement of *C. edentula* but was not the primary factor involved because it is found both within and outside the extinction zone of *C. edentula*.

1.6.1 Distribution history of *Cakile* in Australia

*C. edentula* and *C. maritima* are believed to have been introduced accidentally to the Australian coastline. Both species look similar at first glance and have naturalised in Australia and overseas. The two species were introduced in different locations and even the direction of spread has been different. *C. edentula* was first introduced in Victoria and started spreading to the east and west (Figure 1.4 a), whereas *C. maritima* was introduced first into Western Australia and started spreading towards South Australia (Figure 1.4 b). However, it is possible that there have been multiple introductions, rather than a single incursion that has spread throughout the country. It has been suggested that, in the 1800s, *Cakile* seeds were transported regularly between shipping ports in sand ballast and this is probably the manner by which the two species reached Australia (Rodman 1986).
Both species have the potential for local dispersal via floating propagules that can survive several weeks of immersion in seawater (Oliver 2001) and their detached fruits can be rolled along the beach by wind. *C. edentula* was the first of the two species to be introduced to Australia (Rodman 1974). Herbarium records indicate that *C. edentula* was first recorded in Victoria near Melbourne in 1863 and was well established on beaches along the southern and southeastern coast of Australia by the time *C. maritima* reached the same areas (Rodman 1974). *C. edentula* was later collected in New South Wales near Sydney in 1870. It reached South Australia in 1882 near Adelaide and was further collected in Tasmania in 1893 near Hobart. It was seen in Queensland near Brisbane in 1922 and 40 years later it was collected near Eucla in Western Australia, in 1962 (Rodman 1986).

**Figure 1.4 (a):** Herbarium records of *Cakile edentula* in Australia (fruit illustrated on the upper left). The year of first collection in each State is underlined, from (Rodman 1986).
The earliest known collection of *C. maritima* in Australia was in 1897 in Western Australia (Rodman 1986). Ten years later it was reported that *C. maritima* was spreading from Western Australia towards the east, threatening to quickly occupy the whole of the coast line (Heyligers 1984). In 1918 it was collected in South Australia around Adelaide and, four years later; it was collected from Victoria on Phillip Island. It was later found in New South Wales near Sydney in 1969. It was collected from Hobart, Tasmania in 1979 (Rodman 1986) as illustrated in Figure 1.4 b. In the process of migration it intermingled with *C. edentula* and within a relatively short time it had virtually replaced *C. edentula* along much of the South Australian and New South Wales coast (Thrall 2001). Visits during the course of this study to sites from which there are records for *C. edentula* found only plants of *C. maritima*.

Given the vastness of the Australian beach habitat and the presence of open spaces which are not colonised by other plants, it is difficult to see competitive exclusion as the mechanism of replacement of one species by the other. It is possible that there has been hybridisation and introgression in *Cakile* in Australia, North America, and Canada (Rodman 1986, Boyd, 1993, Cody and Cody 2004) but this has not been tested formally. The main diagnostic character is fruit shape and that of *C. maritima* may be dominant, and thus hybrids might then appear to be plants of *C. maritima* (Barbour 1972; Rodman 1974; Rodman 1986).
Figure 1.4 (b): Herbarium records of *Cakile maritima* in Australia (fruit illustrated on the upper left). The year of first collection in each State is underlined, from (Rodman 1986).

1.7 Purpose of the study

Australia’s sandy beaches provide linear and relatively homogenous habitats for colonization. These are an ideal situation in which to examine the formation of pattern on an almost one-dimensional habitat similar to that assumed by theoretical ecologists. There are relatively few native species that occupy this ecological niche. *C. maritima* does not occupy the entire habitat.

Typically, there are large plants or patches of plants, interspersed by bare sand (Figure 1.5). Initial observations suggested that plant size and pattern varies between sites and that patterns appeared to be regular rather than clumped. Also, though there was considerable variation in leaf shape, this variation appeared to be discontinuous (perhaps three leaf types) and plants with similar leaf types appeared to be aggregated. Why does *C. maritima* display such patterns when it appears capable of colonizing the entire available strandline habitat?
A possible reason the incomplete occupation of the habitat could be constant disturbance by wind or wave action. This may wash away exposed seedlings between plants, while those growing beneath adult plants may be protected. Seeds which are blown away from adult plants might be exposed to predation and harsh conditions that limit establishment of individual plants. Perhaps many seeds are dropped directly under adult plants, thus re-establishing and maintaining these patches over time. Seeds blown further may be trapped by other plants/patches (as illustrated by the arrows in Figure 1.5) where the conditions are conducive for germination and survival.

Hence, a proposed model to explain the distribution of *C. maritima* plants on the shore involves:

- Poor establishment in the harsh conditions of bare sand;
- Better germination and survival beneath existing plants where organic material collects;
- Restricted dispersal of basal pod segments beneath parent plants and hence with progeny usually replacing maternal plants;
- Preferential collection of further dispersing seeds around existing plants;
- Disturbance by wave action removing small, isolated seedlings and a proportion of large plants.

This could result in a pattern of plants/patches of multiple plants with bare areas, which tend to be self-sustaining over time (even though plants themselves may be annuals) both in location and in (maternal) genetic traits.
Figure 1.5: Likely seed dispersal of *Cakile maritima* along a beach. The arrows inserted to show the movement of seeds during dispersal (picture reproduced from; Thrall 2001).

The main objectives of this research were:

(a) To describe and compare the spatial pattern of *Cakile maritima* at three contrasting sites,

(b) To collect information that might confirm or refute the assumptions of the model.

Chapter 2 will discuss plant distributions at three sites on Phillip Island, Victoria, (sheltered, semi-open and open beaches). Analyses examined whether plants are aggregated or regularly spaced, and at what scale.

Chapter 3 examines the extent to which maternal and progeny leaf shapes are similar. If similar, then limited seed dispersal may be resulting in aggregation of plants with similar leaf types.
Chapter 4 examines the soil seed banks beneath and between existing plants, to see whether the spatial pattern of dispersed seed reflects the pattern of maternal plants.

Chapter 5 discusses matters arising from the whole research and the implications of the results on spatial pattern.
CHAPTER 2

SPATIAL PATTERN OF *C. MARITIMA*
2.1 INTRODUCTION

The recognition of spatial patterns has long been of significance to ecologists. Pattern in vegetation will reflect the physical and biological processes that are in action. Hence, understanding the processes leading to particular spatial patterns helps us to interpret our environment. Spatial pattern has been defined as “the arrangements of points, of plants or other organisms, or patches of organisms in space, which exhibits a certain amount of predictability” (Dale 1999). Spatial pattern is a crucial aspect of vegetation that has important implications for the plants themselves and for other organisms which interact with plants. Aggregations of plants will undergo intense competition but will easily cross-pollinate, while the opposite will be true for spaced plants. The analysis of spatial pattern has become a fundamental part of ecology.

There is a range of factors that cause spatial pattern, classified by Dale (1999) as (a) morphological factors based on size and growth pattern of the plants; (b) environmental factors that are spatially heterogeneous; and (c) phytosociological factors that affect the occurrence of another plant species through their interaction. The interaction of the topography, the process of soil formation, vegetation itself and exposure to extreme physical conditions may contribute to variation in spatial pattern and plant size (Dale 1999). Understanding the variability in time and space of a particular process is arguably one of the key goals in ecology. The general importance of spatial effects and scale has been explored extensively in ecology through theoretical models. Spatial aggregation is common in natural plant communities and there is substantial evidence that neither seeds nor plants are uniformly distributed in space (Lortie, Ellis et al. 2005).

Little attention has been given to the formation of pattern in relatively homogeneous habitats. Cakile species grow on sandy shores where the
environment is relatively homogeneous along the shore, plants are at a low density and communities have few species. Under such conditions does spatial pattern form within the *Cakile* population or are plants distributed at random? The spatial pattern of the two *Cakile* species in southern Australia and British Columbia, Canada has been examined previously by Cody and Cody (2004). It was reported that in Canada, *C. edentula* was distributed along the entire length of the lower beach whereas *C. maritima* was mostly restricted to the upper beach region.

The aim of this part of the study was to investigate pattern in *C. maritima* and to see whether the spatial distribution varied between locations. The specific questions were:

- Does the species exhibit particular types of pattern and at what scale?
- Does the spatial pattern differ among sites?
- Are plants of different leaf shapes clustered within the pattern?

2.2 MATERIALS AND METHODS

2.2.1 Location of study sites

An important variable in the ecology of species growing on the sea shore is exposure to wave action. Three sites were selected on Phillip Island of varying exposure. At Cowes, the site was located on a “sheltered” beach where there was minimum exposure to strong winds and hot sun. At San Remo Bridge, the site was located on a “semi-open” beach with exposure to south-easterly winds. Lastly, the Woolamai site was located at an “open” beach facing strong south-westerly wind (Figure 2.1). Each site is described in detail below.
Figure 2.1: Map of Phillip Island showing the location of the three sites where *C. maritima* was mapped. The blue ellipse is a sheltered beach (Cowes); red is a semi-open beach (Bridge); and purple is an exposed beach (Woolamai) (Asia Travel 2001).

2.2.1.1 Sheltered beach

The beach at Cowes is located just over 100 km south east of Melbourne, on the northern side of Phillip Island (38°27′S, 145°14′E) (Wisemen 2005). Figure 2.2 shows the *C. maritima* habitat. The beach faces north and only receives waves coming across Westernport Bay. The plants are growing along the high tide zone, mostly at the base of a low bank. There are very few co-occurring species but there are occasional plants of *Euphorbia paralias* and *Salsola kali*. At the time of mapping, plants were surrounded by litter from seagrass washed up on the shore.
Figure 2.2: The sheltered beach at Cowes, showing the habitat of *C. maritima* along the high tide zone. The brown litter is accumulation of seagrass leaves, which are deposited when the tide is in and then blown by wind.

2.2.1.2 Semi-open beach

"Bridge" beach is at the eastern end of Phillip Island (38°30'S, 145°21'E) and located adjacent to the San Remo Bridge, which links Phillip Island to the mainland (Wisemen 2005). Figure 2.3 shows the habitat of *C. maritima* at the site. The zone containing *C. maritima* is dominated by Marram grass (*Ammophila arenaria*), *Spinifex sericeus*, and *Acacia longifolia var. sophorae* beyond the grass zone. Unlike the sheltered beach, the wind-blown seagrass leaves did not reach the *C. maritima* plants and can be seen close to the water's edge.
Figure 2.3: The semi-open beach showing the habitat of *C. maritima* beyond the shoreline. Arrows show where plants were mostly growing (in a gully) close to the edge of the shrub zone, but some were growing further out among the grasses. None were in the strand line.

2.2.1.3  **Open beach**

Cape Woolamai is located at the tip of the island's south-eastern peninsula, directly south of Newhaven. Woolamai Beach faces out to Bass Strait on the western side of the peninsula. Behind the beach, and to its south, is the Cape Woolamai State Faunal Reserve (Wisemen 2005). The associated plant species are *Senecio pinnatifolius*, *Sonchus oleraceus* and grasses. It receives waves from the southern ocean and the prevailing south easterly winds. It is thus a highly exposed beach. Figure 2.4 illustrates the habitat where *C. maritima* occurs, on the steep sand dune face and at its base.
Figure 2.4: The open beach, showing the habitat of *C. maritima* beyond the shoreline. Plants were growing on the eroded face of the sand dunes and at their base.

2.2.2 Data collection

Mapping of *C. maritima* was done using a NavCom NCT-2000D™ differential Geographical Positioning System (GPS) and a hand-held computer using the Solofield® Version 3.2 software. This is accurate to approximately 20 cm depending on the number of satellites that are detected in the sky. Plants were mapped along a stretch of approximately 100 m at each beach in May 2005. Cowes was mapped on 6 May 2005, Bridge on 10 May 2005 and Woolamai on 16 May 2005. Only mature plants with fruits were mapped. Plants were classified into four leaf form categories: dissected, entire, serrated and unknown. Unknown plants were mature, with fruits or flowers, but had shed their leaves.
In order to be consistent when logging points, the operator’s right heel was placed at the base of the plant which was being logged and directly perpendicular to the GPS satellite receiver. The habitat occupied by *C. maritima* was recorded by logging at frequent spatial intervals around the area within which all plants were situated. The data were downloaded from the hand-held computer as ASCII files. Excel® was used to produce maps.

**Figure 2.5:** Logging points using GPS and hand-held computer at the open beach. The right heel of the logger was placed on the plant perpendicular to the GPS satellite receiver in the backpack.

It was apparent when visiting the sites that plant sizes were different between locations. To quantify this difference, samples of plants were collected and weighed. Fifteen plants were collected at random from the “sheltered” and “semi-open” beaches. No plants were collected from the exposed beach due to the instability of the sand dunes and removing any kind of plant was in
appropriate. The plants were then placed in an oven at 60°C for 48 hours. The weight of each plant was recorded and data were analyzed in MINITAB®.

2.2.3 Data analysis

As it is difficult to calculate plant density for a long string of points, the mean nearest-neighbour distance was calculated instead. The nearest-neighbour distance for any point is defined as “the distance between it and the nearest other point in the pattern” (Boots and Getis 1988) and is inversely related to density. Values at each site were compared using analysis of variance in MINITAB®.

2.2.3.1 Spatial pattern of C. maritima plants

The raw data were the co-ordinates of individual plants. A map of such data is referred to by statisticians as a “point pattern”. Unlike quadrat-based data, there are few methods available for their analysis (although point data can be converted to quadrat counts) (Fortin and Dale 2005). However, in this study only the point pattern was used. The main objectives of the spatial analysis were (i) to determine whether C. maritima plants were significantly aggregated or regularly spaced, and (ii) to determine whether plants of different leaf types were aggregated within the overall pattern.

To determine whether the plants differed from a random distribution, we calculated the O-ring statistic using Programita® (Wiegand 2004). The O-ring statistic super-imposes a ring at a distance r over the mapped points and counts the number of points within the ring. These counts are then divided by the intensity λ of the pattern. The program increases the ring radius incrementally.
until all the points in the study region have been counted. The higher the density of points within each ring, the more aggregated the pattern is at that scale. A maximum ring width of 0.2 m was used during the analysis as this was the approximate accuracy of the GPS.

Programita™ calculates confidence intervals for a random pattern by taking the same number of points and randomizing them within the study area. The O-ring procedure is then used on the randomized data. This is repeated 19 times (Bailey and Gatrell 1995) and the maximum and minimum value for each scale is plotted as the 95% confidence interval for a random distribution (Wiegand 2004). If the observed are above the confidence interval, the pattern is considered to be significantly aggregated at that scale. If the values are below the confidence intervals, the pattern is more regular (over-dispersed) at that scale than a random pattern.

Usually, a rectangular area is used for these randomizations. However, Programita™ allows the user to restrict the randomizations to an irregular area. For example, it is pointless comparing observed results with a pattern that is randomized over an area that includes the sea. We began by confining randomization to the approximate boundary of suitable C. maritima habitat as recorded using the GPS. However, it was apparent that this was an over-estimate of the area occupied. Hence, we then defined our region by constructing a polygon around the observed plants (rather like a convex hull, but done by eye).

2.2.3.2 Spatial pattern of leaf morphotypes within the C. maritima pattern

In order to examine how the different leaf types are arranged relative to each other, Dixon’s nearest-neighbour method was used (Dixon 2002). This method
examines each point (in this case a plant) and lists its leaf type and the type of its nearest-neighbour. Frequencies of all possible pair-wise combinations of plant types are determined and compared with the frequencies expected if plant types were distributed at random among the mapped points. With \( S \) plants, an \( S \times S \) contingency table is created, with the entry in the \( i \)th column, \( N_{ij} \), recording the number of times that the nearest neighbour of plant type \( i \) was a plant type \( j \). In order to perform a test of the departure from expectation for the table as a whole, individual entries are tested using the normal approximation:

\[
Z_{ij} = \frac{N_{ij} - E(N_{ij})}{\sqrt{Var(N_{ij})}}
\]

where \( Z_{ij} \) are Z-scores from the z table, \( i \) and \( j \) are plants leaf types, \( N_i \) and \( N_j \) are the number of plants with leaf type \( i \) and \( j \), \( E \) is the expected counts of plant leaf type \( i \) and \( j \), \( Var \) is variance. If plant leaf type \( i \) is more frequently (or less frequently) found in association with plant leaf type \( j \), then \( N_{ij} \) is larger (or smaller) than the expected count, \( E(N_{ij}) \) (Dixon 2002).

A test of segregation can be calculated to determine whether the number of conspecific neighbours is larger than expected. The index of segregation of the \( i \)th type, based on the excess of within-type nearest neighbours, was calculated as below

\[
S_{ij} = \log \left[ \frac{N_{ij}(N_i - N_{ij})}{N_i(N - N_j - 1)} \right]
\]

where \( S_{ij} \) is a pair-wise segregation index, \( S_{ij} \) is larger than 0 when \( N_{ij} \) the frequency of neighbours of plant leaf type \( j \) around plant leaf type \( i \), is larger than expected at random. \( S_{ij} \) is less than 0 when \( N_{ij} \) is smaller than expected at random. \( N \) is the total number of plants. The analyses were computed by Jose Blanco at the University of Alberta.
2.3 RESULTS

2.3.1 Mapped plants

The distributions of plants of each leaf type are shown in Figure 2.6. The maps show how narrow the *C. maritima* habitat is at all sites. At all sites the plants were situated along a very narrow band, with the exception of a few plants at the semi-open site. There are occasional gaps of up to 10 m at the open site and 5 m at the semi-open site.

![Diagram of plant distributions](image)

**Figure 2.6:** Distribution of *C. maritima* plants based on leaf types at the three sites. Data were collected from an approximately 100 m transect at each site: green squares (entire leaves), blue triangles (serrated leaves) and red diamonds (dissected leaves). Note that (c) is at a different scale. Coordinates are relative to the most southerly and westerly plants at each site.
Generally, all sites had few plants with dissected leaves (Figure 2.7) and these were widely spaced. The results of the nearest-neighbour distance showed a variation in mean distances between plants at the three sites (Table 2.1). The mean of nearest neighbour distance was much higher (1.19 m) at the open site than the sheltered (0.43 m) and semi-open (0.38 m) sites. The open site or more exposed site was more hostile and had a lower number ($n$) of plants than the other two sites (Table 2.1) and there were large gaps between plants (i.e. plants were few and in clusters).

**Table 2.1**: Nearest-neighbour distances at the three sites.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Mean (m)</th>
<th>Mode (m)</th>
<th>Standard deviation</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheltered</td>
<td>0.43</td>
<td>0.43</td>
<td>0.294</td>
<td>253</td>
</tr>
<tr>
<td>Semi-open</td>
<td>0.38</td>
<td>0.08</td>
<td>0.378</td>
<td>253</td>
</tr>
<tr>
<td>Open</td>
<td>1.19</td>
<td>0.01</td>
<td>1.058</td>
<td>67</td>
</tr>
</tbody>
</table>

Although mean distances were almost similar at the sheltered and semi-open sites, the distribution of nearest-neighbour distances included more short-distance and more long-distance plants at the semi-open site, resulting in a shorter modal distance. Plants appeared to be in clusters at a small scale and yet over-dispersed at a larger scale.
Figure 2.7: Frequency distributions of leaf morphotypes at the three sites. (a) Sheltered beach $n = 253$, (b) semi-open $n = 253$ and (c) open site $n = 67$. 
Dissected plants were the least abundant morphotype at all sites. There was variation in the relative frequencies of the other leaf types between the three sites. Entire plants were more frequent at the sheltered (59%) and semi-open site (43%) than at the open site (35%). The open site had the highest proportion of serrated plants (45%) than sheltered (23%) and semi-open (33%) sites. To quantify the variation in plant size a *t*-test on log$_e$ transformed plant weights showed that plants were about eight times larger at the sheltered beach (mean = 42.3) than at the semi-open beach (mean = 5.4), (*P*<0.001, *t* = -4.38, df = 27 and *n* = 15).

2.3.2 Spatial analysis

The results of the *O*-ring statistic (Figure 2.8) show that sites have a similar spatial pattern at each site. Significant departures from a random distribution can be seen at a scale of one metre or less when tested with the *O*-ring statistic (marked with red circles). The red circles above the confidence interval envelope indicate that plants are aggregated at this scale at all sites. The solid blue circles within the confidence envelope indicate that plants were irregularly dispersed. There are a few minor aggregations at larger distances as indicated by the red circles. Although the scales of these secondary peaks are almost similar for the three sites, it is unclear whether this has any biological significance.
Figure 2.8: Univariate analysis of point-patterns using the O-ring statistic (solid blue circles), giving the neighbourhood density of the pattern at each scale \( r \) and confidence intervals (black solid lines). Red circles show significant aggregation at that scale. The ring width was 0.2 m at all sites.
The Dixon test determines whether the nearest-neighbour of a plant is of a particular leaf type more or less often than would be expected from a random distribution of the leaf types. Significant positive Z-scores indicate that the arrangements of plant types is aggregated, while significant negative values indicate that the nearest neighbour is less often associated with a particular leaf type than expected (over-dispersed, or regular).

Table 2.2: Results of the Dixon method: MCLASS; “Match Class” (first letter represents the plant type and second letter represents its closest neighbour) with \( n \times n \) classes. D: dissected, E: entire, S: serrated; \( M_U \): Number of matches; \( E_U \): Expected number of matches; \( V_U \): Variance for the number of matches; \( Z_U \): Z-scores; \( P_U \): Probability of Z-score statistic; \( S_U \): Pair wise segregation index. Significant values are shown in bold type.

<table>
<thead>
<tr>
<th>Sheltered site:</th>
<th>MCLASS</th>
<th>( N_U )</th>
<th>( E_U )</th>
<th>( V_U )</th>
<th>( Z_U )</th>
<th>( P_U )</th>
<th>( S_U )</th>
</tr>
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<tbody>
<tr>
<td>DD</td>
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<td>0.89</td>
<td>-0.68</td>
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<tr>
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<td><strong>38</strong></td>
<td><strong>27.62</strong></td>
<td><strong>12.02</strong></td>
<td><strong>2.99</strong></td>
<td><strong>0.00</strong></td>
<td><strong>2.14</strong></td>
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<tr>
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<td>0.70</td>
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<td><strong>14.38</strong></td>
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<table>
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<td>0.35</td>
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</tbody>
</table>

<table>
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<th>( E_U )</th>
<th>( V_U )</th>
<th>( Z_U )</th>
<th>( P_U )</th>
<th>( S_U )</th>
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<tbody>
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<td>DD</td>
<td>5</td>
<td><strong>2.36</strong></td>
<td><strong>2.69</strong></td>
<td><strong>1.61</strong></td>
<td><strong>0.05</strong></td>
<td><strong>1.03</strong></td>
<td></td>
</tr>
<tr>
<td>DE</td>
<td><strong>2</strong></td>
<td><strong>4.73</strong></td>
<td><strong>2.81</strong></td>
<td><strong>-1.63</strong></td>
<td><strong>0.95</strong></td>
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</tr>
<tr>
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<td>7</td>
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<td>3.11</td>
<td>0.62</td>
<td>0.27</td>
<td>1.17</td>
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<tr>
<td>ED</td>
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<td>4.73</td>
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<td>-1.57</td>
<td>0.94</td>
<td>-1.61</td>
<td></td>
</tr>
<tr>
<td>EE</td>
<td>11</td>
<td>8.36</td>
<td>6.34</td>
<td>1.05</td>
<td>0.15</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td><strong>15</strong></td>
<td><strong>10.91</strong></td>
<td><strong>5.54</strong></td>
<td><strong>1.74</strong></td>
<td><strong>0.04</strong></td>
<td><strong>0.92</strong></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>6</td>
<td>5.91</td>
<td>3.55</td>
<td>0.05</td>
<td>0.48</td>
<td>-0.82</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>11</td>
<td>10.91</td>
<td>5.83</td>
<td>0.04</td>
<td>0.48</td>
<td>-0.21</td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td><strong>8</strong></td>
<td><strong>13.18</strong></td>
<td><strong>7.67</strong></td>
<td><strong>-1.87</strong></td>
<td><strong>0.97</strong></td>
<td><strong>-0.77</strong></td>
<td></td>
</tr>
</tbody>
</table>
The sheltered site had two positive significant associations DE and ED, which means that if a plant is dissected, its nearest neighbour was more often an entire plant and if the target plant was entire, its nearest neighbour was more likely to be a dissected plant than would be expected by chance. A negative association SE indicated that an entire plant's neighbour was less likely to be a serrated plant, i.e. there was a dissociation of these leaf types. At the semi-open site the Z-scores are all non-significant; therefore there are no associations or dissociations.

At the open site, there were two positive significant associations DD and ES, dissected plants thus tended to be aggregated together, while serrated plants were more likely to occur near an entire plant than we would expect by chance. There were also two negative significant associations DE and SS, showing that it was less likely to find a dissected plant alongside an entire plant, while serrated plants were less likely to occur next to each other than we would expect at random.

Although there were some significant associations within two sites, the results were inconsistent: positive associations of plants at one site were negative or insignificant at another site (Table 2.2). For example, the association DE is highly positive and significant at the sheltered site but negative at the open site. It is worth noting that the size of the sample to some extent determined the outcome of the statistical analysis: a larger sample size would yield better results and would be more likely to reveal more positive associations than in a small sample in the future. However the opposite associations might be a result of the different environmental conditions of the three sites.
2.4 DISCUSSION

This chapter has shown that non-random spatial patterns exist within populations of *C. maritima*. Combining the results of the various analyses of spatial pattern, the discussion will be divided into two parts. It will first look at the pattern within the beach, how plants were distributed and at what scale along the three sites. Secondly, how plant types were distributed based on the three leaf types.

*Spatial pattern*

Plants were growing in a relatively very narrow strip at all sites with just a few isolated plants at the semi-open site (Figure 2.6). The width of the strips in the landward direction was less than two metres. It can be speculated that constant disturbance (wave action and wind) would have reduced habitat width by uprooting or burying seedlings and mature plants near the shore. The habitat where the two *Cakile* species occurred at Westlake Shore in South Australia was four metres wide (Cody and Cody 2004), two times the width of the habitats at Phillip Island. Similarly, at the Martinique Beach in Canada (Figure 1.3), *C. edentula* plants were widely distributed across the environmental gradient and growing with grasses beyond the fore-dune up to the landward side of the beach (Keddy 1982). Plant sizes at the sheltered beach corresponded with those at Keddy’s seaward zone, whereas sizes at the semi-open site were very small and crowded, corresponding more closely to his middle-dune plants.

At the three sites on Phillip Island this was not the case, no plants were found growing beyond the middle part of the beach. At the sheltered site most of *C. maritima* plants were growing at the extreme seaward side along the beach where there were few native plants to compete with. At the semi-open site plants were growing with grasses in a ditch at the middle part of the beach. This
was perhaps because of high moisture and shade provided by the shrubs and bushes. Cody and Cody (2004) recorded *C. maritima* plants were growing at the upper beach and *C. edentula* at the lower beach at the Pachena Beach in Canada. At the open site, most plants were found at both lower beach and in the middle part of the cliff, where it intermingled with grasses and other dune plants. The observed pattern is in agreement with Barbour (1970b and 1972) who noted that at the Californian beach no *C. maritima* plants grew behind the fore dune although deliberately-sown seeds germinated there.

The biological mechanisms underlying the observed patterns of *C. maritima* distribution at the three sites are unknown, given that the beach is sparsely populated and only a few native plants can survive in such harsh conditions. The non-existence of *C. maritima* plants at the landward side of the beach could be attributable to environmental factors such as limited landward dispersal, predation, drought and competition from other plants (Barbour 1972). In Australia, temperatures on the sand surface can be extreme in summer and perhaps plants can only survive in habitats that are kept moist, either by tide, spray or in gullies that trap water (for example the semi-open site Figure 2.3). Also, at Phillip Island landward end is usually colonised by shrubs that would shade *Cakile* plants, whereas in Canada the grassy dunes can stretch tens or hundred of metres away from the shore.

The bank along the beach at the sheltered site would act as a physical barrier which limited dispersal beyond high water mark of the beach. At the semi-open site *C. maritima* plants were competing with grasses and shrubs, whereas at the open site the cliff was too high to allow seeds to be dispersed to the landward side of the beach (Figure 2.2, 2.3 and 2.4). Keddy (1982) discovered that although seeds of *C. edentula* were deposited on the landward side, plants did
not survive for long due to low reproductive output which prevented self-sustaining group of individuals from establishing.

Initial observations indicated that the open site had a large number of plants but on the day of mapping a lot of plants had disappeared and only a few plants remained. The disappearance of plants was presumably due to frequent disturbance by strong waves, which washed away plants and unstable cliffs, which buried seedlings and mature plants. That led to the large gaps between plants and a high nearest-neighbour mean distance of 1.19 m (Table 2.1). Similarly, Boyd and Barbour (1993) found that “open beach” habitat only existed seasonally when storm waves destroyed all plants by late autumn or winter along a San Francisco beach.

*Scale of aggregation*

There was aggregation of plants at a scale of less than a metre at all sites and plants were not regularly arranged as originally hypothesised (Figure 2.8 a-c). Since the O-ring statistic is a scale-dependent density function, aggregation indicates that there are also larger gaps within the pattern (Wiegand 2004). Similarly, Keddy (1981) noted that plant density of *C. edentula* was higher near the middle of the gradient than at the landward and seaward side of the beach. Variation in plant density is likely to result from environmental factors such as physical barriers, nutrient status, interspecific competition, exposure and presence of organic matter (Keddy 1981).

Analysing spatial pattern in such a narrow habitat is difficult and choosing the appropriate method involves a technical decision on the width of the rings. In the use of the O-ring statistic, narrow rings in the analysis of pattern with a few plants often produce jagged plots as not enough points fall into the different
distance classes (Wiegand 2004). This can make the O-ring statistic results difficult to interpret. In the case of the three sites, it was not clear whether the significant aggregation detected at a scale of less than one metre was along the strip of plants or up and down the shore. It seems surprising that different beaches with very different plant sizes and nearest neighbour distances would have similar aggregation at a similar scale (Figure 2.8 a-c).

**Plant distribution within the pattern**

At the three sites, plants with similar leaf types did not form clusters within the overall pattern, as would be expected from the proposed model which hypothesises that if leaf shape is inherited and if most seeds fall near parents, leaf types should be aggregated. In most cases, plants with similar leaf types were not found along-side each other any more often that would be expected by chance. More studies on dispersal need to be conducted to ascertain the movements of seeds and how persistent patches are over time. Although there was little aggregation, it was inconsistent and very weak to tell us anything about spatial pattern at the three sites.

All leaf morphotypes (entire, serrated and dissected) were widely distributed at all sites. The proportion of leaf morphotypes was almost similar and the highest proportions were entire and serrated leaves at both the sheltered and semi-open site and the least was dissected leaves at all sites (Figure 2.7). The proportion of dissected leaves was approximately 20% at each site in the current study. On the contrary, Cody and Cody (2004) found low proportions of entire and serrated leaved plants and a high proportion of plants with dissected leaves, 37% from first year plants and 48% from second year plants at Westlake Shores in South Australia. The low relative abundance of dissected leaved plants could be because this trait is recessive in comparison with entire or serrated traits. In

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retrospect, given the results in Chapter 3, perhaps a 1-10 scale should have been used here.

Cody and Cody (2004) discovered that leaf morphotypes at Westlake Shores in South Australia were widely distributed across the lower and high elevation part of the beach. More deeply lobed leaves were distributed on the upper part of the beach and were considered to be more like C. maritima leaves. Less lobed leaves considered to be more like C. edentula were at the lower part of the beach. They also noted that plants with similar leaf types tend not to grow close to each other among the Cakile population at Westlake Shores. However, they did not use formal tests and analysis of point patterns to quantify this. They argued that the observed pattern was a function of the morphological differences of different leaf types. For example, plants may have different demands for light which might alter the leaf morphology as means of adaptation. Plants tend to reduce leaf area under shade conditions (Schlichting 1986) and frequently submerged plants tend to be more dissected (Lynn and Waldren 2001).

Initial observations in the field before measurements were made, were that plants with similar leaf types appeared to be growing in clusters or had plants with similar leaves as nearest-neighbours, but Table 2.2 shows only one association of dissected plants at the open site. Although there were positive significant associations, the results were inconsistent. The casual field observations and classification of leaves did not correspond to the statistical analysis. One problem was that plants with serrated leaves may have been classified as entire due to being modified by abrasion, thus increasing the proportion of entire leaved plants at sheltered and semi-open sites. The number of plants mapped was limited and perhaps the sample size was not big enough to display the anticipated associations.
This research has established that spatial pattern exists in relatively homogenous habitats and among populations of *C. maritima*. Despite the variation in site conditions, the patterns at different sites are similar. Further work is required to determine whether the spatial pattern of *C. maritima* would change over time and whether environmental factors and gene flow contributed to the distribution of leaf morphotypes at all sites. This will need to be known before predictive statements can be made on how environmental factors affect both spatial patterns and distribution of plants leaf morphotypes within the pattern.
CHAPTER 3

DO MATERNA L PLANTS PRODUCE SEEDLINGS WITH SIMILAR LEAF TYPES?
3.1 INTRODUCTION

In the previous chapter, plants growing in the field were classified according to three leaf shapes. This was determined arbitrarily on the basis of a visual impression that there were three discrete categories of plant leaf shape. It was noted that the frequencies of the three leaf types varied from site to site. Considerable variation in leaf form often occurs within individual plants (Jensen 1990) or between plants of the same species, and between old and young leaves of the same species (Newell 1989). Studies have shown that leaf shapes and sizes may be modified by environmental conditions (Radford et al. 2004). Research on leaf morphological variations in tidal marsh species across an environmental gradient has shown that leaves were wider and longer at the highest elevation (Seliskar 1985).

Leaf size and shape characteristics are important in the study of taxonomy, evolutionary processes (McLellan 1998) and measurement of responses of plants to different environmental conditions (West and Noble 1984). Diversity of plant form is mostly attributable to genetic variation of leaf and floral organs, which are modified leaves. The problem of variation has two aspects: first, does the variation actually have discernibly different distributions regardless of the degree of overlap among outliers (are there truly distinct leaf types), and secondly, how severe is the overlap, (is variation useful taxonomically)? A smooth distribution of the variation may indicate pervasive gene flow among leaves and would argue against taxonomic recognition of the extreme forms. Moreover, continuous variation would indicate multigenic control of leaf shape.

Leaf shape is believed to be paternal or maternal, depending on which parent has the dominant gene. Some species exhibit maternal mitochondrial inheritance, whereas others exhibit paternal plastid inheritance (Liu, Mercer et
al. 1996). If clusters of plants have similar leaf types, the chances are that seedlings from underneath the clusters will also have the similar leaf type. On the other hand, seedlings might inherit 50% of the maternal genes but still inherit the leaf type of the paternal parent. Since Cakile is pollinated by insects, paternal plants may be some considerable distance away. Empirical studies of life history variation (Mousseau 1991; Dingle and Mousseau 1994; Mousseau and Roff 1995) made it obvious that many traits expressed by offspring were the result of environmental influences experienced by their maternal parent.

Leaf morphology is often highly variable in the Brassicaceae family and Cakile maritima is an example of this. Cody and Cody (2004) discovered that variation in leaf morphology exists among the Cakile species in South Australia as outlined in Chapter 1 and 2. Leaves of C. maritima in Australia have been described as varying in shape from entire to lobed to deeply lobed (Rippey and Rowland 1995). The variation is suggested to be a result of introgression between C. edentula and C. maritima. However, this research did not study introgression.

Given the variation in leaf shapes between plants of C. maritima, the current research investigated whether (i) there are discrete leaf shapes or continuous variation, (ii) whether progeny have similar leaf type to their parent. This may help in determining whether there was limited dispersal. Moreover, if there is limited dispersal and progeny have similar leaf types to parents, we would expect aggregation of different leaf types within a population (see Chapter 2).

The aims of this chapter are:

(a) To examine whether parent plants produce progeny with similar leaf types;
(b) To define leaf characters which adequately describe shape characteristics;
(c) To determine whether variation in leaf morphology is discrete or continuous without studying genetics.

3.2 MATERIALS AND METHODS

3.2.1 Seed collection and germination

The analysis of leaf shape variation was carried out on fruits collected from Cowes, Philip Island (sheltered beach) on 6 May 2005. Plants were categorized into three leaf forms: entire, serrated and dissected leaf (Figure 3.2). Fruits were collected from 30 plants at random (10 with each leaf shape category) and the leaf forms of neighbouring plants within a radius of 1 metre were noted. Seeds were extracted from the pods and nicked before sowing to break dormancy and thus improve germination.

All parents were given random numbers and the experiment was completely randomized with each parent treated as a replicate. Germination trays were filled with seed mix soil, which composed of 5 pt medium pine bark, 5 pt fine pine bark, 1 pt coarse mined sand, 1 pt sieved corn peat and 1000/m² of dolomite. Sowing took place on 25 May 2005. Fifty seeds were planted for each parent; one tray with 100 cells was divided into two blocks of 50 cells per parent. A thin layer of horticultural vermiculite grade 3 was sprinkled on top of the trays to cover the seeds. Trays were then placed in a green house running at maximum temperature of 26°C and minimum of 14°C. On 30 May 2005 seedlings started to emerge and were grown until their third leaves had fully developed. Since the media did not have enough nutrients to support seedlings, seedlings were treated with 10 litres of Aquasol fertilizer 23:4:18: NPK once a week until the experiment ended.
Figure 3.1: Examples of the three different leaf types as categorized in the field. (a) Entire, (b) Serrated and (c) Dissected leaves.

3.2.2 Visual assessment of progeny leaf morphotypes

Progeny leaves were assessed visually at the third-leaf stage and the proportion of each leaf type produced per parent plant was recorded. The third leaf stage was used because progeny leaves already portrayed the distinctive leaf types of mature plants as classified in the field. A one-way analysis of variance (ANOVA) was used to determine the significance of differences between the progeny and parent leaf types. Parent leaf type was the “treatment” and proportion of the progeny within each leaf type was the observed variable. The 300 progeny leaves were then scored using Cody and Cody (2004) classes of leaves on a scale of 1-10 (Figure 3.2). Histograms of proportions of each leaf type were obtained in Excel®.
Figure 3.2: Leaf morphotypes in *Cakile* species classified in a scale of 1-10 used in the assessment of progeny leaf shapes of 30 maternal plants of *C. maritima*. Figure is reproduced from (Cody and Cody 2004).

3.2.3 *Leaf sampling and Digitization*

The third leaf from ten randomly selected seedlings of each of the thirty parents was removed without their petiole. In total there were 300 leaves used for shape analysis (10 from each of the ten replicates of the three parent leaf type). They were then attached to paper with a clear sticky-tape (Figure 3.3). The leaves were then scanned individually into a Desktop computer using a Canon Scan N12400™ at a resolution of 600 dpi and in gray-scale. Images were then saved separately for leaf measurement and Fourier analysis. tpsDig (Rohlf 1990b) was used for measuring leaf length, leaf perimeter, leaf area, maximum width, lobe length and other measurements.
Figure 3.3: Digitized images of ten representative leaves from one parent (S1).

3.2.4 Leaf measurements

Measurements (Figure 3.4) were taken from each of 300. The character set included 12 measured variables computed from the digitized leaf images and five derived describing leaf shape.

Figure 3.4: An image of a leaf illustrating eight characters measured using the tpsDig (Rohlf, 1990) program and later used in the multivariate analysis. A is (area), P (perimeter), L (length), $W_L$ (width of largest left lobe), $W_R$ (width of largest right lobe), Lobe width.
All of the sixteen characters and five derived indices were analysed using S-plus® version 6.0 which produced scatter plots and PCA biplots. Those that explained a significant amount of variance were selected and those which gave us similar information were excluded from the final principal component analysis. Of the 16 characters which were initially considered only the six highlighted below and two derived indices were the measures ultimately used in the analysis making a total of eight variables. These were:

1) Leaf length (L), measured from the leaf base to the leaf apex;
2) Leaf perimeter (P), measured by fitting an outline around the leaf image using the tpsDig program;
3) Leaf Area (A) measured by fitting an outline around the leaf image and determining the area using the tpsDig program;
4) Total number of lobes/segments on the leaf margin;
5) Distance from the base of the leaf to the first lobe/segment (average of left plus right measurements) or to the point of maximum width for entire leaves;
6) Maximum width, the average width of the largest lobe on the right ($W_r$) plus largest lobe on the left ($W_l$) measured from the midrib;
7) Maximum of width of the first lower section of the leaf measured from the midrib
8) Maximum of width of the middle section of the leaf measured from the midrib
9) Maximum of width of the top section of the leaf measured from the midrib
10) Length of the largest lobe in the 1/3 lower section of the leaf on the left;
    Length of the largest lobe in the 2/3 middle section of the leaf on the left;
    Length of the largest lobe in the 3/3 top section of the leaf on the left;
11) Length of the largest lobe in the 1/3 lower section of the leaf on the right;
Length of the largest lobe in the 2/3 middle section of the leaf on the right;
Length of the largest lobe in the 3/3 top section of the leaf on the right;
12) Ratio of leaf length to maximum leaf width;
13) Form factor;
14) Perimeter for per millimetre of leaf length;
15) **Leaf shape index**
16) **Dissection index**

The two derived indices which were finally used in the analysis to describe leaf shape were calculated from the measured variables. The first one was the *dissection index* (DI) which is based on leaf perimeter and leaf surface area;

\[
DI = \frac{\text{Perimeter}}{\sqrt{\text{Area}}}
\]

(Kincaid 1998; McLellan 1998). The minimum value of DI is that of a rectangle, for which DI = 1.0 (Lynn and Waldren 2001). The value of DI for a circularly shaped entire leaf is slightly larger than 1.0 and the more deeply lobed, dissected or lanceolate a leaf, the larger the value of DI. Although DI is a variable derived from a ratio, it is usually normally distributed in samples of leaves and that was so in this study.

The second index was *leaf shape index* (LSI); which is a mathematical measure of the “complexity” of a leaf’s outline;

\[
LSI = \frac{\text{Area}}{W \times L}
\]

where *W* is maximum leaf width and *L* is leaf length (McLellan 1998). For instance, LSI = 1 for any ellipse of oval shaped leaves with relatively smooth
margins (Kincaid 1998). The more complicated the shape of the leaf, relative to the best fit of the ellipse, the greater the value of LSI. DI and LSI may or may not be correlated because each variable describes different aspects of leaf shape, independent of leaf area.

3.2.5 Multivariate analysis

To determine if leaf shape varied within the progenies of the three maternal plants, Principal Component Analysis (hereafter, referred to as PCA) was done using the eight measured leaf variables of the 300 hundred progeny leaves. PCA involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible (Sundberg 1989).

All the eight variables were selected and analyzed by principal component analysis using the multivariate statistical (MVSP®Plus version 3.1, KCS, 1986-2001). The eight variables were: perimeter, area, maximum width, leaf length, and number of lobes, leaf shape index, dissection index and distance from the base of the leaf to the first lobe or point of maximum width. Values of PC 1 and PC 2 were used. We also used Fourier coefficients as variables in a separate multivariate analysis (McLellan 1993). Data were analysis were analysed based on correlation matrices of the coefficients to examine the extent to which leaf shapes were defined by this method, and further used the values of the first (PC 1) and the (PC 2) second principal components as variables in comparison with other metrics of leaf shape. In both multivariate analyses, graphs of PC axes were examined visually to see whether or not variation was continuous. Biplots also indicated the leaf traits having most influence on the data.
Elliptic Fourier analysis was performed by putting an outline around the digitized leaf image with a program written to save $x$ and $y$ coordinates starting at the first point in each image at the juncture of the leaf base and the lamina. A total of 21 harmonics (Figure 3.5) were fitted on one leaf and four coefficients were produced for each harmonic. We decided to fit only 21 because the shape of the transformed was very similar to the original leaf shape after fitting the 21 harmonics.

Data were analyzed using the EFAWIN® program. To remove differences in size and orientation, coefficients were normalized to the first harmonic as given by (Kuhl 1982). Another program TOMINI® was used to convert the coefficients to a file which could be read by MINITAB®. Since MVSP® could not accommodate large files of leaf coefficients, a maximum of 25 coefficients were analysed by principal component analysis.

Figure 3.5: Outline of a leaf summarized using 1, 5, and 10 harmonics (elliptic Fourier analysis carried out using EFAWIN) on outline data obtained with tpsDIG (Dickinson 2001).
3.3 RESULTS

3.3.1 Visual assessment of all progeny leaves

The progeny leaf types were classified to the three paternal leaf types. There was a high proportion of serrated progeny regardless of the parent’s leaf type. However, there was a trend for more progeny to be of the same leaf type as their parent. For example, 83% of serrated progeny were from serrated parents, compared to 77% from entire and 68% from dissected parents. Significantly more entire progeny were from entire parents: 8% compared to 3% from serrated and 2% from dissected parents. Similarly, a significantly greater proportion of dissected progeny were from dissected parents: 30% compared to both entire and serrated parents 14%. The ANOVA table is shown in Appendix 1.1.

Table 3.1: Means of the percentage of cash leaf morphotypes in the progeny of parents collected from the field. Bold figures indicate a significantly greater proportion of progeny with that leaf type.

<table>
<thead>
<tr>
<th>Progeny Parents</th>
<th>Entire %</th>
<th>Serrated %</th>
<th>Dissected %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire</td>
<td>8.44</td>
<td>77.03</td>
<td>14.53</td>
</tr>
<tr>
<td>Serrated</td>
<td>2.83</td>
<td>82.94</td>
<td>14.23</td>
</tr>
<tr>
<td>Dissected</td>
<td>1.80</td>
<td>68.24</td>
<td>29.96</td>
</tr>
<tr>
<td>Standard Error</td>
<td>4.77</td>
<td>14.91</td>
<td>12.61</td>
</tr>
</tbody>
</table>

3.3.2 Scoring of progeny leaves

The frequencies of the 300 progeny leaves on the 1-10 scale as used by Cody and Cody (2004) (Figure 3.6). Leaf morphotypes of the progeny from each of the three parent types presented a wide variation of shape with every class
represented. Classes 1-3, 4-6 and 7-10 corresponded approximately with the above classification of entire, serrated and dissected leaves respectively.

Figure 3.6: Frequency distribution of leaf morphotypes in the progeny of plants from 3 maternal leaf types from the sheltered beach using the Cody and Cody (2004) 1-10 scale. Blue is entire leafed parent, maroon is serrated leafed parent and yellow is dissected leafed parent.

There was a significant leaf shape variation among the progeny of *C. maritima* (*P < 0.05*). The mean parent leaf types indicated that progeny leaves visually classified as dissected had the highest mean (5.18), followed by serrated (4.62) and entire (4.00) on the Cody and Cody scale. The mean differences were minimal, reflecting the considerable overlap in the leaf morphotypes of the progeny from each parent type.
3.3.3 Leaf measurements

These results show four of the eight variables that were highly significant in explaining leaf shape variation among the progeny (Table 3.3). These were perimeter, length, area, and dissection index.

![Graphs showing relationships between variables](image)

**Figure 3.7:** (a) The association between leaf perimeter and length from plants grown from seeds in the glasshouse, (b) Dissection Index and perimeter (c) perimeter and area. Red squares are those which were classified visually as entire, blue triangles are serrated and pink diamonds are dissected morphotypes.
Most of the leaves classified visually as dissected had a high perimeter, large Dissection Index and were longer than the other two leaf types.

### 3.3.4 Statistical analysis of leaf measurements

The means and standard deviations of the eight measured variables (Table 3.2) used to define leaf shape, (see Appendix 1.2 for ANOVA tables). The results showed that there is a significant difference between the leaf shapes of the progeny of the three parent leaf types as expressed by the eight variables.

**Table 3.2**: Descriptive statistics for 300 progeny leaves classified visually as entire, serrated and dissected. The first row of figures is progeny means regardless of their parent leaf types and second row in parentheses is their standard deviations.

<table>
<thead>
<tr>
<th>Variables Leaf types</th>
<th>Length</th>
<th>Perimeter</th>
<th>Area</th>
<th>Width</th>
<th>No. of Lobes</th>
<th>Base to 1st lobe</th>
<th>LSI</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire (n = 100)</td>
<td>30.31</td>
<td>69.82</td>
<td>159.78</td>
<td>13.62</td>
<td>2.00</td>
<td>0.54</td>
<td>0.46</td>
<td>5.58</td>
</tr>
<tr>
<td>(n = 100)</td>
<td>(8.69)</td>
<td>(18.65)</td>
<td>(60.57)</td>
<td>(5.24)</td>
<td>(0.00)</td>
<td>(0.29)</td>
<td>(0.24)</td>
<td>(0.62)</td>
</tr>
<tr>
<td>Serrated (n = 100)</td>
<td>32.56</td>
<td>91.57</td>
<td>219.72</td>
<td>13.12</td>
<td>10.92</td>
<td>0.42</td>
<td>0.56</td>
<td>6.31</td>
</tr>
<tr>
<td>(n = 100)</td>
<td>(6.16)</td>
<td>(22.41)</td>
<td>(84.81)</td>
<td>(4.76)</td>
<td>(3.37)</td>
<td>(0.27)</td>
<td>(0.24)</td>
<td>(1.03)</td>
</tr>
<tr>
<td>Dissected (n = 100)</td>
<td>36.56</td>
<td>160.24</td>
<td>230.91</td>
<td>13.23</td>
<td>9.32</td>
<td>0.36</td>
<td>0.50</td>
<td>10.56</td>
</tr>
<tr>
<td>(n = 100)</td>
<td>(8.36)</td>
<td>(52.06)</td>
<td>(97.00)</td>
<td>(4.42)</td>
<td>(2.57)</td>
<td>(0.20)</td>
<td>(0.18)</td>
<td>(1.90)</td>
</tr>
</tbody>
</table>

Some of the individual variables contributed significantly to the discrimination of the different leaves, whereas some of them did not. It was evident that in terms of length, leaves classified visually as entire and serrated were significantly shorter than dissected ones. A larger proportion of dissected leaves also had larger perimeter, Dissection Index and perhaps surprisingly, area. Serrated leaves had a significantly larger number of segments compared to
entire and dissected leaves. Apparently leaf width, leaf shape index and distance from the base to the first lobes did not contribute much to leaf variation.

### 3.3.5 Multivariate analysis

The first two PC (Axis 1 and 2) gave significant loadings on the axes, while the third PC had insignificant loadings. Length, perimeter, area, and Dissection Index had large positive loadings on PC 1, whereas maximum width and Dissection Index had large negative loadings on PC 2.

#### Table 3.3: Correlation between individual leaf variables and ordination axes for principal component analysis morphological variables.

<table>
<thead>
<tr>
<th>Leaf variables</th>
<th>Axis 1</th>
<th>Axis 2</th>
<th>Axis 3</th>
</tr>
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<tbody>
<tr>
<td>Length</td>
<td>0.518</td>
<td>0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>Perimeter</td>
<td>0.511</td>
<td>-0.177</td>
<td>-0.306</td>
</tr>
<tr>
<td>Area</td>
<td>0.479</td>
<td>0.169</td>
<td>0.291</td>
</tr>
<tr>
<td>No. of lobes</td>
<td>0.112</td>
<td>0.005</td>
<td>0.514</td>
</tr>
<tr>
<td>Maximum width</td>
<td>-0.065</td>
<td>-0.605</td>
<td>0.27</td>
</tr>
<tr>
<td>LSI</td>
<td>0.219</td>
<td>0.632</td>
<td>0.015</td>
</tr>
<tr>
<td>DI</td>
<td>0.342</td>
<td>-0.306</td>
<td>-0.544</td>
</tr>
<tr>
<td>Base to the 1st lobe</td>
<td>-0.244</td>
<td>0.285</td>
<td>-0.42</td>
</tr>
</tbody>
</table>

#### Table 3.4: The eigenvalues and covariance percentages for the principal component analysis of the eight variables of the 300 progeny leaf morphotypes.

<table>
<thead>
<tr>
<th></th>
<th>Axis 1</th>
<th>Axis 2</th>
<th>Axis 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalues</td>
<td>3.04</td>
<td>1.88</td>
<td>1.22</td>
</tr>
<tr>
<td>Percentage</td>
<td>37.99</td>
<td>23.49</td>
<td>15.23</td>
</tr>
</tbody>
</table>
Covariance showed the contribution of PC 1, PC 2 and PC 3 in terms of percentages of the total variation. Almost half of all the variance was in the first principal component (37.99%). PC 1 was plotted against PC 2 as illustrated by Figure 3.8. The loadings in the eigenvectors indicated that many of the variables are closely related and significant, especially the hand-measured variables with the exception of maximum width.

![PC biplot](image)

**Figure 3.8:** PCA biplot for eight leaf variables of 300 progeny leaves. Each symbol represents an individual. Red squares are leaves scored visually as entire, blue circles serrated and green triangles dissected leaves.

Graphs of principal components axes 1 and 2 showed almost no segregation of individual leaf types into distinct groups (Figure 3.8). Leaf types were overlapping, entire leaves on the left and centre, serrated leaves across the graph and dissected leaves around the centre and far right. Multivariate analysis using the Fourier parameters shows clustering of all leaf types at the centre of the graph, with a few extreme outliers. This pattern confirms the continuous variation of leaf types as illustrated by Figure 3.8.
Figure 3.9: The first two principal component axes of 25 coefficients from the Fourier analysis of leaf shapes. Red squares were progeny classified visually as entire, blue circles are serrated and green triangles are dissected leaves.

3.4 DISCUSSION

Continuous variation

Leaf morphology is often highly variable in the Brassicaceae family, specifically in *C. maritima* (Barbour and Rodman 1970; Rodman 1974; Boyd 1988; Cody and Cody 2004). There was continuous leaf shape variation among the progeny of *C. maritima* based on Cody and Cody (2004) (Figure 3.2) and based on our own observations (Figure 3.3, 3.8). This suggests that the use of only three leaf types is not appropriate. The proportions of leaf types vary in their frequencies, as also found by Cody and Cody (2004) with the serrated types being the most frequent (types 3-5) and dissected types being the least common.
**Introgression**

It has been suggested that hybridisation via introgression contributes significantly to the morphological variation in *C. maritima* in South Australia (Cody and Cody 2004). However, this study did not look at introgression so there is no scientific evidence to support their suggestion. The herbarium specimens collected by Eichler (1965) showed some intermediate characteristics which suggested that hybridisation occurred in a mixed population of *C. edentula* and *C. maritima* in South Australia. The variation might be due to the different breeding system of the two species. *C edentula* is self-compatible and *C. maritima* is mostly cross-pollinated (Rodman 1974). There appears to be no hybridisation in northern California, where the two species have been growing together and naturalised. Leaf shapes were able to be clearly assigned to their respective species (Barbour and Rodman 1970). However, Rodman (1974) concluded that intermediate morphologies and the pattern of character variation in mixed population of *Cakile* species suggest that natural hybridisation does occur.

**Progeny and parent similarity**

In our study, it was apparent that seedlings do not always produce leaves similar to their parents. This is probably due to genetic variation within a species and individual plants. Pollen may come from plants a considerable distance away. Cross pollination perhaps contributed to mixed genes in the seed, which means progenies resembled the dominant leaf type of either parent. Helenurm and Schaal (1996) acknowledged that non-reciprocal effects of maternal and paternal parents on offspring phenotypes have long been recognised in both genetics and biology. They further noted that genetic variability is a common
feature of out-breeding plant populations and genes controlling morphological and biochemical characters can also be highly variable.

*Leaf characters which defined leaf shape*

Eight of the measured leaf variables contributed significantly in the discrimination of leaf shape among the progeny leaves. Five of the eight measured variables were found to explain much of the variation within multivariate clusters (Figure 3.8a). However, measurements often overlapped among groups of leaves. Studies have shown that leaf length, perimeter, width, area and dissection index play a significant role in shape discrimination (Newell 1989; MacLellan and Endler 1998; Lynn and Waldren 2001; Cody and Cody 2004; Burton 2004).

The sampling of parent types in the field was biased, because fruits were collected from specific plants of the three leaf types instead of a random sampling. The leaf types of the paternal plants were not known at the time of sampling, only of the maternal plants. The growing medium was different from the field in terms of nutrients and porosity. There was also less competition between the plants since each seedling was grown in a separate cell within the trays. Leaf shape changes throughout plant development, and the choice of a particular leaf for a study is arbitrary. The first leaves tend to be more entire in shape, while later leaves may express greater variation in shape. In field studies, the leaves studied may be from a wide range of developmental stages.

Without disputing the good work done by Cody and Cody (2004) on developing the scale of 1-10 for leaf shape classification among leaves of *Cakile* species, some leaves were difficult to classify because they did not fall into any of these
classes. This led to the conclusion that leaves might not be in discrete classes as initially observed (Figure 3.2). McLellan (1998) noted that leaf shape is the most obvious trait that changes during heteroblastic development. In many plants there is gradual transition from juvenile traits, soon after germination to adult traits at the time of flowering. She concluded that the change from juvenile to adult leaf shape was a product of change in genes which determine leaf shape.

*tps Dig*

The tpsDig® program was very useful because it allowed us to take as many measurements as possible within a short space of time. It also enabled storage of large files with detailed leaf shape data in a regular format. It was easy to manipulate the images in preparation for calculation of the leaf parameters. However, calculation of many leaf characters required measurements to be done perpendicular to the midrib, for example maximum leaf width. In some cases this was not possible because leaf segments/lobes were alternating, so that is why two measurements of the largest lobes/segments were taken on the left and right to calculate the average maximum leaf width. It was also difficult to take leaf length measurements where the apex of the leaf was not perpendicular to the leaf base (e.g. Figure 3.4).

Although the Fourier analysis was used to determine leaf shape and indicated continuous variation in leaf shape, the method appeared to be sensitive and often produced outliers than from the PCA of measured variables. However, it can be concluded that there is no distinct groups of leaf shapes but continuous variation among the population of *C. maritima.*
Leaf morphology is simply a marker and cannot be used to study gene flow between plants and their progeny. Future research could study genetic variation in natural populations to infer the degree of genetic relatedness among individuals in wild populations, which are then in turn used to estimate the heritability of quantitative traits (Meagher and Thompson 1987). This approach would allow one to examine long-lived species in a wild setting without the need for any form of manipulation (other than to collect tissue for DNA fingerprinting).
CHAPTER 4

DO DISPERSED SEEDS COLLECT AROUND EXISTING PLANTS?
4.1 INTRODUCTION

The previous chapters reported that *C. maritima* plants grow in a very harsh environment of blowing sand and salt spray where there are few native plants to displace. In spite of the unoccupied space, *C. maritima* plants appeared to be aggregated at a small scale at all sites. In the model proposed in Chapter 1, it was suggested that aggregation will occur from seeds dropping directly below the parent, thus being trapped there, and that seeds dispersing away from parents would preferentially collect beneath other plants, forming a seed “bank”.

A seed bank is an aggregation of un-germinated seeds potentially capable of replacing adult annuals or perennials plants (Leck, Parker et al. 1989). The potential to replace adult plants is crucial. If seeds are buried too deeply they fail to be an effective seed bank. Seeds are important both for the maintenance and growth of existing populations and for the initiation of new ones (Fenner and Thompson 2005). Many populations of herbaceous perennial plants contain seeds stored in a soil seed bank. A seed bank can be transient (can last for one year or less) or persistent (usually lasting for one to five years) (Silvertown 2001). However, the emergence of a plant from a burial deposit primarily depends on the depth of burial and energy reserves in its storage organs (Bastida 2002).

*C. maritima* is one of the sand dune plants that can withstand blowing sand and burial, and still germinate the following season (Barbour 1970a; Rodman 1974; Rippey and Rowland 1995). High seedling density around buried plants was noted in Canada (Keddy 1980). However, germination rates of *Cakile maritima* are difficult to obtain because individual seeds cannot easily be followed in the field. The long-term fates of individual seeds in the soil are difficult to monitor,
especially where seeds are very small. *Cakile maritima* plants produce small seeds that are dispersed into the sand, but the existence of a viable seed bank has not been confirmed experimentally. Studies have shown that buried seeds of *C. edentula* have the ability to better tolerate fore-dune conditions and survive for two to three years (Barbour 1972; Rodman 1974; Heyligers 1984; Zhan and Maun 1994), but there are no data on *C. maritima*.

Therefore, the research questions asked in this study were: do seeds of *C. maritima* accumulate around existing vegetation and do they retain viability to be recruited to the next generation? This study therefore, examined the quantity and viability of seeds in the soil seed bank under plants and in unoccupied space between plants. It was hypothesized that the soil seed bank would primarily be beneath the *C. maritima* plants as a result of many seeds falling within the canopy.

### 4.2 MATERIALS AND METHODS

#### 4.2.1 Study site

Cowes is located 140 km south of Melbourne, at 38°27′S, 145°14′E and on the northern side of Phillip Island. The site at Cowes consists of a sheltered beach with a series of vegetation types aligned parallel to the shore. Large numbers of *C. maritima* plants can be found along the base of a small bank at the high tide zone.
Figure 4.1 The sheltered beach at Cowes where the soil samples were collected. Most plants were surrounded by sea-grass leaves (brown litter).

4.2.2 Data collection

In order to determine whether a seed bank existed for *C. maritima*, ten random soil samples were collected using a hand trowel from the top 5 cm of the soil (after first removing the layer of fresh organic matter). Five samples were from beneath individual plants and five were from un-occupied spaces with plants at least one metre away on either side. The soil samples were spread into trays and kept at room temperature to dry. The volume of each sample was measured and sieved through four different sized sieves (2.36, 1.00, 0.8, and 0.6 mm). Soil samples from beneath plants were taken within the circumference of a plant. The material collected from each sieve was assessed and the seeds and fruit segments were retrieved. The seeds were extracted from the segments, (which were mainly the distal segments of the fruit) and then assessed.
4.2.3 Viability test

The viability of recovered seeds was tested using the standard tetrazolium test (TZ) (ISTA 1985). Seeds were removed from the fruit segments and imbibed in water for 24 hours. The embryos were then removed from the seed coat and soaked in 2, 3, 5, Triphenyl-2H tetrazolium chloride (TZ) for 2 hours at 20°C. Seeds were removed from the chemical and rinsed with distilled water. Seeds were then assessed under a microscope to determine the degree of staining. Data were analysed by a t-test with unequal variances using MINITAB® and graphs were produced in Excel®.
4.3 RESULTS

4.3.1 Viability test

More seeds were recovered from beneath plants than in the un-occupied space between plants (Figure 4.3).

Figure 4.2 Seed quantities from un-occupied spaces between plants and beneath plants. Graph bars are fitted with error bars.

Regardless of where the seeds came from, they were all viable. Most of the seeds came from the distal segments (top part of the fruit) and very few of the proximal (lower part of the fruit) had seeds. The results showed that the quantity of seeds which could be found beneath plants was significantly higher than between plants, $P < 0.05$, $t = 3.52$ and d.f = 4.
**Figure 4.3:** An example of a Petri dish with viable seeds from beneath plants subjected to a standard TZ test. All embryos stained bright red and were interpreted as viable.

All of the seeds from both locations were well stained. Extreme red spots were not abnormalities, but a result of injuries incurred during the removal of the seed coat. There were small white spots as well, presumably dead cells inflicted during seed extraction. The size, location and nature of injuries and other imperfections occurring within seeds determine whether independent embryos are capable of producing a normal seedling. Sound tissues tend to stain red gradually and uniformly, from the exposed surfaces inward (ISTA 1985). Generally, all the embryos were red and sound, which was a sign of viable seeds.

### 4.4 DISCUSSION

Seeds are more abundant under existing plants than in soil between plants (Figure 4.3). This may have been caused by the amount of sea-grass litter which collected beneath the plants possibly trapping a lot of seeds beneath existing plants. In addition, the plants were relatively large and decumbent (Eichler
1965) which allowed them to trap seeds under their branches. Sand and sea
grass blowing on to mature plants buried whole branches still attached to parent
plant. Another reason for the lack of seeds in open spaces could be due to
exposure to predation either by rodents (Boyd and Barbour 1993) or birds
(silver gulls) (Rippey and Rowland 1995) along the beach. The lack of mulch or
obstacles in the open spaces also increases the chances of fruits dispersing away
from maternal plants. Such fruits are probably likely to being washed away by
the next tide or blown away by wind, thus limiting their chance of being buried
in the soil.

There were more distal segments retrieved from the soil seed bank beneath
existing plants than proximal segments. It was observed that this was because
some of the proximal segments were still attached to the maternal plants and
technically not part of the seed bank. Other authors have reported that distal
segments are often blown further than proximal segments (Barbour and Rodman
1970; Barbour 1972; Keddy 1981; Donohue 1997; Donohue 1998). In our case,
the majority of the distal segments did not necessarily get blown away,
especially where there were very large plants. Therefore, it is important to note
that the distance at which the distal seeds move can depend on the availability
of debris around the maternal plant and the size of branches. More debris, large
branches and decumbent plants will trap and limit fruit dispersal.

However, single samples of seed bank cannot distinguish between fruit
segments from the immediate plant and those that have been blown in (if the
system is in equilibrium, immigration and emigration may balance). Genetic
methods are needed to determine the sources of seeds underneath each plant
(Meagher and Thompson 1987). Although the sample size was relatively small,
it was apparent that seeds tended to accumulate under existing vegetation at
much higher densities than in un-occupied sites. However, it was not easy to
determine whether the seeds were from the current parent or from previous generations of plants. Further studies should pay attention to three aspects, (i) the number of samples; (ii) different sampling depth and (iii) seed dispersal. In dune systems, seed distribution is known to be uneven and ideally requires the use of a more intensive sampling than was used in this preliminary study, and over different seasons.

In conclusion, the results of this study clearly demonstrate that even if seeds are buried, they can still germinate if the conditions are suitable for germination. Further studies are required to determine the mortality rate of seeds and hence whether the seed bank is transient or persistent (Fenner and Thompson 2005). The number of seeds retrieved probably means that the site is stable enough to produce seedlings to form the next generation. It can be inferred that patches would persist or increase over time if seeds are continuously lodged under existing plants. This would result in an aggregated spatial pattern within the population of *C. maritima*. Although seeds accumulate under plants, often from the maternal parent patches of *C. maritima*, the progeny would often not have similar leaf types as observed in Chapters 2 and 3. This is because seeds come from different parents with different leaf types. Although seeds were retrieved from a shallow depth in our study, a detailed analysis of the soil seed bank would require a larger volume of samples from various depths given the dynamic nature of populations in the sand dune systems.
CHAPTER 5

UNDERSTANDING THE SPATIAL PATTERN OF C. MARITIMA
5.1 INTRODUCTION

The aims of this thesis were to determine whether spatial pattern exists among populations of *C. maritima*, what the distribution of plant leaf morphotypes is like within the pattern and whether seeds are more likely to be associated with existing vegetation or unoccupied sand. The experiments presented in this thesis examined whether the spatial distribution of plants predicted the distribution of the plants in the following seasons, if there is aggregation of plants with similar leaf types, whether there is discrete or continuous variation of leaf shape within a population of *C. maritima* and if the existing seed bank can tell us anything about persistence of patches over time. The major findings were:

- *C. maritima* was present as a long narrow aggregated strip of plants along the shore at all sites with aggregation at a scale of less than one metre;
- There was only a weak (and inconsistent) association of plants with similar leaf types within the pattern and leaf types do not persist in clusters;
- Continuous variation in leaf shape exists within populations of *C. maritima*;
- The three leaf type categories used for classification in the field are therefore not justified and do not reflect a simple genetic leaf shape system;
- Seeds collected under existing plants and viable seeds were greater directly under plants than between plants but it was not possible to ascertain the origin of the seeds.

This chapter will discuss a range of issues arising from the research based on the proposed model in Chapter 1.
5.2 GENERAL DISCUSSION

Plants of *C. maritima* did not cover the whole beach but were confined along upper edges of the sand dune where the high tide ends (Figure 2.2 and 2.4) with the exception of the semi-open site where plants were growing in a depression (Figure 2.3). There was evidence of gaps between plants (Chapter 2, Figure 2.6) especially at the open site. The mechanism governing the observed pattern of *C. maritima* is not known. However, it has been noted that the spatial pattern of a plant population is determined by physical and biological variables (Powell 1990) and these varied with beach conditions. A few of these factors noted in this thesis were physical barriers which limited dispersal of seeds beyond the high tide zone at all sites, disturbance which involved washing of plants closer to shore and burial of seedlings and mature plants, thus reducing the width of the strip of plants and increasing gaps between plants, for example the open site had largest mean nearest-neighbour distance of 1.19.

A single or different mechanism can produce regular, random and aggregated spatial pattern (Pielou 1960). The spatial pattern can be created by recruitment, (which creates either a regular or aggregated pattern) pre-reproductive mortality (which may create a regular pattern) and post-reproductive mortality (an aggregated pattern) (Powell 1990). It is likely that the observed spatial pattern among *C. maritima* populations was due to the same processes. The findings support the proposed model in Chapter 1 that there is poor establishment of seedlings between existing plants due to harsh environmental conditions of bare sand.

A greater number of seeds were found under existing plants at a sheltered beach than in un-occupied places between plants (Chapter 4, Figure 4.3). This agrees with the model that there is preferential collection of seeds around existing
plants. However, since fruits can be blown away until they were trapped by obstacles (Barbour and Rodman 1970; Barbour 1970 a and b: Rodman 1974; Keddy 1981), it was not easy to determine the origin of seedlings. There is a possibility that the distal fruits came from neighbouring plants and, therefore, it is not clear whether progeny replace maternal plants because most of the proximal fruits were still attached to the maternal plants.

However, if there is better survival of seedlings around existing plants due to availability of organic matter, high moisture content, and protection from wind, then patches are likely to persist over time. Research has shown that germination of seeds varies with depth (Adair, Higgins et al. 1990). Seed banks in a wide range of habitats are generally clumped, at least at the horizontal scale and sometimes at much finer scales (Thomson and Grime 1979; Lortie, Ellis et al. 2005). Fine scale spatial variation in the distribution of seeds has the potential to directly influence the plant community by providing gaps which can affect the spread of species within a community (Lortie, Ellis et al. 2005), influence local seed density within patches which at least partially determines the final plant density or spatial pattern of plants, or affect competitors within a local neighbourhood (Figure 5.1). Further studies could look at the persistence of the soil seed bank over time.

Although patches are likely to be formed and sustained over time, successive plants are likely to have different leaf types if the parental type is low in frequency. This would probably be due to cross pollination, which is a norm among plants of *C. maritima* (Barbour 1972; Rodman 1974; Rodman 1986; Boyd and Barbour 1993; Cody and Cody 2004), all have one paternal plus one maternal set. Therefore, the existence of patches does not guarantee that patches will maintain similar leaf types.
From Chapter 3 of this thesis, it was apparent that there is continuous variation of leaf shapes among populations of *C. maritima* (Figure 3.8) as similarly noted by Barbour (1972), Rodman (1974), Rodman (1986), Boyd and Barbour (1993), and Cody and Cody (2004). The variation has been suggested to be a result of introgression between *C. maritima* and *C. edentula* (Rodman 1974; Heyligers 1984; Boyd and Barbour 1993; Cody and Cody 2004). Similar variation in leaf morphotype was recoded by Rodman (1974) between hybrids of *Cakile* species in Florida and Gulf of Mexico. The main need is to examine leaf types in populations in land of origin, also in herbarium specimens before the second species arrived, to decide whether the range of variation in Australia is greater than *C. maritima* in Europe. If not, there is no evidence of introgression.

Environmental factors played a significant role in the establishment of plants and their spatial pattern (Pielou 1961; Powell 1990; Lortie, Ellis et al. 2005). Through disturbances the width of the habitat was not so wide, for example (Cody and Cody 2004) at all of the three sites. Erosion of the beach can negatively impact beach ecology by removing habitat (Kephart 1997). Plant burial or erosion increased gaps between plants, for example at the open site. Mode of dispersal like wind and water, strong winds and waves (Figure 5.1) may deposit fruits to unfavourable sites where survival of seedlings and mature plants is limited (Keddy 1982; Donohue 1998).

The depth at which seeds are buried usually determines whether seeds will germinate and be recruited to the existing population, thus increase plant density (Cheplick and Demetri 1999) or merely replace the previous generation. High temperatures and low moisture in the soil may lower the germination rate of seeds from the soil seed bank (Figure 5.1) and reduce population size. The interaction between environmental factors and genotypes can also contribute to modification of leaf morphology and plant size (Schlichting 1986). Different
plants respond differently in different environments. All these components might in one way or the other contribute to the arrangements of *C. maritima* plants along the beach.

**5.3 CONCLUSION**

Whilst *C. maritima* exists along side the native species, it is not a threat to native plants or any coastal ecosystem. This is due to the fact that *C. maritima* colonises empty sites and observations from this study showed that only a few native species can survive immersion in salty water at high tide and abrasion due to tide action except for *Spinifex sericeus*. Nonetheless, much can still be gained from studying its spatial pattern at different sites with different level of exposure over a longer period of time. Many researchers have studied spatial pattern along environmental gradients. However, this thesis has demonstrated that spatial pattern also exists along a relatively homogenous habitat. The findings from this thesis will provide the base information for a genetic study to determine the relatedness between parents and their progenies in a natural population and find out whether the seeds that occur between clumps spaces are disadvantaged in their establishment.
Figure 5.1: Factors contributing to the spatial pattern of *C. maritima*. (a) A proposed model in Chapter 1. (b) A conceptual model explaining spatial pattern as per the outcome of the research. Solid arrows represent factors affecting the spatial pattern process. Dashed lines are factors that still need to be researched on.
REFERENCES

Asia Travel (2001). Reservation Network.


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Appendices

Appendix 1. Chapter 3: *Visual assessment of all progeny leaves*

**Appendix 1.1:** ANOVA results of leaf progeny morphotype scores from 30 maternal plants.

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F</th>
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<th>P value</th>
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<td>6.968</td>
<td>3.484</td>
<td>6.35</td>
<td>0.006</td>
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<tr>
<td>Error</td>
<td>27</td>
<td>14.812</td>
<td>0.5486</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>21.78</td>
<td></td>
<td></td>
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**Appendix 1.2:** ANOVA tables testing the significance of progenies with similar leaf types as parent plants.

(a) *Entire progeny versus Parent*

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<th>Mean Square</th>
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<tr>
<td>Parent</td>
<td>2</td>
<td>287.8</td>
<td>143.9</td>
<td>6.32</td>
<td>0.006</td>
</tr>
<tr>
<td>Error</td>
<td>27</td>
<td>614.8</td>
<td>22.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>902.6</td>
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(b) *Serrated progeny versus Parent*

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<tr>
<td>Parent</td>
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<td>1056</td>
<td>528</td>
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<tr>
<td>Error</td>
<td>27</td>
<td>5997</td>
<td>222</td>
<td></td>
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<tr>
<td>Total</td>
<td>29</td>
<td>7054</td>
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</table>
(c) Dissected progeny versus Parent

<table>
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<th>Mean Square</th>
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<tr>
<td>Total</td>
<td>29</td>
<td>6026</td>
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</tr>
</tbody>
</table>

Appendix 1.3: ANOVA tables of the statistical analysis of the eight leaf variables on 300 progeny leaves.

(a) Length versus progeny

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progeny type</td>
<td>2</td>
<td>987.1</td>
<td>493.6</td>
<td>10.50</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>297</td>
<td>13965.5</td>
<td>47.0</td>
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<td></td>
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<tr>
<td>Total</td>
<td>299</td>
<td>14952.6</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Perimeter versus progeny

<table>
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<th>Source</th>
<th>D.F</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
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<td>270662</td>
<td>135331</td>
<td>133.15</td>
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<tr>
<td>Error</td>
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<td>1016</td>
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<td>Total</td>
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<td>572517</td>
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</table>
(c) **Area versus progeny**

<table>
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<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
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<td>55598</td>
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</table>

(d) **Maximum width versus progeny**

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<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
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</tbody>
</table>

(e) **Number of lobes versus progeny**

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<th>Mean Square</th>
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<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
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</table>
(f) Base to the first lobe versus progeny

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<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Parent</td>
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</table>

(g) Leaf shape index versus progeny

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<th>Mean Square</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

(h) Dissection index versus progeny

<table>
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<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
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