Chapter 1 Introduction

Macadamia integrifolia Maiden and Betche (Family Proteaceae) is a medium-sized tree endemic to notophyll vine forests of South-Eastern Queensland, Australia, as well as a major horticultural nut crop. As a result of extensive clearing within its natural range, the majority of known populations of *M. integrifolia* in the wild are present as small, isolated remnants, predominantly located on private property (Hardner 2004). The species is subsequently listed as vulnerable under the EPBC Act 1999 (Cth), classifying it as likely to become extinct within the next 20 to 50 years (Briggs & Leigh 1988). In addition to being an Australian iconic species and the only major food crop to be domesticated from the Australian flora, wild *M. integrifolia* populations may be valuable to the Australian macadamia nut industry as a germplasm resource for the development of new cultivars. Conservation of wild genetic diversity may be important for providing a competitive edge to the Australian industry over international producers.

Gross (1995) identified the major threats to macadamia as clearing, trampling by livestock, introduction of exotic plant species, and fires originating in nearby eucalypt forest or grassy woodland. Many of these are associated with or exacerbated by habitat fragmentation, and threaten both the long- and short-term viability of remnant populations (Saunders et al. 1991). Fragmentation can be defined as the reduction of continuous habitat into several smaller spatially disjunct remnants (Young et al. 1996), and along with habitat destruction, has been suggested as the major cause of the increased rate of species extinction observed in recent decades (Groombridge 1992).

Given the importance of conservation of the species in the wild and the highly fragmented and disturbed state of the majority of known populations, this study aims to investigate both the long- and short-term viability of fragmented *M. integrifolia* populations in an endeavour to provide background information necessary for an effective management strategy (if required) to be developed for the species. In addition, the complex geological history and recent anthropogenic threats to its habitat makes the study of *M. integrifolia* survival in fragments of particular interest and provides the opportunity of exploring the consequences of fragmentation for a unique Australian rainforest tree species.

Section 1.1 in this chapter is an introduction to Australian rainforest flora and its dynamics. A background to *M. integrifolia* natural history and its conservation status in the wild is provided in Section 1.2, and a general introduction into habitat fragmentation and its basic physical consequences is presented in Section 1.3. Sections 1.4, 1.5 and 1.6 cover in more detail the consequences of habitat fragmentation on plant population demographics, mating system, and population genetics; and the aims of this study and outline of the thesis are presented in Section 1.7.

1.1 Introduction to Australian Rainforests

As for many regions of the world, rainforests in Australia have undergone range expansion and contraction over geological history (Bush & Colinvaux 1990; Flenley 1979; Kershaw et al. 1991; Street 1981; Webb & Tracey 1981). During the early Miocene, rainforests are thought to have dominated most of the Australian continent with substantial contractions in range beginning in the mid-Miocene as a result of major local and global climatic changes (Rossetto et al. 2004). Range decline continued through the Plio-Pleistocene due to the onset of seasonality, general aridity (Trusswell 1990) and altered fire frequencies (Bowman 2000). During the late Pleistocene, rainforests are thought to have been restricted to topographically protected refugia before expanding again in the Holocene (Kershaw et al. 1991).

As a result of the range contractions during the Miocene and associated loss of species diversity, Australian rainforests are comparatively rich at the genus and family level but poor at the species level (Adam 1992; Hopkins et al. 1993). Patterns of genetic diversity across the geographic range of species also likely reflect the history of large-scale, natural habitat fragmentation and expansion events over geological time (Ellstrand & Elam 1993; Joseph et al. 1995).

In more recent times, anthropogenic disturbance in the form of logging, clearing and urbanisation has also led to extensive fragmentation of rainforest habitat in Australia, particularly lowland rainforests (Connelly & Specht 1988). Occurring at a rate far more rapid than the natural fragmentation events previously described, anthropogenic disturbance is a threatening process for broad-scale species diversity and a major concern today (Hobbs & Hopkins 1990). While rainforest presently represents less than one percent of Australia's total vegetation, it contains a considerable proportion of the continent's biodiversity, including over 550 plant genera (Webb & Tracey 1981).

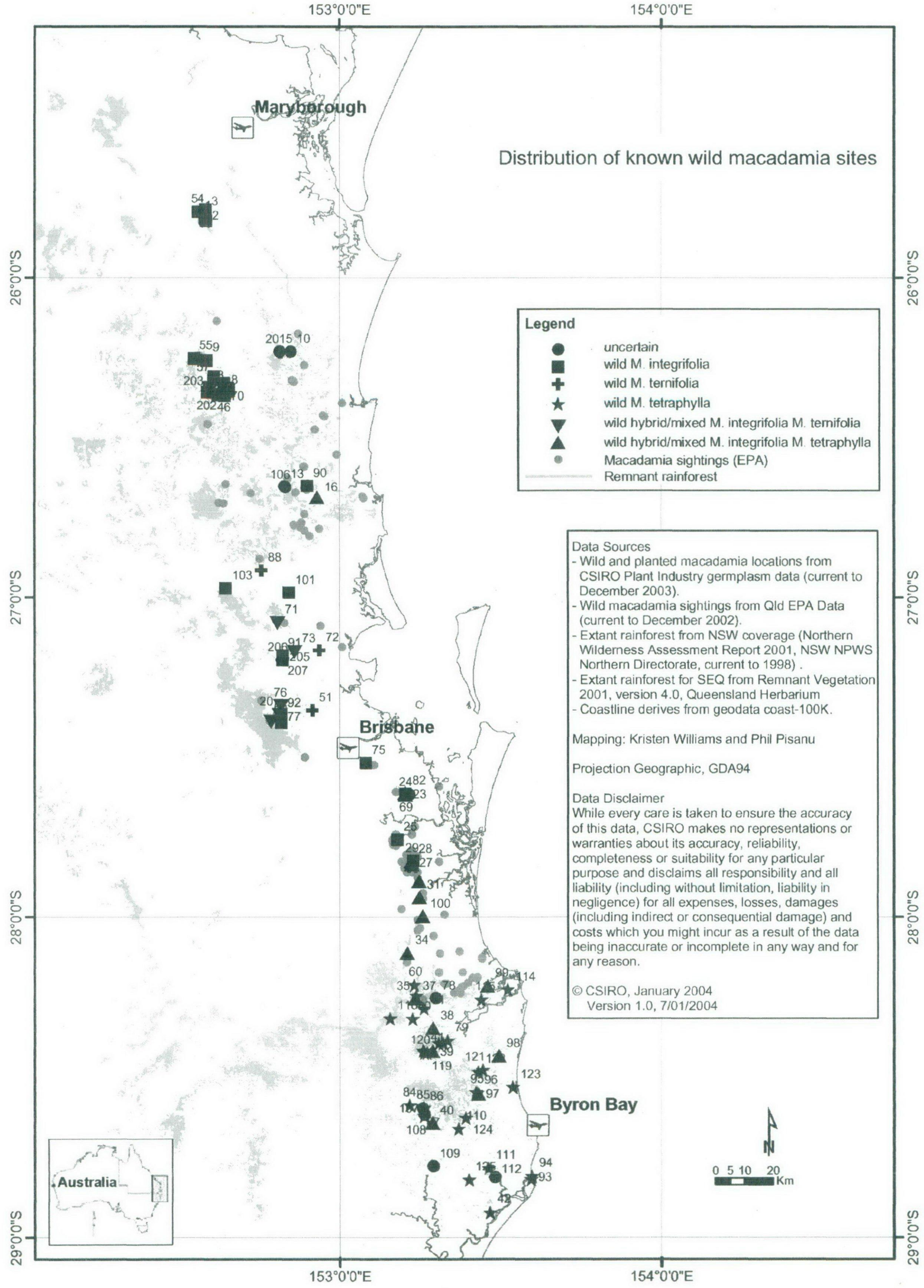
1.2 Background to M. integrifolia *Natural History*

The genus *Macadamia* is one of the few rainforest representatives of the Gondwanan family, Proteaceae (Gross 1996). Nine species are present in the genus, seven endemic to subtropical and tropical Eastern Australia (*M. claudiensis* C.L.Gross and B.Hyland, *M. whelanii* (Bailey), *M. grandis* C.L.Gross and B.Hyland, *M. tetraphylla* L.Johnson, *M. integrifolia* Maiden and E.Betche, *M. ternifolia* F.Muell and *M. jansenii* C.L.Gross and P.Weston), and two endemic to Sulawesi, Indonesia (*M. hildebrandii* Steenis and *M. erecta* J.A.McDonald and Ismail) (Gross 1996; Gross & Hyland 1993; Gross & Weston 1992; McDonald & Ismail 1995).

M. integrifolia (Figure 1.1) is endemic to notophyll vine forest, complex notophyll vine forest and Araucarian notophyll vine forest of subtropical South-East Queensland, Australia (Hardner 2004). Its distribution prior to anthropogenic disturbance is unknown, however at present, known populations are scattered from Mount Bauple in the north to Currumbin Valley near the Queensland/New South Wales border (Barry & Thomas 1994, Figure 1.2). It is found on high nutrient volcanic (mostly basalt and diorite) and alluvial soils of pH 5.5-6.5, that range in texture from clayey sand through various loams to silty clay (Hardner 2004). All sites are well drained, many with exposed rock fragments or substrate on the surface

(Hardner 2004). Annual rainfall is reasonably even across the range of the species and is presented in Table 2.1.

Figure 1.1 Left: *M integrifolia* juvenile on a creek bank in Amamoor State Forest, 2002 (site 128). Right: mature coppiced tree in the Samford Valley, 2003 (site 20).



Data Sources
- Wild and planted macadamia locations from CSIRO Plant Industry germplasm data (current to December 2003).
- Wild macadamia sightings from Qld EPA Data (current to December 2002).
- Extant rainforest from NSW coverage (Northern Wilderness Assessment Report 2001, NSW NPWS Northern Directorate, current to 1998).
- Extant rainforest for SEQ from Remnant Vegetation 2001, version 4.0, Queensland Herbarium
- Coastline derives from geodata coast-100K.

Mapping: Kristen Williams and Phil Pisanu

Projection Geographic, GDA94

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Figure 1.2 Distribution of verified macadamia records and unverified sightings from Queensland EPA databases in South-Eastern Queensland (Hardner 2004).

Six to 18 metres tall (Gross 1996), mature *M. integrifolia* trees frequently possess multi-stemmed trunks, potentially as a response to damage and/or stress. Adult leaves are laminate ovate to obovate in whorls of three, and 6.5-14 cm long and 2-6.5 cm wide (Gross 1996). The pale cream florets are bisexual and borne on racemes 8.5-25 cm long (Gross 1996) in numbers of 100 to 300. Similar to most Proteaceae, *M. integrifolia* exhibits a secondary pollen presentation system where pollen from the anthers is deposited directly onto a specialised subapical region (pollen presenter) of the style prior to flowers opening (e.g. Gross & Caddy 2006). During anthesis, elongation of the style causes the pollen-laden pollen presenter to burst out of the flower (Johnson & Briggs 1975). Self pollen must then be removed before incoming pollen can be deposited on the stigma (e.g. Gross & Caddy 2006). Fruit are globose follicles 3-4 cm long and 2-4.5 cm wide (Gross 1996; Strohschen 1986) that mature six months after fertilisation (Stephenson 2005). As the husk dries, it splits along a single suture to release the nut, which consists of a thick, light-tan seed coat enclosing the kernel (Stephenson 2005).

M. integrifolia is pollinated primarily by stingless native bees (*Trigona carbonaria*) and imported honey bees (*Apis melifera*) (Vithanage & Ironside 1986), and characterised by a partial gametophytic self-incompatibility system under orchard conditions (Heard 1993; Heard & Exley 1994; Sedgley et al. 1985). Little is known of the mating system of the species in the wild, however trees in orchards have been observed to rarely produce selfed seed (Ito & Hamilton 1969; Sedgely et al. 1990). Seeds are potentially dispersed by gravity and water, with some evidence of dispersal by native rodents (Pisanu 2001). The introduced black rat *Rattus rattus* is a common seed predator in macadamia orchards, however the role of this species in dispersal may be limited as caches of cultivated macadamia nuts were found to contain no undamaged nuts in a study by Elmouttie and Wilson (2005).

1.3 Background to Fragmentation

Habitat fragmentation has been defined by Li and Reynolds (1993) as "the processes of increasing the number of landscape pieces, decreasing interior habitat area, increasing the extent of forest opening edges, or increasing isolation of residual forest patches". Fragmentation threatens many plant species (Young et al. 1996), however in general, tropical trees are particularly susceptible due to their low densities, self-incompatibility systems, and typically high rates of outcrossing (Cascante et al. 2002). The widespread clearing of vegetation in the subtropics has resulted in extensive fragmentation of remnant forest (Catterall & Kingston 1993; Coiacetto 1995). Most remaining subtropical forests are less than ten hectares in size, separated by cleared or modified landscapes (Lott & Duggin 1993, Figure 1.3). Local extinctions typically occur at some time following fragmentation as habitat conditions become unsuitable for species, or small population size and/or isolation from other patches reduces fecundity or local fitness. This section discusses the physical consequences of habitat fragmentation, in particular the general implications of reduced patch size and increased isolation.

Figure 1.3 Fragmented *M. integrifolia* population (site 76, bottom centre) within a pasture matrix in the Samford region, 2002.

1.3.1 Habitat Area

A reduction in habitat area associated with fragmentation typically has implications for local species viability. Consequences often include reductions in population sizes as well as alterations in biotic and abiotic conditions within the patch due to edge effects.

1.3.1.1 Small Population Size

Through the fragmentation of previously continuous habitat into smaller, isolated patches, population sizes of plant species can be severely reduced. A major decrease in population size can impact on breeding structure, genetics and evolutionary dynamics of plant and animal species (Mustajarvi et al. 2001). Island biogeography theory predicts that random extinctions are likely to increase with decreasing habitat size due to an increasing proportion of species being represented by populations too small to sustain themselves. Species that maintain mutualistic relationships with other species, such as those between plants and their pollinators and seed dispersers, may be particularly at risk of extinction (Tewksbury et al. 2002), as the random loss of one species may have deleterious effects on the others (Aizen & Feinsinger 1994a; McKey 1989; Rathcke & Jules 1993; Tewksbury et al. 2002). Small populations are also more susceptible to loss of genetic diversity through genetic drift, which can lead to increased levels of inbreeding and reduced population fitness (Ellstrand & Elam 1993). Stochastic processes may have greater impact on the dynamics and genetic composition of small populations (e.g. Barrett & Kohn 1991; Ellstrand & Elam 1993; Schemske et al. 1994), while small populations of plants are likely to be less attractive to pollinators than large populations, and may suffer from insufficient pollen transfer and consequently lower seed set (e.g. Agren 1996; Fischer & Matthies 1998; Jennersten 1988; Lamont et al. 1993).

1.3.1.2 Edge Effects

The other major consequence of the reduction in habitat area through fragmentation is the increased exposure of organisms remaining in the patch to the environmental conditions of the surrounding matrix (Saunders et al. 1991). Edge effects appear to be a major cause of ecological change in plant communities (Laurance et al. 1998a), and are the result of an interaction between two adjacent ecosystems when the two are separated by an abrupt, artificial boundary (Laurance 2002; Murcia 1995). Initial consequences of edge effects are a change in the abiotic conditions within patches followed by changes in forest structure and dynamics (biotic effects) (Murcia 1995).

1.3.1.2.1 Abiotic Effects

Abiotic changes associated with edge effects typically involve modifications to incident light, soil and air humidity and temperature, wind and frequency of fires (Benítez-Malvido & Martinez-Ramos 2003). The matrix outside fragments is commonly characterised by low biomass and structural complexity, such as crops or pasture (Murcia 1995). These environments typically receive large amounts of radiant light at ground level during the day and much heat is lost from the ground at night, resulting in large diurnal temperature fluctuations (Murcia 1995). In comparison, forest microclimate at ground level is usually moister, cooler and more uniform, and where edges have been created between these two environments a gradient of temperature and moisture perpendicular to the edge is likely to be found (Murcia 1995). Conditions near edges can be altered by disturbances originating in the matrix, such as fire or the movement of chemical compounds, e.g. fertiliser or herbicides from crops (Murcia 1995). Strong turbulence created when wind strikes forest edges may also increase rates of wind throw and cause structural damage to trees (Ferreira & Laurance 1997; Laurance 1991).

1.3.1.2.2 Biotic Effects

Changes in the physical and chemical environment caused by edge effects may also directly affect forest structure and dynamics. Increased incident light near edges can promote plant growth (Williams-Linera 1990), and higher stem densities and basal areas have been observed within 20 m of edges in a variety of tropical- and temperate-zone forests (Murcia 1995). A range of responses in seedling density has been recorded in tropical ecosystems, with increases (Laurance et al. 1998b), decreases, and no change. Tree mortality has also been observed to increase near edges relative to patch interiors (Chen et al. 1992), and in Amazonian forests, elevated mortality was observed within 100 and 300 metres of edges due to wind turbulence and edge-related desiccation (Laurance et al. 2000; Laurance et al. 1998a). Such heightened mortality levels and damage near edges will tend to promote gap and building phases of forest dynamics, and can have important impacts on ecosystem processes such as carbon cycling (Laurance & Bierregaard 1997) with reduced tree biomass and an increase in the production of wood debris and litter adjacent to edges (Laurance 2002).

Distribution of plant species relative to distance from habitat edge may be altered due to differences in the physiological tolerances of species (Benítez-Malvido & Martinez-Ramos 2003; Murcia 1995). Some forest species are absent near edges or show lower densities, while others show higher densities, or no density change at all (Murcia 1995). The varied responses of species to abiotic conditions can also result in localised shifts in species composition (Dirzo & Miranda 1990; Matlack 1994; Scariot 1999). For instance, edges of fragmented tropical ecosystems often possess an abundance of vines, lianas and secondary vegetation (Laurance 1991), and some tropical forests may be susceptible to invasions from exotic or other generalist plant species (Benítez-Malvido & Martinez-Ramos 2003; Laurance & Bierregaard 1997). Cases have been found, however, where species composition has remained constant with distance to edge (Murcia 1995).

Finally, edge-related changes in forest environment and structure have the potential to alter species interactions and dynamics such as herbivory, predation and pollen and seed dispersal (Murcia 1995). Most studies to date have focussed on nest predation and brood parasitism in birds (Murcia 1995), however a study in an Oak-Hickory forest in Michigan found lower seed predation and higher herbivory near edges than in the interior (Sork 1983), and in a tropical rainforest in Queensland, animal-dispersed seeds from the matrix were moved up to 80m into the forest (Willson and Chrome, 1983).

1.3.1.2.3 Contributing Factors

The magnitude of edge effects is largely dependant on the size and shape of a patch, its orientation, physiognomy, and the surrounding matrix (Benítez-Malvido 1998; Laurance & Bierregaard 1997; Murcia 1995; Saunders et al. 1991). Areas immediately adjacent to edges are usually the most impacted; although effects can penetrate up to several hundred metres from the edge (Curran et al. 1999; Laurance 2000). Consequently, small, irregularly shaped remnants are often the most strongly affected due to their large edge to area ratio (Laurance et al. 1998a). Compass orientation of edges influences the amount of exposure to solar radiation, which in turn has a large role in determining the strength of some physical edge effects (Murcia 1995). Changes in physiognomy with edge age are also important in determining the magnitude of edge effects. Newly created edges tend to be structurally open and permeable to light, temperature and moisture fluxes (Kapos et al. 1997; Young & Mitchell 1994), at which time seedlings of pioneer, secondary, or exotic ruderal species will often establish (Benítez-Malvido 1998; Chen et al. 1992; Sizer &

Tanner 1999). As edges age, however, they usually become "sealed" by dense secondary growth (Didham & Lawton 1999), buffering the forest from matrix conditions (Benítez-Malvido & Martinez-Ramos 2003).

1.3.2 Isolation

As continuous habitat is fragmented, remnants on average also tend to become more spatially isolated from other similar remnants. The major implication of increased isolation between populations or subpopulations is often a reduction in inter-population gene flow and an increase in the severity of inbreeding (Severns 2003). Reduced gene flow can result in strong genetic differentiation between populations and loss of genetic diversity within populations through increased genetic drift. Lower rates of pollen flow may also negatively affect the quality and quantity of seeds in small, inbred populations if plant species possess self-incompatibility mechanisms, as does *M. integrifolia* (Cunningham 2000a).

1.4 Demographic Consequences of Fragmentation

Population dynamics in predominantly sexually-reproducing species such as *M. integrifolia* are dependent on levels of recruitment, mortality, immigration and emigration (Silvertown & Charlesworth 2001), all of which may be impacted by habitat fragmentation. The consequences of altered patterns of recruitment and mortality in fragmented habitats are frequently a shift in the demographic structure of a population, the number of individuals, and plant density (Mustajarvi et al. 2001). Changes to demographic patterns may ultimately alter susceptibility of fragmented populations to local extinction from stochastic events or population decline (e.g. Barrett & Kohn 1991; Ellstrand & Elam 1993; Lande 1988; Schemske et al. 1994). Population growth and demographic structure are consequently closely associated with population viability, and are discussed in more detail below.

1.4.1 Population Dynamics

Population growth and dynamics can be described using the equation

$$N_{t+1} = N_t + B - D + I - E$$

where N_t is the number of plants in a population at a set point in time, N_{t+1} is the number of plants at a later time, B is the number of births, D is the number of deaths, I is the number of immigrants into the population, and E is the number of emigrants (Silvertown & Charlesworth 2001). While immigration and emigration are important for gene flow and colonisation of new populations, the abundance and distribution of tree populations is determined primarily by seedling recruitment and mortality (Connell et al. 1984). Most emphasis in this thesis will therefore be placed on these two areas.

1.4.1.1 Recruitment

Local species extinction as a result of habitat fragmentation is common (Cardoso da Silva & Tabarelli 2000; Turner et al. 1995; Turner et al. 1994), and it is frequently hypothesised that breakdown in seed production and recruitment are major factors, leaving populations prone to random fluctuations in population size (Benítez-

Malvido & Martinez-Ramos 2003; Bond 1995; Scariot 1999; Tabarelli et al. 1999). Reproductive success of plants is reliant initially on the extent of flowering and fruiting within populations. Decreased flowering, potentially as a result of unsuitable abiotic conditions, has been observed in fragments compared with continuous forest (e.g. Aizen & Feinsinger 1994a; Nason & Hamrick 1997). There is also some evidence, however, that flowering of tree species in fragments can be positively affected, presumably due to an increase in available resources. In a study into the tropical dry forest tree *Pachira quinata* (Bombacaceae), Fuchs et al. (2003) observed that isolated trees produced significantly more flowers per individual than those from continuous populations. Similarly, Osunkoya (1999) found that *Gardenia actinocarpa* (Rubiaceae) plants produced more flowers in canopy gaps than under the forest understorey.

The amount of fruiting in populations is dependent on many factors, and empirical evidence demonstrates that habitat fragmentation can negatively affect plant reproduction by reducing pollen availability (extent of flowering), pollinator activity (Aizen & Feinsinger 1994b; Cunningham 2000a), pollen deposition (Aizen & Feinsinger 1994a; Cascante et al. 2002; Cunningham 2000a; Ghazoul et al. 1998) and pollen quality. Mutualisms between plants and their pollinators may be disrupted in small, isolated populations which may reduce plant reproductive success (Aizen & Feinsinger 1994b; McKey 1989; Olesen et al. 1994; Rathcke & Jules 1993). In addition, pollinator behaviour may change in small populations which may result in plants not being visited at all (Allee effects, Allee et al. 1949), or increased within-plant movements and geitonogamous selfing (De Jong et al. 1993). Self-incompatible plants not visited by pollinators are expected to not produce any seeds at all, while species with mixed mating systems will be forced to self-pollinate spontaneously, leading to low seed set and increased selfing rates (Oostermeijer et al. 2003). Stochastic events and genetic drift in small populations of self-incompatible plants may result in the loss of mating types or skewed relative frequencies of the different mating types (e.g. Byers & Meagher 1992; Heuch 1980; Les et al. 1991). Availability of compatible pollen may therefore become limited, most especially for plants of the most common mating type, and may reduce reproductive output of these plants significantly (Agren 1996). Resources available for seed production, i.e. light, nutrients and water (Zimmerman & Pyke 1988) may also have a large impact on plant reproductive success. Numerous studies have observed reduced seed set in rainforest tree species associated with forest fragmentation (e.g. Cascante et al. 2002; Ghazoul & McLeish 2001; Hall et al. 1996; Nason & Hamrick 1997), however several cases report no change or even increases (e.g. Boshier et al. 2004; Dick 2001). In a study into the related subtropical rainforest species *M. tetraphylla*, Pisanu (2001) observed that reproduction was higher and more consistent between years in highly disturbed sites compared with less disturbed populations.

While pollination and seed production are considered to be particularly important in buffering populations from local extinction (Bond 1995; Kearns et al. 1998; Rathcke & Jules 1993), the modification of several post-pollination processes can also impact on seedling abundance (Cunningham 2000b; Jules & Rathcke 1999). For instance, abiotic conditions near forest edges or in small fragments are often hotter and drier than in continuous forest (Benítez-Malvido 1998; Bruna 1999; Bruna & Kress 2002), and seeds may be produced and dispersed but fail to germinate if these conditions are unsuitable (Bruna 1999). Similarly, germination and seedling survival may also be compromised by increased levels of seed predation (Curran & Leighton 2000), or if decreases in population size have led to inbreeding depression (Menges 1991; Young et al. 1996). Seedling growth and survivorship in fragmented habitats can also be detrimentally affected by many factors including increased herbivory (Benítez-Malvido 2002), disturbance (Benítez-Malvido 1998), competition (Sizer & Tanner 1999), litterfall (Bruna 1999, 2002; Janzen 1983; Sizer et al. 2000), fungal infection (Benítez-Malvido et al. 1999), and damage from falling debris (Scariot 2001).

The effects of fragmentation on seedling germination and establishment are not uniformly detrimental, however, and increases (Dick 2002; Ferreira & Laurance 1997; Laurance et al. 1998b; Sizer & Tanner 1999), decreases (Benítez-Malvido 1998; Bruna 2002; Bruna & Kress 2002), and no observable changes (Harrington et al. 1997) have been reported. This variation in response to fragmentation has been thought to reflect spatial variability in microclimatic changes (Kapos et al. 1997) and litter production rates (Bruna 2002).

1.4.1.2 Mortality

Fragmentation-related mortality can be separated into two distinct areas: adult mortality and seedling mortality, each of which are likely to occur for differing reasons. Mortality of adult trees in tropical rainforest fragments is likely to be due to at least three factors: (i) stress caused by sudden changes in microclimate associated with edge creation (Lovejoy et al. 1986; Sizer et al. 2000); (ii) wind damage to trees, where winds accelerate over cleared land and strike fragment edges, creating increased turbulence and windshear (D'Angelo et al. 2004); and (iii) increase in liana abundance near edges, reducing tree growth and survival (Appanah & Putz

1984). Other factors, such as increased herbivory, disease or vulnerability to fire may also contribute to elevated mortality levels in fragments (D'Angelo et al. 2004).

All of the above factors are likely to be problematic during the first few years following fragmentation. During this time forest edges are structurally open, and permeable to fluxes of heat and light (D'Angelo et al. 2004). Trees exposed to forest edges may also be poorly adapted biomechanically for increases in wind velocity associated with fragmentation (Holbrook & Putz 1989; Putz et al. 1983). After several years, fragment edges tend to become sealed by lianas and secondary growth, reducing the intensity of edge-related microclimatic gradients (Camargo & Kapos 1995). Mortality near edges may also decrease as intolerant species die or become physiologically acclimatised to the changed conditions (Laurance et al. 2001). Mortality due to wind damage may increase at this stage and remain high, however, as wind-tunnel models indicate that downwind turbulence should increase as edge permeability is reduced (D'Angelo et al. 2004).

In contrast, seedling mortality in forests is likely to be caused by: (i) lack of resources; (ii) damage by predators or parasites; (iii) interference by other plants; (iv) failure to establish required symbiotic relationships; and (v) chance events (Nadolny 1999). The essential resources required by seedlings include water, light and mineral nutrients. While water may be limited in forest fragments, particularly towards the drier edges, light is unlikely to be limited under these circumstances, and the large seed size of *M. integrifolia*, with their substantial carbohydrate and mineral reserves, would make lack of nutrients unlikely during the seedlings' first two or three years (Nadolny 1999). Vertebrate browsers, invertebrate folivores, and fungal pathogens are likely to influence seedling mortality in forest fragments (Nadolny 1999). Interference may be caused by adult trees or other seedlings, however in fragments this is most likely to be the result of an increased abundance of weed species, especially near fragment edges. Trampling by introduced ungulates such as cows can also have major impact on the growth and survival of seedlings and understorey plants (Nadolny 1999, Figure 1.4).

Figure 1.4 Cows underneath tree 20-157 in site 20 (2002). Many fragmented *M. integrifolia* populations exist within a pasture matrix, and trampling by introduced ungulates has the potential to impact seedling mortality levels.

1.4.2 Demographic Structure

The demographic structure (or stage structure) of a population usually reflects previous variation in the population dynamic processes of recruitment, mortality, immigration and emigration (Harper 1977). In addition, because vital demographic rates in plants are usually stage-dependent (Harper 1977), a population's stage structure may also be indicative of future demographic dynamics (Bruna & Kress 2002) and as such can be an important tool in estimating future viability of fragmented populations in situations where long-term demographic monitoring is unfeasible.

1.5 Mating System Consequences of Fragmentation

The mating system of a population or species can be defined as the genetic relatedness and patterns of pairings between gametes (Ward et al. 2005), and is distinct from breeding systems, being the morphological and physiological characteristics of pairing (Neal & Anderson 2005). The low population densities typical of tropical rainforest trees (Hubbell & Foster 1983) originally led population ecologists to estimate that these species would be largely selfing or inbred, under the premise that pollinators would be unlikely to traverse the relatively long distances between conspecifics (Ward et al. 2005). As more empirical studies become available, however, this view has been completely revised, with many tropical tree species displaying high levels of dioecy, self-incompatibility in hermaphroditic and monoecious species (Bawa et al. 1985), high rates of outcrossing, and long-distance pollen dispersal (e.g. Boshier et al. 1995; Nason et al. 1998; Stacy et al. 1996).

Outcrossing rate and pollen dispersal distances are usually sensitive to ecological factors such as population density and pollinator abundance and composition, and may vary over spatial and temporal scales (Dick et al. 2003; Nason & Hamrick 1997). Landscape changes, such as those that occur with habitat fragmentation, may alter population size, demographic structure, plant density, phenology and pollinator abundance, diversity and behaviour (Aldrich & Hamrick 1998; Dick 2001; Lowe et al. 2005), and may therefore influence mating systems. Dispersal of pollen by insect pollinators typically takes place over a few hundred metres (Chase et al. 1996b; Nason et al. 1997), and may be reduced by fragmentation. In Amazonian forests, Powell and Powell (1987) observed that a 100 m-wide clearing proved an effective barrier to the movement of euglossine bees. In contrast, evidence of long-distance pollen flow has also been observed in fragmented terrain (Chase et al. 1996a; Nason & Hamrick 1997), and spatially isolated trees may be important for maintaining gene flow between populations (Levin 1995). Negative consequences of a shift in the mating system of plant populations typically involve either a rise in inbreeding in cases where pollen flow is limited, or an increase in outcrossing distances that may disturb locally-adapted genotypes (Young et al. 1996). These issues are described in more detail below.

1.5.1 Inbreeding

Increased inbreeding, i.e. selfing or mating between relatives, is often a major deleterious outcome of habitat fragmentation and degradation (Lowe et al. 2005). Fragmented populations typically experience a decline in the number of breeding individuals and a reduction in inter-population gene flow (Dudash & Fenster 2000). Matings between individuals in these populations are consequently more likely to represent selfing and/or biparental inbreeding, resulting in inbred offspring (Dudash & Fenster 2000).

The primary genetic consequence of an increase in inbreeding is the redistribution of individual allelic variation, reducing the proportion of heterozygotes in the population (Lowe et al. 2005). The subsequent increase in homozygosity may then lead to the increased expression of deleterious recessive alleles in inbred progeny, which may translate into a decline in fitness, i.e. inbreeding depression (Aizen & Feinsinger 1994a; Heschel & Paige 1995; Menges 1991; Ouborg & Van Teuren 1994). Over successive generations, continued inbreeding, combined with genetic drift and selection, may also result in allele fixation and reduced genetic diversity (Hall et al. 1996; Severns 2003; Young et al. 1996). Erosion of genetic diversity at loci controlling self-incompatibility may also reduce mate availability (Buza et al. 2000). Inbreeding depression is most likely to occur for species where normal levels of inbreeding are relatively low, and which have only recently experienced increased inbreeding (Husband & Schemske 1996). Populations and species that historically self or mate with close relatives may have purged some of their genetic load, substantially reducing the likelihood of inbreeding depression (Lienert et al. 2002). If inbreeding occurs for a sufficiently long period of time, recently fragmented populations experiencing inbreeding may theoretically adapt to the change in mating system through the purging of deleterious alleles, however empirical evidence for this is rare (Young et al. 1996).

Inbreeding depression may be exhibited through fitness components such as adult fecundity, germination, juvenile survival and growth/reproduction of the offspring (e.g. Charlesworth & Charlesworth 1987; Husband & Schemske 1996), pollen and ovule production (Dudash et al. 1997), physiological traits (Norman et al. 1995), and long-term survival (e.g. Jimenez et al. 1994). Comparisons of progeny fitness in plant populations (e.g. Dudash 1990; Hardner 1998; Sakai et al. 1989) suggest that the magnitude of inbreeding depression experienced is often great.

1.5.2 Outcrossing

In contrast to inbreeding depression, outbreeding depression is the phenomenon where outcrossed offspring possess lower relative fitness than their parents (Lynch & Walsh 1998; Waser 1978). Typically the result of crosses between distantly related individuals or different inbred lines (Severns 2003), initial outcrossed offspring may show signs of elevated fitness, or heterosis, however in the F2 and F3 generations, mean progeny fitness may decrease to levels below the parent populations (Lynch & Walsh 1998). This reduction in progeny fitness may be attributed to the disruption of locally adapted genotype-by-environment interactions through unfavourable epistatic gene interactions (Lynch & Walsh 1998; Waser & Price 1994). Outbreeding depression may occur in fragmented populations due to changes in pollinator behaviour as discussed above, or as potentially the case for *M. integrifolia*, due to the establishment of distantly-related plants in close proximity to natural populations, in the form of orchards or garden plants. This may have negative consequences not only for progeny fitness, but also may erode genetic population structure in fewer generations than inbreeding (Severns 2003), and has serious implications for long-term viability of fragmented populations.

1.6 Population Genetic Consequences of Fragmentation

The primary predicted genetic consequences of habitat fragmentation for plant populations centre on the implications of small population size and increased spatial isolation of fragments (Young et al. 1996). The main forces involved include genetic bottlenecks from the initial reduction in population size (and potentially from later stochastic events), and increased levels of genetic drift and inbreeding associated with both small population size and spatial isolation. These changes can result in the loss and continued erosion of genetic diversity, and increased genetic differentiation between populations (Young et al. 1996).

1.6.1 Genetic Diversity

The loss of genetic diversity (heterozygosity and allelic diversity) is potentially one of the most threatening consequences of habitat fragmentation (Bawa 1990; Charlesworth & Charlesworth 1987; Ellstrand & Elam 1993). Loss of genetic diversity in fragmented populations may reduce both short-term fitness through decreased heterozygosity, and long-term adaptability via the loss of alleles. It is also potentially associated with decreased colonising ability (Barrett & Kohn 1991), and increased susceptibility to pathogens and pests (Ouborg et al. 2000; Schmid 1994). Positive feedback between demographic and genetic factors may ultimately force small, isolated populations into an “extinction vortex” (Gilpin & Soule 1986).

Genetic diversity may be lost from fragmented plant populations in two ways: (i) immediate loss via an initial reduction in population size and inbreeding effects;

and (ii) genetic erosion over time. For populations where the initial fragmentation event decreases population size significantly, alleles may be lost due to sampling effects if they are not represented in the remnant trees. Genetic bottlenecks via habitat loss are likely to be amplified if fine-scale spatial genetic structure is present (Section 1.5.2), such as is the case for many rainforest tree species (Hubbell & Foster 1983), as remnant trees are more likely to share similar alleles than if genotypes were randomly distributed across the population. The severity of a genetic bottleneck is also dependant on the severity of habitat clearing, that is, the extent and duration, or the reduction in population size (Barrett & Kohn 1991). Rare alleles are likely to be lost first, and if populations remain small and isolated over many generations, more common alleles may also be lost (Lande 1988). Similarly, if populations remain small and isolated for multiple generations, erosion of genetic diversity may occur due to increased genetic drift. Increases in homozygosity as a result of inbreeding in small populations has also been observed in the majority of studies of neotropical tree species reviewed by Lowe et al. (2005).

1.6.2 Spatial Genetic Structure

In addition to the amount of genetic diversity, the spatial distribution of variation among individuals and populations is also an important characteristic of species. Spatial genetic structure, the non-random distribution of genotypes in space, may develop naturally in plant populations due to their sedentary nature and spatial dependency on gene flow (Vekemans & Hardy 2004). Structure of neutral genetic diversity may occur at a variety of spatial scales, and its strength and spatial magnitude determined primarily by gene flow, mating system, genetic drift and historical events such as vicariance and dispersal (Dutech et al. 2004; Epperson & Li 1996; Epperson & Li 1997). Similarly, the presence of spatial structure has the potential to feed back into these processes. Most studies into this phenomenon in a fragmentation context focus on two spatial scales: the distribution of variation within populations, and the partitioning of variation among populations.

1.6.2.1 Fine-Scale Spatial Structure

Within-population spatial genetic structure depends primarily on patterns and distance of pollen and seed dispersal (Luna et al. 2005). Local structure is predicted when gene flow is spatially dependent, and individuals are likely to show increasing relatedness as spatial distance between them decreases (Hardesty et al. 2005). Conversely, if gene flow is widespread within populations, genotypes are predicted to be randomly distributed. Spatial genetic structure is also predicted to be strongest for species with low densities of reproducing adults, as is the case for many rainforest trees (Hubbell & Foster 1983). Seed dispersal has a greater impact on spatial genetic structure than pollen flow, and may even overcome restricted patterns of pollen flow to neutralise structure if seed flow is widespread (Hamrick & Loveless 1986; Kalisz et al. 2001).

Due to its relationship with the gene flow and consequently mating system, the fine-scale spatial genetic structure of a population is susceptible to change from habitat fragmentation (Hamrick et al. 1992; Loveless & Hamrick 1984; Nason et al. 1997), which may in turn have implications for both population demographics and genetics. A reduction in reproductive tree density following fragmentation may strengthen genetic structure, potentially leading to increased levels of inbreeding and loss of genetic diversity.

1.6.2.2 Partitioning of Variation Among Populations

The partitioning of genetic diversity among populations of a species is often dependent on evolutionary factors such as historical events, as well as the demographic and population genetic processes of gene flow between populations and genetic drift (Young & Brown 1996). As such, the decrease in population size and increase in isolation frequently associated with habitat fragmentation have the potential to increase population differentiation as levels of gene flow between populations decline, and genetic drift and loss of alleles occurs (Slatkin 1987; Young et al. 1996).

1.7 Aims of this Study

As an iconic Australian species and an important resource for the Australian macadamia industry, the major aim of this study is to assess the vulnerability of *M. integrifolia* populations to local extinction and provide basic management recommendations, if necessary, to ensure future viability of the species. After consideration of important processes and empirical evidence from the conservation biology literature, questions based in three areas of investigation are proposed:

(1) What is the impact of fragmentation on patterns of flowering, fruiting, recruitment and mortality in wild *Macadamia integrifolia* populations?

(2) Has habitat fragmentation altered the mating system of *Macadamia integrifolia* populations?

(3) Have levels of genetic diversity and spatial genetic structure within and among wild *Macadamia* populations been impacted by habitat fragmentation, and how?

The thesis is presented in seven chapters. Chapter 2 details the site selection process and study site characteristics. Chapter 3 describes the molecular techniques used in this study and marker application and assessment, and Chapter 4 examines the impact of habitat fragmentation on the demographic structure and dynamics of wild *M. integrifolia* populations. Chapter 5 assesses the breeding and mating systems in wild *M. integrifolia* populations. Chapter 6 investigates the impact of habitat fragmentation on the genetic diversity and spatial genetic structure of wild *M. integrifolia* populations; and Chapter 7 provides a summary of important findings in the context of the above hypotheses, and recommends basic management strategies and future research directions for the species.

Chapter 2 Site Selection and Description of Study Sites

A series of study sites were selected for the array of experiments undertaken in this thesis. This chapter describes the process of site selection for this study and site characteristics.

2.1 Aims of Site Selection

The premise of site selection in this study was to specify a number of fragmented sites as 'treatment' sites, and larger, more intact sites as controls. Habitat area was selected as the major classifier of fragmentation severity, a reduction in area being one of the defining characteristics of habitat fragmentation and frequently associated with the detrimental effects of reduced population size and increased disturbance via edge effects. An increase in the isolation of populations is also a common attribute of fragmented sites, however many of the known *M. integrifolia* sites show evidence of clumping, often separated by only a few kilometres. Intermittent gene flow between these populations is feasible, and isolation was therefore incorporated into analyses as a secondary site characteristic only.

Ten study sites were chosen from two regions within the natural distribution of *M. integrifolia*, in order to incorporate some degree of geographic variation into statistical analyses. Extensive searches were performed in these regions to locate potential sites, and the short-listed sites selected to fall within three fragment area classes (referred to as site classes herein): small (less than one hectare), medium (1-

10 ha), and large (greater than 100 ha). Small and medium site classes were used as 'treatment' sites, with two scales of fragmentation severity. Sites greater than 10 ha are relatively rare, however several 'large' sites were known from conservation areas and were included in this investigation as controls.

2.2 Site Selection and Census

Three to four sites per area class were chosen from two regions within the natural distribution of *M. integrifolia*: Amamoor and Samford (Figure 2.1). Small and medium sites are represented in both locations, however sites larger than 10 ha are only known to occur in Amamoor and so all large study sites are from this region.

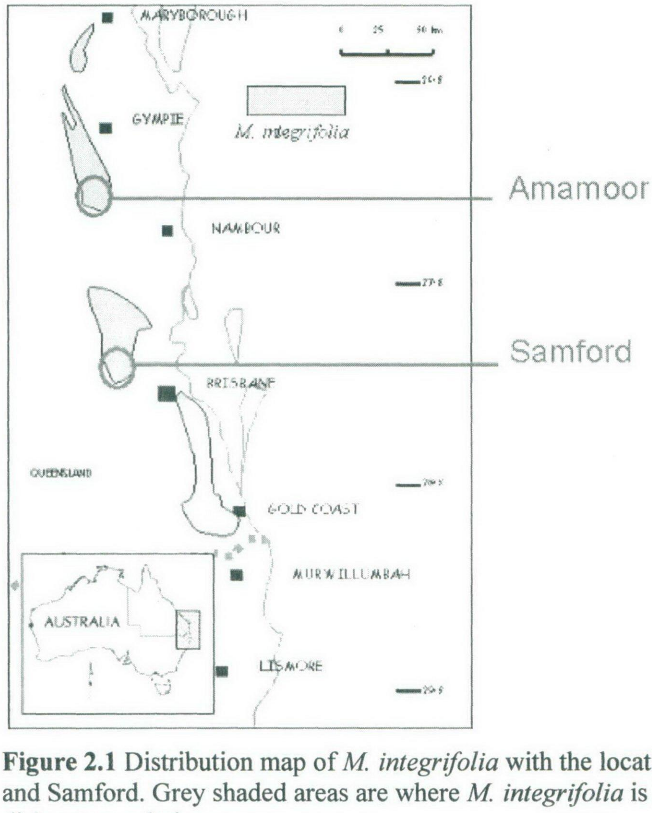

Figure 2.1 Distribution map of *M. integrifolia* with the locations of the two study regions: Amamoor and Samford. Grey shaded areas are where *M. integrifolia* is known to occur, typically in scattered, disjunct populations.

Site area was determined from aerial photographs and topographic maps. Adobe Photoshop version 6.0 was utilised to overlay a 10 ha block on the aerial photographs, and comparison between pixel numbers in the block and the study sites was used to calculate site area. Using this technique, three small, four medium, and three large sites were selected from the Amamoor and Samford regions (Figure 2.2).

Where possible, census work was undertaken in the entire area of small and medium sites. Only in site 8 was this not possible, due to the large number of individuals present and considerable time requirement necessary to locate, map and monitor all plants. One arm of the population, randomly selected, was therefore omitted from census, but was included in the estimation of site area. Searches of random sections within the large study sites suggested that the species is distributed more or less continuously throughout their area, with slight clumping observed. Sampling of large sites was therefore undertaken in two randomly located, four-hectare square plots per site. Four hectares was selected as this was roughly comparable in size to the medium sites, allowing more straight-forward comparison between sites for the statistical analyses undertaken in this study.

Once established, the sampled areas of each site were systematically searched and all *M. integrifolia* individuals allocated unique identifying numbers and labelled with aluminium tags. The number of new plants discovered decreased substantially in successive visits to each site, and so it is believed that the majority of individuals present were recorded. The locations of all individuals were mapped relative to a point for which the GPS location was determined using a Garmin eTrex. From this point, compass direction and horizontal distance to the closest tree were estimated, and in this manner all trees in each of the sites/plots were mapped. Distances between trees were measured using a Digital Hypsometer Forestor Vertex to an accuracy of 0.1 m, and multiple linkages were made between trees where possible to help reduce cumulative error. Based on this data, tree locations within each site were mapped by hand and recorded as Australian Map Grid Coordinates.

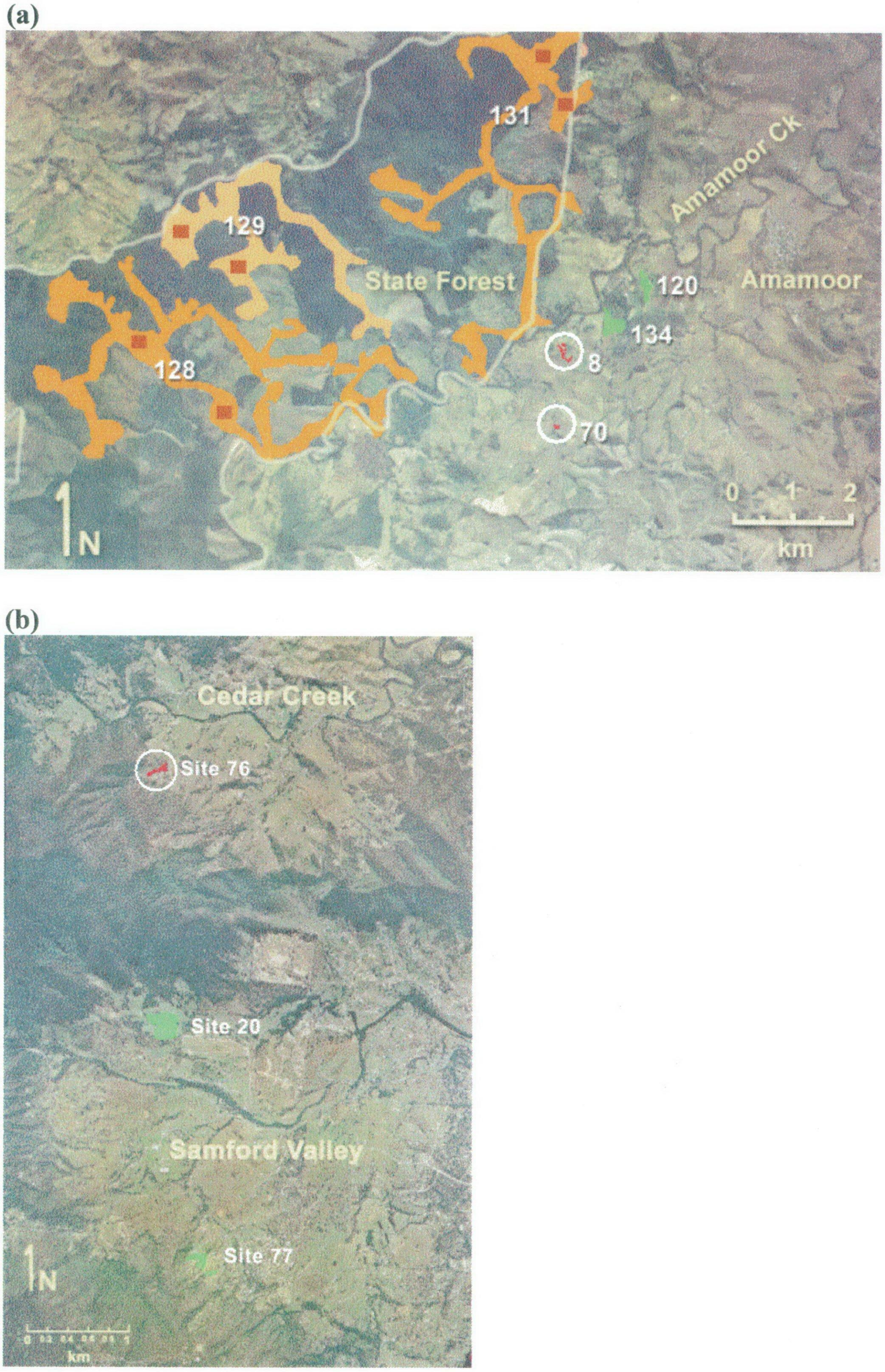

Figure 2.2 Aerial photographs of (a) the Amamoor region, and (b) Samford region, Queensland, showing the locations of study sites (source: Queensland Department of Natural Resources and Mines). ‘Small’ study sites are represented in red (and circled in white), ‘medium’ sites are light green, and the three ‘large’ sites are shown in orange. Brown squares represent the size and location of six 4 ha quadrats within the large sites (two quadrats per site).

2.3 Site Characteristics

Characteristics of the study sites are presented in Table 2.1. Patch area varied from 0.24 ha to 361 ha, and isolation (distance to the closest known habitat also containing *M. integrifolia*) was found to vary between 150m and 3000m. The total number of individuals within sites ranged from 94 to approximately 10500 across the study populations. The majority of small and medium sites were located within a pasture matrix, with one immediately adjacent to a macadamia orchard (site 120). All large sites were surrounded by a natural sclerophyll forest, and on some borders, *Araucaria cunninghamii* (Araucariaceae) plantations.

Site history was ascertained as far as was possible from aerial photographic records (source: Queensland Department of Natural Resources and Mines). Records for the three Samford sites (20, 76 and 77) only extend as far as 1955, at which time sites 20 and 77 were still fragmented but slightly larger than their current size. Site 76 was similar in size and degree of fragmentation as today. Photographic records of the Amamoor region date back to 1940, and no substantial changes in the area or connectivity of sites 70, 120 or 134 has occurred since this time. Site 8, however, was completely cleared prior to 1940, and the site as it currently exists is regrowth from sometime between 1940 and 1958. Aerial photographs of the Amamoor State Forest suggest that the distribution of rainforest, and therefore the large sites, has changed little since 1940.

Table 2.1 Study site characteristics. Total number of individuals has been extrapolated from the number of individuals in the sampled area of each site. Distance to closest patch is the distance to the nearest known habitat containing *M. integrifolia*.

Site area class	*Site No.*	*Location*	*Patch area (ha)*	*Sampled area (ha)*	*Estimated total no. of individuals in site*	*Distance to closest patch (m)*	*Disturbance level*	*Matrix type*	*Aspect*	*Slope (%)*	*Annual rainfall (mm)*	*Soil type*
Small (<0.5 ha)	8	Amamoor	0.49	0.36	153	150	high	Pasture	N/NE	0-5	1100-1150	red podzolics
	70	Amamoor	0.24	0.24	135	600	high	Pasture	SW	0-5	1150-1200	yellow podzolics
	76	Samford	0.45	0.45	96	3000	high	Pasture	NE	11-24	1100-1150	red-yellow podzolics
Medium (1-10 ha)	20	Samford	6.57	6.57	176	2500	high	Pasture	E	0-5	1100-1150	red-yellow podzolics
	77	Samford	2.63	2.63	115	2500	high	Pasture	NE	11-24	1100-1150	red-yellow podzolics
	120	Amamoor	1.19	1.19	94	2400	high	Orchard	N	6-10	1150-1200	alluvial
	134	Amamoor	1.73	1.73	249	150	medium	Pasture	W	0-5	1100-1150	red podzolics
Large (>100 ha)	128	Amamoor	361.41	2 x 4ha plots	10526	250	low	Forestry	SE	11-24	1100-1300	red podzolics
	129	Amamoor	193.18	2 x 4ha plots	5677	300	low	Forestry	SE	11-24	1150-1300	red podzolics
	131	Amamoor	251.87	2 x 4ha plots	8941	300	low	Forestry	SW	0-24	1150-1300	red podzolics

Before the major analyses of the impacts of site area on population viability could be investigated, sampling areas within sites were examined to determine the comparability of the number of individuals within sites/plots. Within site classes the total number of individuals in the sampled regions of sites varied significantly for small and medium classes [*G*=6.64, P<0.05; *G*=89.68, P<0.005, with Williams (1976) correction factor] but not for large [*G*=3.26, P>0.15 with Williams (1976) correction factor] (Figure 2.3).

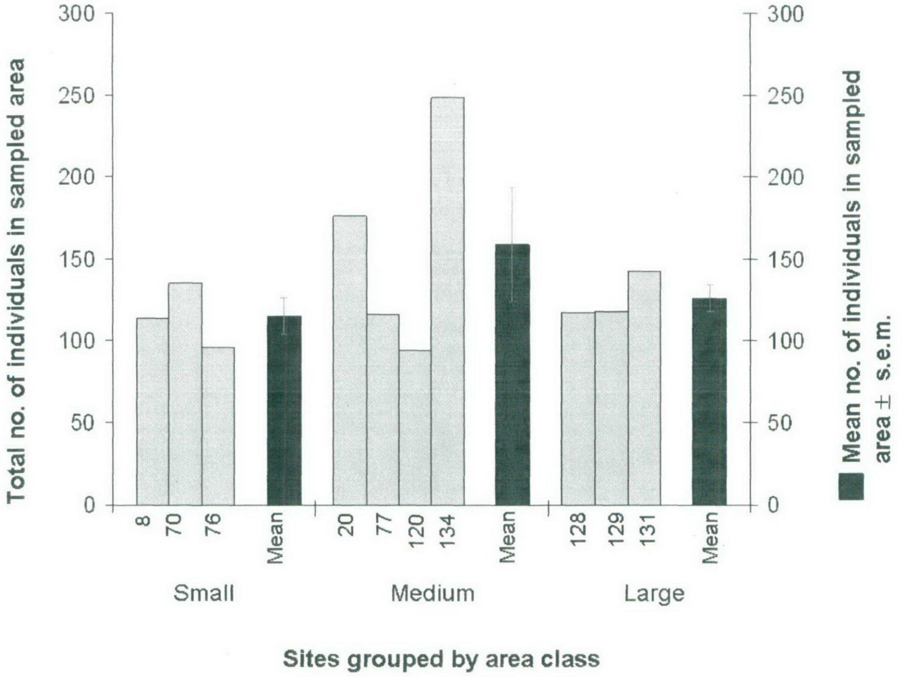

Figure 2.3 Number of individuals in the sampled region of each site, grouped by site class (small = <0.5 ha, medium = 1-10 ha, large = >100 ha). Site numbers are shown on the x-axis, and the sampled areas for each site are given in Table 2.1. Black bars represent the mean number of individuals (± s.e.m.) for each site class.