No sex, will travel? The association between invasiveness and mating systems in introduced Iridaceae to Australia

Submitted by
Wun Thye Ho
B. Sc. Cons. Bio. Ecol. (Hons)
La Trobe University

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Department of Ecology, Environment and Evolution
School of Life Sciences
College of Science, Health and Engineering

La Trobe University
Victoria, Australia

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Abstract

Invasive species have become a global problem, by threatening native biota, ecosystem services and human health. Since its inception more than half a century ago, the field of invasion biology has made great strides in the understanding of invasion dynamics. As a demographic and geographic phenomena, biological invasion events are underpinned by reproduction, as population spread is contingent on population persistence and dispersal. While the role of reproduction has been recognised early on, how reproductive modes can change during invasion has received less attention.

In this study, the association between invasiveness and mating system was explored using five Iridaceae taxa with specialised pollination systems and different levels of self-incompatibility that were introduced to Australia after colonial settlement. Anecdotal evidence pointed to the absence of seed production in some introduced species, which raised the question of invasive spread with the loss of propagule assurance. It was also hypothesised that invasiveness would be related to mating strategy, as asexual species would lack genetic variability to adapt to novel environments, thus constraining invasive spread.

Response to the loss of co-evolved pollinators was species-specific. While there was recourse to aseuxal reproduction in the Iridaceae, one species co-opted native and introduced pollinators to maintain outcrossing in Australia. Other species circumvented the loss of sex by vegetative fragmentation via cormels and aerial cormels, or through self-fertility. Although regrowth in cormels was low compared to seeds, their abundant production assured local spread. Of the five taxa, three were found to be predominantly asexual, one predominantly sexual and the last one facultatively autogamous.

Molecular data, while inconclusive, corroborated findings from mating system studies, where asexual and selfing species were found to be fairly genomically homogeneous. Intriguingly, the data also suggested polyploidy in two Iridaceae species, which has never been recorded for these species. Polyploidy was also suspected in an accession from South Africa, suggesting that polyploidisation may be occurring in the native range, although the evidence is tenuous.

Species distribution modelling and ecological niche modelling again demonstrated niche shifting in all focal taxa. Contrary to expectations, there was

no support for the association between invasiveness, as measured by niche occupancy, and mating strategy. Both niche conservation and divergence were inferred in two asexual species, while a high degree of niche shifting (but inconclusive niche divergence) was detected in the selfing species. Finally, niche conservation was inconclusive for the sexual taxon.

In general, mating system was found not to predict invasiveness. It was hypothesised that stochastic factors, such as human dispersal, might continue to mediate the spread of invasive species. It is therefore recommended that weed assessment protocols incorporate modelling approaches and native-range information that may reveal cryptic niche evolution in introduced species.

Chapter 1

General introduction

1.1 The role of reproduction in biological invasions

Invasion biology is the study of contemporary relocation of species around the world, and of their impacts upon indigenous biota and environment. Although the natural movement of species has long been an ongoing process, the degree and rate of redistribution of biota by humans has no parallel in geological time (Ricciardi, 2007). However, the majority of introduced biota fail to thrive and persist in novel environments (Williamson and Fitter, 1996; Zenni and Nuñez, 2013), it is widely acknowledged that successful colonisers more than not have a pernicious effect on indigenous species, and consequently on ecosystems and human health (Fritts and Rodda, 1998; Pejchar and Mooney, 2009). As such, timely management of these species and their effects is important (Leung et al., 2002).

Once an introduced species has become abundant, it is often prohibitively costly to eradicate (Rejmanek and Pitcairn, 2002; Leung et al., 2002). Therefore, it makes more economic sense to identify taxa capable of becoming invasive, and to prevent these taxa from making landfall in the first place. Such an aspiration is enshrined in risk assessment protocols (e.g. Pheloung et al., 1999). However, the number of translocated species shows no sign of abating (Seebens et al., 2017), and scant resources need to be optimised for managing these species (Bogich et al., 2008). One way of doing this is to base research and management needs on the magnitude of invasion to understand what makes a species invasive or not. Various schemas exist that differ nominally and structurally, but most visualise an invasion event as a series of stages starting from transportation to a foreign region, and ending in a full-fledge colonisation of that region (Williamson, 2006).

One such schema that has found wide reception is the model by Blackburn et al. (2011). Here, an invasion process is described as four stages along a con-

tinuum: transport, introduction, establishment, and spread. Different barriers act on each stage. If an introduced population succumbs to any barrier thus far, the invasion process fails. For example, an introduced population comprising a sole individual may be unable to reproduce if it lacks mating partners. If a population is able to reproduce in the adventive region, it is considered *naturalised* (Richardson et al., 2000b). Moreover, if the population manages to spread and become widespread, it is defined as *invasive* (Colautti and MacIsaac, 2004), although the criteria differ among authors (Falk-Petersen et al., 2006). Here, the circumscription by Blackburn et al. (2011) is adopted: a fully invasive species is one where its individuals can survive, reproduce and disperse from multiple sites across habitat types in an adventive range.

It is also useful to consider biological traits, that is, the suite of attributes that enable an organism to survive and flourish in a particular environment (Pyšek and Richardson, 2007). Plant traits have been used to explain ecological phenomena from vegetation patterns (Venn et al., 2011) to ecosystem functioning (Quétier et al., 2007). In invasion biology, a key goal is the prediction of species invasiveness (Kolar and Lodge, 2001). This typically involves the identification of traits associated with invasiveness (Rejmanek and Richardson, 1996; Pyšek and Richardson, 2007), for example via comparison between invasive and native, or invasive and non-invasive taxa (van Kleunen et al., 2010a).

While many attributes can be linked to performance from individuals to ecosystems, traits should measure fitness in terms of growth, reproduction, and survival at an individual level (Violle et al., 2007). Reproductive traits are important, as they dictate how genetic information is passed from one generation to the next, how genetic content is structured among populations, and the quality and quantity of offspring, which in turn drive capacities for dispersal and adaptation (Barrett, 2011; Bock et al., 2015).

Different traits have been associated with different stages along the invasion continuum (Richardson et al., 2000b; Dietz and Edwards, 2006; Williamson, 2006; Datta et al., 2017). Traits as predictors need to scale across space, time, and ecological organization (Drenovsky et al., 2012). While naturalisation is by definition a local process, an invasion proper takes place over a larger geographical extent, and very likely over different abiotic and biotic values, where distinctive traits may govern population dynamics (van Kleunen et al., 2015). For example, Baker (1955) theorised that self-compatible species were predisposed to long-distance colonisation, as they were not reliant on mates (that might not have been dispersed to the same location) for reproduction (Baker's rule: Stebbins, 1957). However, the importance of self-compatibility along the invasion continuum is unknown (Rambuda and Johnson, 2004). Given that plant reproductive systems are changeable over both deep evolutionary (Barrett, 2013) and

shallow evolutionary time frames (Bodbyl Roels and Kelly, 2011), it is surprising that few have explored how reproductive diversity has propelled naturalised populations to invasive ones (Barrett et al., 2008; Moravcová et al., 2015).

1.2 Sexual reproduction: forms and consequences

Plants—terrestrial and aquatic—boast myriad ways of reproduction (Richards, 1997). A fundamental distinction of reproductive modes is that between asexual and sexual reproduction; however, there is no agreement on the classification criteria (for example, self-fertilisation as a form of uniparental reproduction can be considered asexual, or sexual as sex cells undergo syngamy) (Fusco and Minelli, 2019, p. 13). As this work is concerned with the evolutionary consequences of reproductive strategy in introduced plants, a genetic definition is adopted, rather than an embryological one (*sensu* Archetti, 2010). Here, sexual reproduction is defined to be the process whereby new individuals are produced from pre-existing individuals, and which necessarily involves a two-division meiosis, recombination and syngamy. Conversely, asexuality is defined as a form of eukaryotic reproduction that does not contain all the elements comprising sexual reproduction (Bengtsson, 2009; Stelzer, 2015).

The evolution of sexual reproduction is considered an enigma, as it raises a 'twofold cost of sex' paradox in anisogamous species (Fusco and Minelli, 2019, p. 107): sexual females can only have half the number of second-generation descendants compared to asexual females, who do not waste resources bearing males. In addition, the processes of recombination and syngamy during sex conjure new genotypes that may result in lower fitness in the offspring. Then, *Why sex?*

Forty years after the publication of Charles Darwin's theory of natural selection, Weismann (1889) explained that sexual reproduction generates variation in a population, such that one fit individual can compensate for other less well-adapted ones. In other words, sex engenders additive genetic variation on which directional selection can act (Burt, 2000). This has been borne out empirically. Working with facultatively outcrossing rotifers, Becks and Agrawal (2012) found an increased rate of sexual reproduction in new environments, with progenies having an average level of fitness below those of asexual rotifers, but some sexual offspring performed well above the latter. In another experiment, Lachapelle and Bell (2012) introduced the green alga *Chlamydomonas reinhardtii* from a freshwater to a saline environment, and found that lineages with high genetic diversity and obligate sex were better poised to avoid extinction compared to asexual lineages.

Sex can also mediate response to biotic pressures. The Red Queen hypothesis—

first proposed by Van Valen (1973)—posits that organisms need to constantly adapt and evolve to avoid being out-competed by other organisms (Liow et al., 2011). Evolutionary rescue by sexual reproduction has been observed in host–pathogen systems (Morran et al., 2011; Lynch et al., 2018) and plant–herbivore interactions (Strauss and Karban, 1994; Johnson et al., 2009). Another benefit of sexual reproduction is that recombination and random assortment expose singular genetic mutations, allowing natural selection to sift beneficial from deleterious mutations, and accelerate adaptation compared to asexual lineages (Goddard, 2016; McDonald et al., 2016).

In non-indigenous and isolated populations, the role of sex in pushing the naturalization–invasion transition is not so clear. Mathematical models suggest that sexual reproduction yields higher population mean fitness in gradually varying environments, but not in randomly varying ones (Charlesworth, 1993a,b; Lande and Shannon, 1996). This presents a potential problem to colonising populations: on introduction, organisms face environments that are different from those in their source ranges (Maron et al., 2004), or may fail to disperse (Mateo et al., 2015), thereby hampering population spread. For example, in a series of *in vitro* simulations, Gray and Goddard (2012) found that sexual reproduction facilitated adaptation in yeast subjected to harsh environments, but had no effect under benign conditions, in agreement with Goddard et al. (2005). By contrast, the Arctic asexual–sexual herb *Bistorta vivipara* produced more flowers across an edaphic gradient with greater nitrogen and organic content, resulting in higher sexual output in quality habitat (Bills et al., 2015). Modelling demonstrated that the rate of adaptation to environmental changes increased with recombination rate, with small populations being more vulnerable to extinction, even when environmental change is slow (Bürger and Lynch, 1995; Neher et al., 2010). While high local abundance often defines invasiveness (Catford et al., 2016), genetic variability can be drastically reduced by founder events and bottlenecks during range expansion (Grapputo et al., 2005). The partitioning of genetic variability among populations can result in a deficit in observed heterozygosity (Wahund effect: Wahlund, 1928, cited in Dharmarajan et al., 2013).

Thus, a large population size can be beneficial for an incipient population, particularly for accidental introductions. Here, self-fertilisation bypasses an Allee effect due to mate limitation, thus providing reproductive assurance (i.e., Baker's rule). In a global study, Razanajatovo et al. (2016) showed that naturalised autogamous and self compatible plants occupied larger ranges in their adventive range, mirroring the result by Grossenbacher et al. (2015) for indigenous forms. Similarly, van Kleunen et al. (2008) suggested that self-compatible species were more likely to become naturalised or invasive in Iridaceae. In China, invasiveness in Asteraceae was linked to self compatibility (Hao et al., 2011).

The latter result was surprising in two ways. The assessment of invasive species goes beyond Baker's original circumscription of incipient colonization. However, Baker's rule may still apply if subsequent spread occurs by 'jump dispersal' (Wilson et al., 2009), such that edge populations are isolated by long-distance dispersal events and suffer mate limitation as a result (Pannell and Barrett, 1998).

The transition from outcrossing to selfing (e.g. following colonisation) at once increases the frequency of genotypes that are homozygous for rare deleterious alleles, resulting in inbreeding depression (Charlesworth and Charlesworth, 1987). Mathematical modelling shows that selfing reduces the effective population size through background selection, which in turns lowers the adaptive potential of a population (Glémin and Ronfort, 2013). Partial selfing also exposes a population to selective interference, or the capacity to fix multiple beneficial alleles simultaneously, again limiting adaptation (Hartfield and Glémin, 2016) and therefore potential for invasion.

The short-term sacrificial gain in demography at the expense of long-term evolutionary pliability prompted Stebbins (1957) to label the transition to self-fertilisation an evolutionary "dead end". Once the path to selfing is committed, it is very likely to be irreversible (Takebayashi and Morrell, 2001; Igic et al., 2006), and environmental upheaval can drive the species to extinction. On the other hand, selfing ensures a higher fidelity in gene transmission across generations, and more efficient purging of deleterious alleles (Glémin, 2007), and fixation of beneficial ones ('Haldene's Sieve': Hartfield, 2016).

Currently, theoretical and empirical questions remain on the evolutionary consequences of pure and partial selfing (Glémin and Galtier, 2012; Wright et al., 2013; Hartfield, 2016). In a meta-analysis on angiosperms, Clo et al. (2019) reviewed quantitative trait heritability in 68 studies, and found that additive genetic variation decreased with selfing rate; however, the loss of additive genetic variation could be compensated by non-additive components of genetic variance, which could become selectable under inbreeding. While Lande (1977) showed by mathematical modelling that the purging of deleterious alleles by selection could offset the gain in homozygosity, Noël et al. (2017) found empirical evidence that the purging of inbreeding depression failed to improve response to selection. Given the disadvantages of selfing, it is difficult to predict the long-term demographic trajectory of invasive plant populations over increasingly variable habitats. It may boil down to their capacity to consistently reproduce and disperse despite low population density and pollen limitation that they can fend off extinction (Randle et al., 2009). Naturally, evolutionary rescue in genetically-depauperate selfing populations can occur through episodic sex (Maron et al., 2018).

With the exception of autonomous self-fertilisation, sexual reproduction in plants requires a pollen vector to accurately transfer pollen, and pollination modes are rich and varied (Faegri and Van Der Pijl, 1979). Pollination can occur abiotically (for example, via wind or water) or biotically (for example, via insects or birds). After long-distance dispersal, an outbreeding plant population may also face pollen limitation when its pollinators are absent in the new range (Larson et al., 2002), or when an Allee effect arises from low population density in colonising populations (Davis et al., 2004). As the reproductive success of introduced plants depends on their breeding system and pollination ecology, both aspects of reproduction need to be considered to understand and to predict invasions (Harmon-Threatt et al., 2009).

1.3 Asexual reproduction: forms and consequences

If one may ask, *Why sex?*, then it makes sense to query the converse. Asexual reproduction does not entail the costs associated with sex: gene transmission across generations is 100%, and individuals do not need to incur risk and resources seeking mates (Stelzer, 2015). Despite these advantages, sexual reproduction is the dominant breeding system among eukaryotes: about 0.1% of animals are obligate asexuals (Vrijenhoek, 1998), and a similar paucity of such practitioners may hold across other taxa (Bell, 1982; Bengtsson, 2009, but see Tibayrenc et al., 2015). However, clonality is prevalent in plants: over 70% of all plant species practise a form of clonality (Klimeš et al., 1997).

There are two broad classes of *ex*-sexual reproduction: gametic and agametic, with and without the formation of gametes (Archetti, 2010). While vegetative propagation—the formation of new individuals by mitosis (e.g. budding)—was previously considered a form of asexuality based on embryology (Nogler, 1984; Asker and Jerling, 1992), within a genetic-based framework it is now relegated to an auxiliary mode of reproduction, along with sporophytic apomixis (the formation of an embryo from a somatic cell of the ovule) and apospory (the formation of an unreduced embryo sac from a somatic cell of the ovule) (Archetti, 2010).

Depending on the levels of recombination and fusion, asexual individuals are expected to show lower levels of linkage disequilibrium compared to outcrossed individuals, as they are more likely to inherit longer contiguous stretches of a haplotype. Under complete clonality, selection effectively acts on the entire genome, thereby reducing the effective population size (Glémin and Galtier, 2012). In addition, asexuality results in the loss of the capacity to mask recessive deleterious mutations (loss of complementation: Archetti, 2004). Previous studies on the biogeography and population dynamics of asexual taxa (e.g. Ho-

jsgaard et al., 2014; Dellinger et al., 2016) may need to be refined in light of the potentially variable levels of recombination and fusion that may affect the amount of additive genetic variability in a population, as a genetic approach is more relevant here.

Agametic propagation can be broadly classified into two forms: clonal integration and vegetative fragmentation. Clonal integration is a form of propagation or growth where physiological connections exist between a mother plant and its daughters. These lend population and genetic structure, such that the *genet* (the sum of all individuals from a single zygote: Harper and White, 1974) is clumped, and more genetic variability is found among populations than within a population (Hamrick and Godt, 1990). As long as the physical links to the daughter plants are not severed, a mother plant is able to mediate their competitiveness in unfavourable habitats (Alpert and Stuefer, 1997; Herben, 2004) and to modulate competition among them (Herben and Novoplansky, 2010).

Greenhouse studies (Yu et al., 2009; Wang et al., 2017) and a meta-analysis (Song et al., 2013a) associated clonal integration with invasiveness, but the authors did not explain how an introduced clonal population might eventually attain abundance or occupancy over a wider geographic extent. In a literature review, Widén et al. (1994) uncovered a range of clonal sizes from 10 m to 1 km (in the fern *Pteridium aquilinum*), which suggested a local limit to clonal spread. However, a notable exception to limited clonal size is a single extant clone of quaking aspen (*Populus tremuloides*) that covers 44 ha in Utah via root suckering (DeWoody et al., 2008), with the oldest ramets estimated to be around 10000 years (Ally et al., 2008). However, clone size does not generally scale with clone age (Ally et al., 2008); some ancient clones have been found to spread 1–15 km (Lynch et al., 1998; McAuliffe et al., 2007; Arnaud-Haond et al., 2012). Under shallow evolutionary time frames, it is likely that facultatively clonal plants employ seeds from sexual reproduction to achieve long-distance propagation, for example in *Phragmites australis* (Kirk et al., 2011; Albert et al., 2015) and in *Spartina alterniflora* (Xue et al., 2018). Alternatively, long-distance spread can arise from dispersal of vegetative propagules (Zhang et al., 2010).

Vegetative fragmentation—the abscission of a somatic plant part to form a new individual (ramet)—is prevalent in aquatic plants, as the removal of water stress allows most of their vegetative parts to become propagules, and the alleviation of mechanical support meant that tissues are fragile and easily broken by media or by herbivores (Li, 2014), and contributes greatly to aquatic invasion success (Barrett, 2015; Eckert et al., 2016). In terrestrial plants, greenhouse experiments have shown that invasiveness could be explained by vegetative fragmentation (Dietz et al., 2002; Weber, 2011; Li et al., 2013); however, in a study of 39 stoloniferous species, Song et al. (2013b) showed that a capacity for veg-

etative fragmentation was not associated with invasiveness, perhaps due to the their bulk and lack of dispersive structures (Eckert, 2002). By contrast, Roiloa et al. (2017) showed in a field experiment that colonisation extent was positively correlated to stolon length in *Carpobrotus edulis*, as larger fragments promoted greater above-ground growth and competitiveness. Furthermore, dispersal inertia in vegetative fragments can be ameliorated by agents such as water (Truscott et al., 2006) and humans (Bentley and Mauricio, 2016).

However, some forms of vegetation fragmentation do produce apomictic-like disseminules, for example, bulbils and cormels; however, there are few studies on spatial and demographic population structure for these modes. In *Dioscorea japonica*, which produces seeds and bulbils, Mizuki et al. (2010) found an attenuated spatial genetic structure, with ramets clumping around maternal ramets; seed and bulbil dispersal distances were similar (*c.* 10 m). Bulbil dispersibility also approached that of seed in *Allium vinale* (Ronsheim, 1994); however, the introduced yam *Dioscorea oppositifolia* achieves invasive spread by casting its bulbils in water, but which restricts colonisation to riparian habitats (Thomas et al., 2005). In its adventive range, the dominant cytotype of the heterostylous geophyte *Oxalis pes-caprae* was the sterile pentaploid, which precluded sexual reproduction and compelled regeneration by bulbils (Ornduff, 1987; Rottenberg and Parker, 2004), with spread primarily restricted to human-disturbed habitats (Ross et al., 2008). More recently, sexual reproduction due to a partial breakdown in the morph-incompatibility system was uncovered among rarer tetraploid populations of *O. pes-caprae* in the western Mediterranean Basin, resulting in seed set and viable offspring (Costa et al., 2014). As the recruitment rate from seed in its adventive populations was not known (Costa et al., 2017), the contribution of sexual reproduction to morph and genetic diversity in natural populations, and in particular to the genetic variability of the pentaploid cytotype remained unclear (Ferrero et al., 2015, 2020). Clearly, there is at present a gap on how vegetative seed-like disseminules invite colonisation and invasion of non-riparian, terrestrial habitats, and how genetic variability is associated with the spatial and environmental extent of colonisation.

1.4 Comparative approaches to invasion biology

Comparative methods are often used to draw out elements of invasiveness or community invasibility. Comparisons can be made at an organismal level (for example, indigenous vs. non-indigenous, and between levels of invasiveness) or at a geographical level (for example, between native and introduced ranges). Tests of invasiveness are best performed between invasive and non-invasive taxa in their introduced range (van Kleunen et al., 2010a). However, there are many

caveats with this approach.

One, populations or species may have been introduced at different times, which can result in different residential time at the point of study. This can affect their progression along the invasion spectrum, as populations may need time to overcome their lag phase (Pyšek and Jarošík, 2005; Phillips et al., 2010; Schmidt et al., 2017, but see Gallagher et al., 2015). Two, introduction pathways can affect invasion success (Wilson et al., 2009; Pyšek et al., 2011b). Three, geographic origin is of importance, because this dictates the ecological amplitude and degree of contact with humans and chance of transportation (Casado et al., 2018). The fourth problem concerns the level of comparison on the organismal hierarchy. Ecological generalities are best exposed by multi-species experiments (van Kleunen et al., 2014), but such experiments run the risk of including taxa with divergent evolutionary histories, so that the perceived variation in invasiveness or invasibility may be confounded by phylogeny (Westoby et al., 1995; Pyšek and Richardson, 2007; Grotkopp et al., 2010). These problems can be alleviated by selecting congeneric or confamilial study taxa that have been introduced around the same time, from the same region and for similar purpose.

1.5 The genera *Gladiolus* and *Watsonia*, Iridaceae

The monocot family Iridaceae, with 1900 species in 65 genera, has a worldwide distribution, but is particularly concentrated in southern Africa, where over 1050 species have been recorded (Goldblatt and Manning, 2006). With few exceptions, Iridaceae are deciduous herbs with underground perennating organs such as bulbs, corms or rhizomes (Goldblatt and Manning, 2006). The geophytic life form is well adapted to the mediterranean-climatic biome of the Greater Cape Floristic Region (GCFR), where seasonal hot summers and wet winters prevail (Goldblatt, 1978). Bearing attractive flowers, many southern African Iridaceae are of horticultural importance, and over 20% have naturalised elsewhere, including Australia (van Kleunen et al., 2007). By contrast to the Cape region, Australia has five indigenous Iridaceae genera (two endemic) with 26 species, with all bearing a rhizomatous habit (Cooke, 1986). The paucity of the cormous habit in Australian Iridaceae offers an intriguing scenario where they may be phylogenetically close but ecologically distant to African Iridaceae.

The choice of *Gladiolus* (Goldblatt and Manning, 1998) and *Watsonia* (Goldblatt, 1989) as model systems for investigating the relationship between reproduction and invasiveness ameliorate the problems raised in Section 1.4. Firstly, both genera display a great variation in flower structure and generalist and specialist pollinator guilds. Pollinators common to both genera include bees,

butterflies, hawk moths, long-tongued flies and sunbirds (*Nectarinia* spp.), and in *Gladiolus*, beetles (Goldblatt, 1989; Goldblatt and Manning, 1998), thus facilitating comparisons among pollination modes. Secondly, both genera can reproduce asexually by vegetative offsets, although sexual seed set is paramount for species persistence (Goldblatt and Manning, 2008). This trait enables the intraspecific and interspecific comparisons of sexual and asexual modes of reproduction. Thirdly, many species in both genera were introduced to Australia as ornamentals around the same time from the Cape region (Table 1.1, thereby alleviating variation due to residence time, pathway and source region. Fourthly, both genera are closely related, and belong to the subfamily Crocoideae (Goldblatt et al., 2008). Fifthly, the extent of colonisation is variable among taxa (Figs. 1.3 and 1.4). Lastly, the reproduction modes of naturalised and invasive Iridaceae in Australia is largely anecdotal, although seed production has been assumed to operate in these populations (van Kleunen and Johnson, 2007b).

Five Iridaceae taxa with variable reproductive modes and pollination syndromes are studied in this work: *Gladiolus gueinzii* Kuntze, *Gladiolus tristis* L., *Gladiolus undulatus* L., *Watsonia meriana* (L.) Mill. var. *meriana* and *Watsonia meriana* (L.) Miller var. *bulbillifera* (J.Mathews & L.Bolus) D.A.Cooke.

Gladiolus gueinzii Kuntze (Fig. 1.1A) is unusual in the genus: it inhabits coastal dunes (only one of two species to do so) along the southeastern coast of South Africa, straddling both winter– and summer–rainfall regions. *Gladiolus gueinzii* is facultatively autogamous, with 80% of flowers setting viable seeds in the absence of pollen transfer (Goldblatt et al., 1998). It also has near-actinomorphic flowers, possibly in association with facultative autogamy (Goldblatt and Manning, 1998). Flowers are mauve in colour, with perianth tubes 10–12 mm long (Goldblatt et al., 1998). The identity of pollinators in its native range is unknown, but is thought to consist of long-tongued bees (Goldblatt and Manning, 1998). Flowering time spans October to December in the winter–rainfall region and November to January in the summer–rainfall one. The species inhabits coastal dunes in south-eastern Australia (Fig. 1.3A), and produces seeds and cormels (Fig. 1.1B) (Heyligers, 1999). The chromosome number is $2n = 30$ (Goldblatt and Manning, 1998). *G. gueinzii* is accorded Least Concern conservation status in South Africa (Victor et al., 2005), and a sleeper weed in Australia (WWF Australia, 2006). It is not a declared weed in Australia (Australian Government, 2019b), but is listed as an invasive species in Victoria (State of Victoria (Agriculture Victoria), 2019).

Its common name, marsh Afrikaner, belies the habitat affinity of *Gladiolus tristis* L. (Fig. 1.1C). One of the more widespread winter–rainfall species in the genus, *G. tristis* occupies seasonally or perennially wet areas from lowland to high elevations in the Greater Cape Floristic Region (GCFR) (Goldblatt and

Manning, 1998). Flowers are creamy in colour with a long perianth tube 40–60 mm in length, and open in the late afternoon, concurrently emitting a spicy clover scent that attracts crepuscular pollinators (Goldblatt and Manning, 2002). Scent production starts at 1600h and peaks at 2000h; scent compounds include terpenoids with linalool as the overall primary component (37.3%), and various benzenoids such as methyl benzoate (29.4%) and phenylacetaldehyde (6.9%) (Suzuki et al., 2008). Flowering time takes place from September to November, or later with elevation. Goldblatt and Manning (2002) observed *Cornutiplusia circumflexa* L. (Noctuidae) as a pollen vector, corroborating a moth pollination syndrome with large nectar volume (8.5–12.4 μL) and high sugar concentration (mean ± SD: 36.4 ± 2.1%). This species is self-incompatible, with stylar pollen tube inhibition, suggesting a gametophytic system of self-incompatibility (Ohri and Khoshoo, 1981). The chromosome number is $2n = 30$ (Ohri and Khoshoo, 1985). *G. tristis* is an ornamentally important plant, and forms the basis for many cultivars (Cantor and Tolety, 2011). *G. tristis* is accorded Least Concern conservation status in South Africa (Victor, 2005), a significant environmental weed in Victoria and an environmental weed in Western Australia (WWF Australia, 2006) (Fig. 1.3B). It is not a declared weed in Australia (Australian Government, 2019b). The species is naturalised, and considered weedy in Victoria, Tasmania, South Australia and Western Australia, and it has also naturalised in south-western USA (Biosecurity Queensland, 2016).

Gladiolus undulatus L. (Fig. 1.1C) occupies mesic habitats in the winter-rainfall region of the African continent. Flowers are creamy with long perianth tubes 55–70 mm, but unlike *G. tristis* they produce no scent, and are visited by diurnal insect pollinators, rather than crepuscular moths. *G. undulatus* was observed to be pollinated by the long-tongued fly *Philoliche rostra* (Tabanidae) (Goldblatt and Manning, 1999). Nectar volume was found to be 6.6–10.6 μL, and sugar concentration 25.3 ± 2.6% (mean ± SD) (Manning and Goldblatt, 1997). In another study, Goldblatt and Manning (1999) reported perianth length of 52–60 mm, and nectar volumes of 1.8–4.1 μl and 6.6–10.6 μL in two populations, and nectar concentrations of 24.8 ± 5.1% and 25.3 ± 2.6% (mean ± SD). Flowering time stretches from mid-November to late December. *G. undulatus* is self-incompatible (Goldblatt and Manning, 1999), and chromosome number is $2n = 30$ (Goldblatt and Manning, 1998). *G. undulatus* is accorded Least Concern conservation status in South Africa (Foden and Potter, 2005a). Despite not being declared or considered noxious by any Australian state government authorities (Australian Government, 2019b), it is considered a significant environmental weed in Victoria, South Australia and Western Australia (WWF Australia, 2006) (Fig. 1.3C).

Watsonia meriana is widespread in the GCFR, extending from Bredasdorp

district in the south to the drier Namaqualand in the north, where it persists in mesic areas (Goldblatt, 1989). Flowers are zygomorphic with variable colouring, from orange to red, and even yellow or purple (the latter were not observed in Victoria, Australia); perianth tube is 22–25 mm long at the lower part (Goldblatt, 1989), and 47.1 mm long in total (Geerts and Pauw, 2009). Nectar volume and sugar concentration were not reported, but the flowers were visited by the long-billed malachite sunbird *Nectarinia famosa*, while generalist feeders with shorter and uncurved beaks (Cape white-eye *Zosterops virens* and southern double-collared sunbird *Cinnyris chalybea*) engaged in nectar robbing (Geerts and Pauw, 2009). Flowering time starts in September and ends in November in southern Africa. *W. meriana* is partially self-incompatible, and is capable of setting seeds in the absence of pollen transfer (Horn, 1962).

For *Watsonia meriana*, species taxonomy was at first unclear, as some individuals were observed forming aerial cormels in stem and leaf axils (*Watsonia vivipara* Mathews & L.Bolus), or along the entirety of the flowering stalk (*Watsonia bulbillifera* Mathews & L.Bolus). Goldblatt (1989) considered the presence of aerial cormels (also known as cormlets or cormils; Fig. 1.2D) to be of no taxonomic significance, and merged *W. bulbillifera* and *W. vivipara* under *Watsonia meriana*. However, because distinctive reproductive modes in this species complex may explain differential levels of invasiveness, an infraspecific taxonomy following Conran et al. (2003) is applied in this work. *Watsonia meriana* (L.) Mill. var. *meriana* is the sexual form, and reproduces by seed only (Fig. 1.2A and B). Both *W. bulbillifera* and *W. vivipara* are subsumed under *Watsonia meriana* (L.) Miller var. *bulbillifera* (J.Mathews & L.Bolus) D.A.Cooke, as axillary cormels constitute the primary type of propagule.

Chromosome numbers are $2n = 18$ for *W. meriana* var. *meriana*, and $2n = 27$ for *W. meriana* var. *bulbillifera*, which is a sterile triploid (Goldblatt, 1989), but occasionally produces viable seeds (Goldblatt and Manning, 2008). In a study of five sites with seed-setting *W. bulbillifera* morphotype in the Adelaide Hills in South Australia, Conran et al. (2003) found that specimens produced infertile pollen (with the exception of one site at Hahndorf), and low yields of seed set (1.8–3.9 per capsule, but all viable). Surprisingly, the authors identified all examined seedlings as diploid, and suggested that fertile offspring were produced by aneuploidy, with the chance removal of the superfluous chromosome set. Both varieties are accorded Least Concern conservation status in South Africa (Foden and Potter, 2005b,c). On the other hand, the species is considered an environmental weed in Victoria, New South Wales and Tasmania, and a significant environmental weed in South Australia and Western Australia (WWF Australia, 2006) (Fig. 1.4). *Watsonia* spp. are potential weeds of national significance (Australian Government, 2019a).

The locations of all the study sites are reported in Appendix A.

Table 1.1: Study species and their characteristics in southern Africa: propagule types produced, self-incompatibility system, pollinator guild, and earliest herbarium record in Australia (sources: (GBIF.org, 2018a, 2017, 2018b,d,c) in the order of taxa listed.)

Species	Propagules	Self-incompatibility system	Pollinator guild	Earliest record
Gladiolus gueinzii	Seeds, cormels, corms	Self-compatible	Long-tongued bees	1950
Gladiolus tristis	Seeds, cormels, corms	Self-incompatible	Hawk moths	1841
Gladiolus undulatus	Seeds, cormels, corms	Self-incompatible	Long-tongued flies	1879
Watsonia meriana var. *bulbillifera*	Seeds, corms, aerial cormels	Self-incompatible	Sunbirds	1900
Watsonia meriana var. *meriana*	Seeds, corms	Weak self-compatibility	Sunbirds	1899

1.6 Aims and structure of thesis

My overarching objective is to investigate the association between reproductive strategy and invasiveness in irids introduced to Australia, in the light of breakdowns in plant–pollinator mutualism after introduction. I hypothesise that the species will rely on asexual means for invasive spread, but that population expansion will be arrested by eventual environmental mismatch, as asexual organisms are not expected to evolve rapidly to changing conditions. In particular, I ask these questions:

1. What are the reproductive modes of introduced Iridaceae in south-eastern Australia?
2. Are there shifts in reproductive strategy following introduction?
3. What are the levels of genetic diversity of introduced Iridaceae, and how do these relate to their reproductive strategies?
4. How do their niche dynamics and level of invasiveness correlate to their reproductive modes?
5. What is the role of vegetative fragmentation in invasive spread?

Using a comparative approach, I employ a combination of field studies, greenhouse trials, genetic assays and computer modelling to answer these questions.

In Chapter 2 I evaluate the reproductive mode and pollination ecology of the study taxa in Victoria and New South Wales in south-eastern Australia. In

addition, I estimate the reproductive output and germination capacity of their propagules.

In Chapter 3, I assess the genetic variability of *Gladiolus* spp. using microsatellite markers. I also conduct chromosome counts by root-tip squashing to ascertain polyploidy levels.

In Chapter 4, I use species distribution modelling to predict the potential distribution of the study taxa, and ecological niche modelling to elucidate their niche dynamics. Here, I also use a new statistic to measure model performance.

In Chapter 5, I employ a expectation-maximisation to estimate the contribution of anthropogenic spread to the extant distribution patterns of the study species, and to estimate the dispersal kernel for naturally-dispersed populations.

In the final chapter, I present a general discussion of the results reported in this thesis, and their contribution in addressing the key hypothesis. Recommendations are also made to progress the field.

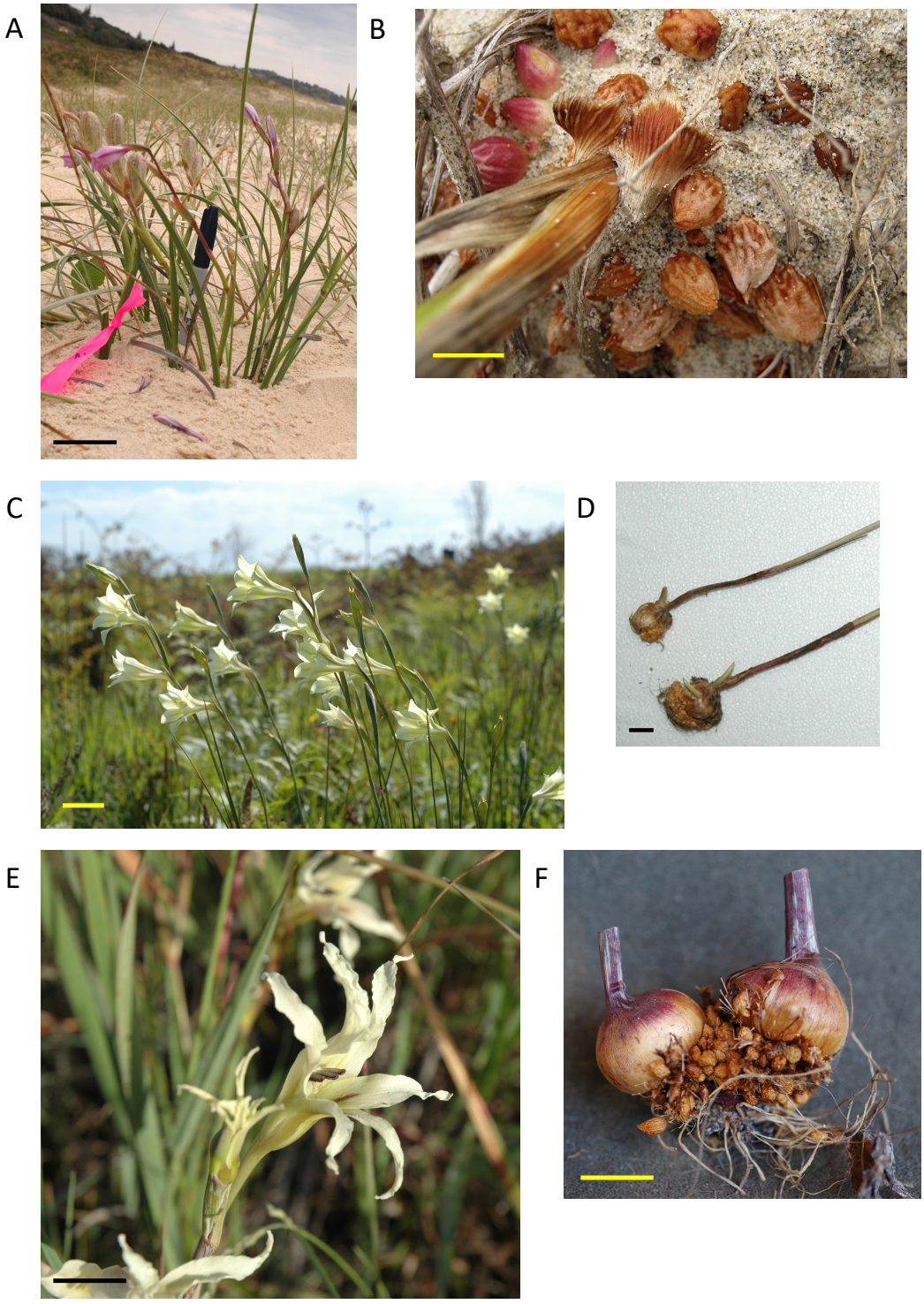

Figure 1.1: Habit, underground storage organs and propagules of *Gladiolus* spp. **A**: Late-flowering *Gladiolus gueinzii* flowering with unripe capsules on a dune in Bermagui, New South Wales 20 November 2011. **B**: Exposed cormels of *G. gueinzii* in Narooma, New South Wales. These cormels are buoyant and are dispersive structures. **C**: *Gladiolus tristis* flowering in Potilla, Victoria on 5 October 2013. **D**: Corms of *G. tristis* excavated mid-October 2012. Note the paucity of cormels. **E**: *Gladiolus undulatus* flowering along the Glenelg Highway in western Victoria, 29 December 2013. **F**: Profuse cormel production in *G. undulatus*. Corms were excavated mid-February 2013. Scale values = 5 cm for **A**, **C** and **E**, 1 cm for **B** (estimated), **D** and **F**. Photo credit: All photos were taken by the author, except for **B** by HankyHelper reproduced under a Creative Commons licence (CC BY-NC-ND 2.0; creativecommons.org/licenses/by-nc-nd/2.0/).

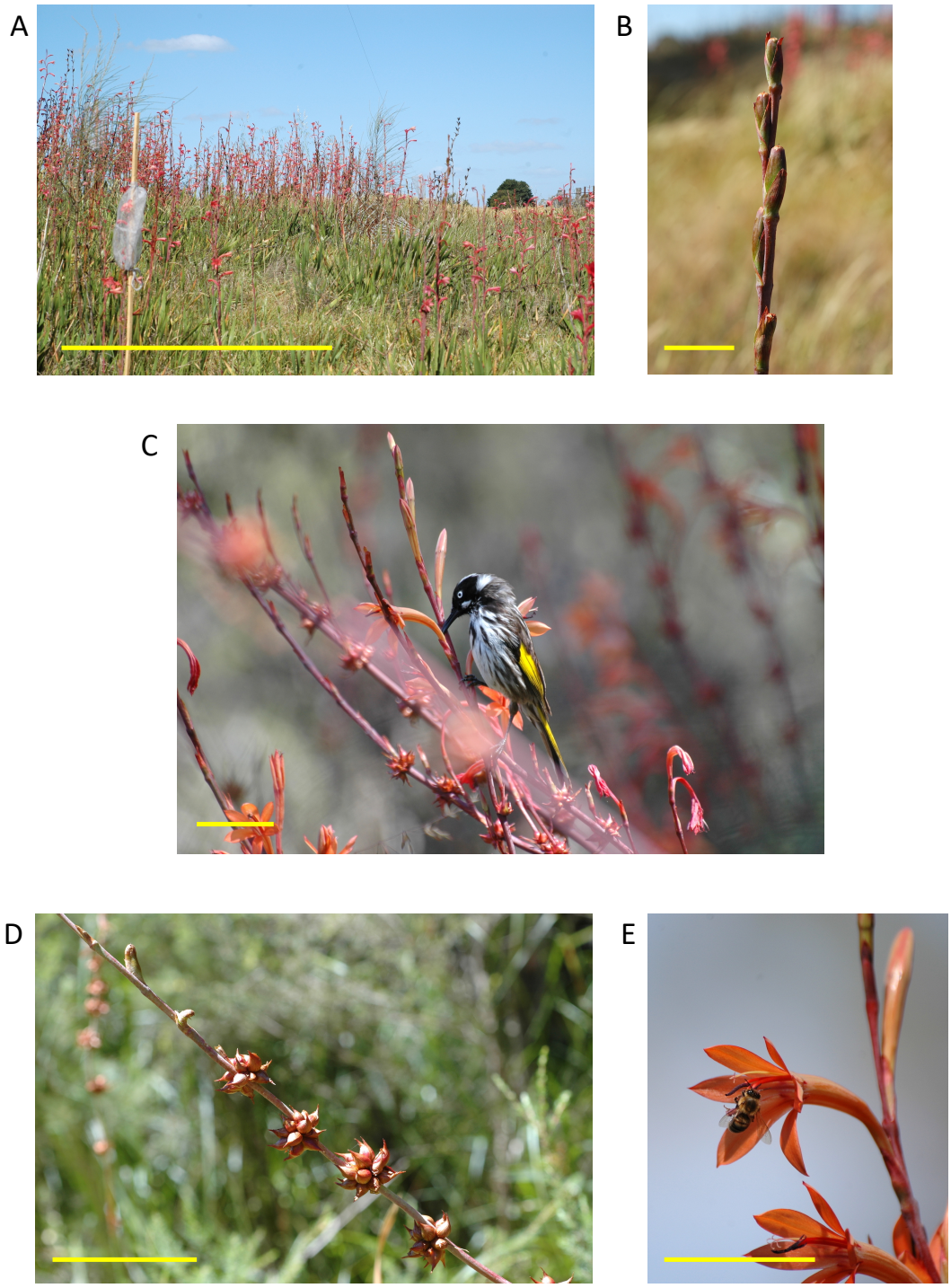

Figure 1.2: Habit, capsule and pollinators of *Watsonia meriana* varieties. **A**: *Watsonia meriana* var. *meriana* in Cape Clear, Victoria on 21 November 2012. A flowering scape has been bagged for seed collection to the left of the photo. Some capsules had already formed (**B**). **C**: A New Holland honeyeater (*Phylidonyris novaehollandiae*) robbing nectar from *Watsonia meriana* var. *bulbillifera* in Chetwynd, western Victoria on 14 December 2013. This species does not always engage in nectar robbing—on other occasions, individuals were observed dipping their beak into the flower tube. Aerial cormels ("bulbils") can be seen on some scapes. **D**: Mixed propagules on *W. meriana* var. *bulbillifera* in Jervis Bay, New South Wales (10 December 2013), with capsules to the left of the photo and aerial cormels to the right. Capsules are typically stunted with fewer viable seeds compared to *W. meriana* var. *meriana*. **E**: A European honeybee (*Apis mellifera*) harvests pollen from *Watsonia meriana* var. *bulbillifera* in Chetwynd, Victoria (14 December 2013). Scale values = 100 cm for **A**, 3 cm for **B**, and 5 cm for **C**, **D** and **E**. All photos were taken by the author.

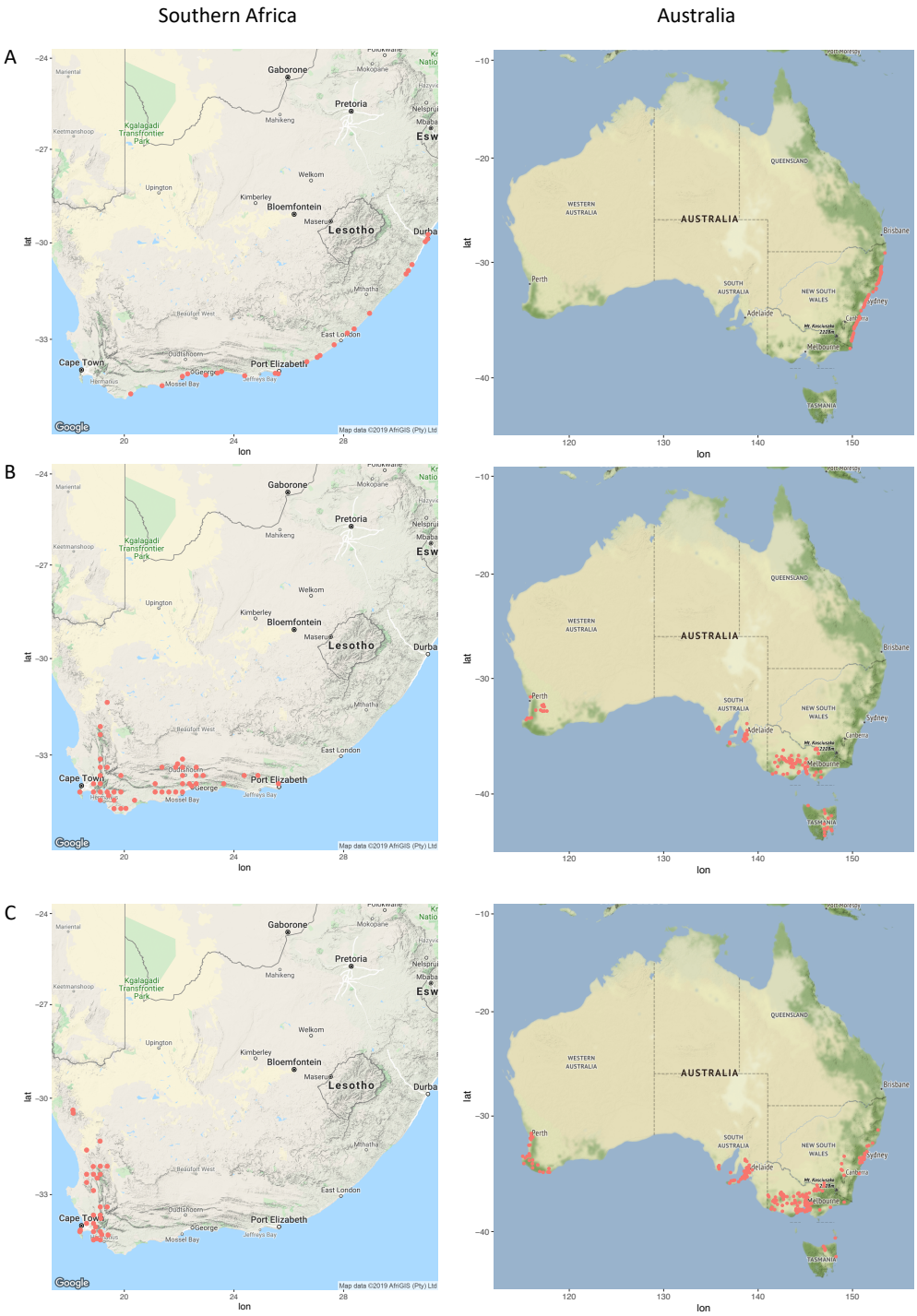

Figure 1.3: Ocurrence records for *Gladiolus* spp. in southern Africa (left column) and Australia (right column). **A**: *Gladiolus gueinzii*, **B**: *Gladiolus tristis* and **C**: *Gladiolus undulatus*. Records were downloaded from Global Biodiversity Information Facility (www.gbif.org) for African accessions, and Australasian Virtual Herbarium (avh.chah.org.au) for Australian accessions in May 2018, and thinned (see Chapter 4 for details on thinning). Maps were downloaded from Google Maps in November 2019.

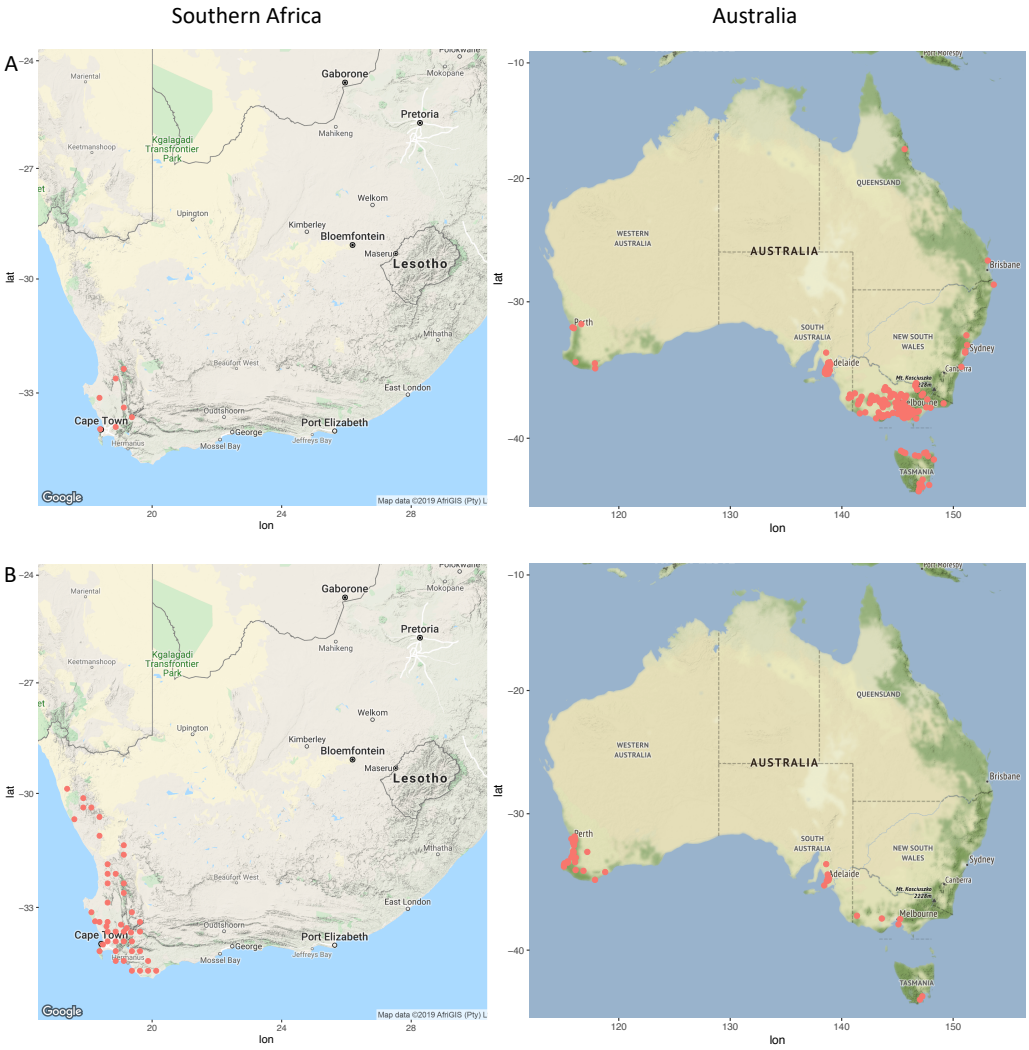

Figure 1.4: Ocurrence records for *Watsonia meriana* varieties in southern Africa (left column) and Australia (right column). **A**: *Watsonia meriana* var. *bulbillifera* and **B**: *Watsonia meriana* var. *meriana*. Records were downloaded from Global Biodiversity Information Facility (www.gbif.org) for African accessions, and Australasian Virtual Herbarium (avh.chah.org.au) for Australian accessions in May 2018, and thinned (see Chapter 4 for details on thinning). Maps were downloaded from Google Maps in November 2019.

Chapter 2

Reproductive ecology of invasive Iridaceae in Australia: less of the same?

2.1 Introduction

Plant reproductive strategies are rich and varied (Richards, 1997). A fundamental dichotomy is the sexual system (whether a plant regenerates asexually or sexually), which affects the fidelity of genetic transmission from a parent to its offspring, with asexual reproduction resulting in a higher fidelity of transmission in the timespan of a generation (see Section 1.2). In turn, the mating system describes the pattern in which pollen grains are transferred from an anther to a stigma, for example allogamy (cross-fertilisation) and self-fertilisation within a flower (autogamy) or among flowers on an individual plant (geitonogamy). Many plant species possess mixed-mating systems (cross- and self-fertilisation: Lu, 2000; Goodwillie et al., 2005) and mixed sexual–asexual systems (Lui et al., 2005; Eckert et al., 2006). Furthermore, plant mating systems are neither fixed in time nor space, and their expression is mediated by abiotic, biotic, demographic, genetic and stochastic factors that can vary spatially or temporally (Hargreaves and Eckert, 2014; Whitehead et al., 2018).

The process of biological invasion starts with the transport of plant individuals (or of their diaspores), usually to an area outside of their normal dispersive capacity. Typically, these are few in number (Wilson et al., 2009, but see Cook and Dias, 2006), and demographic bottlenecking invokes a density-dependent Allee effect. This results in a reduced number of mating partners, which is compounded in self-incompatible (SI) populations by a loss of allelic diversity in the *S*-locus (Busch and Schoen, 2008), and in heterostylous populations by the loss of flower morphs (Eckert et al., 1996). Apart from mate limitation, long-distance transportation can disrupt mutualistic relationships. A key step in an

outcrossing mating system is the transfer of pollen from the androecium to the stigma, which can become a weak chain in species with specialised pollination requirements. Pollen limitation—a reduction in fruit and/or seed production because of inadequate pollen receipt—is prevalent in endemic species, with 63 % of 482 study records showing reduced fruit set (Knight et al., 2005), and is expected to be more severe for introduced plant species with specialised pollinator syndromes or seed dispersal vectors (Richardson et al., 2000a).

Baker recognised many years ago that it is rare for conspecific propagules to end up in the same local area following long-distance dispersal, and he hypothesised that taxa capable of uniparental reproduction had better reproductive assurance (Baker, 1955). Baker's rule (as proclaimed by Stebbins, 1957) has been validated by comparative studies in introduced populations (Rambuda and Johnson, 2004; van Kleunen and Johnson, 2007a; Hao et al., 2011; Ward et al., 2012; Razanajatovo et al., 2016, but see Sutherland, 2004; van Etten et al., 2017), which demonstrated that self-compatible (SC) or apomictic species had higher establishment or invasive success, compared to self-incompatible ones. However, in a study of three partially-SC invasive plant species spanning their native and introduced ranges, Petanidou et al. (2012) were unable to link invasiveness to selection for self-fertility; indeed one species (*Solanum elaeagnifolium*) had greater SC in its native range. The authors postulated that SI was favoured in this instance to allocate more resources to vegetative spread in a novel environment.

In general, populations that are obligately outcrossing (that is, possessing a strong SI system) appear to retain their compatibility mechanism in their introduced range (Li et al., 2012) when pollen delivery is not interrupted (Zhang et al., 2011, 2017). Few studies have managed to capture the evolution from strong/obligate SI to SC in a non-indigenous species' introduced range, which can be caused by duplications of the S-locus or mutations of incompatibility alleles for gametophytic SI (GSI) (Stone, 2002; Hauck et al., 2006) or mutations at modifier loci controlling pollen rejection for GSI and sporophytic SI (SSI) (Vallejo-Marín and Uyenoyama, 2004). Among colonised populations of the highly outcrossing *Centaurea solstitialis*, Sun and Ritland (1998) uncovered a marginal population with significant parental inbreeding, but neither its mating system in its native range, nor the mechanism causing SI breakdown was assessed. In another study, Colautti et al. (2010b) inferred SC evolution in North American populations of *Lythrum salicaria*, an invasive plant with a trimorphic SI system.

While support for Baker's rule is convincing (see above), the pattern of greater SC in naturalised and invasive populations may simply be an artefact of sampling—that is, humans are transporting more plants with self-compatible (or weakly self-incompatible) systems already in place, or SI populations are

failing to establish (van Kleunen et al., 2008; Petanidou et al., 2012). It is therefore unlikely that strong SI populations will evolve self-compatibility after introduction, although the injection of SC individuals can result in the loss of self-incompatibility (Voillemot et al., 2019).

This pattern of mating system consolidation appears to hold in species with mixed sexual and asexual reproductive modes, with introduced populations falling back on clonal reproduction in Japanese knotweed (*Fallopia japonica,* Hollingsworth and Bailey, 2008) and *Oxalis pes-caprae* (Ornduff, 1987). However, recent analyses showed sexual reproduction in these taxa, which might have arisen from introduction of compatible mates (Bzdega et al., 2012; Castro et al., 2016). By contrast, with molecular markers Amsellem et al. (2000) deduced a transition from sexuality to apomixis in introduced populations of *Rubus alceifolius*. In some taxa, invasiveness has been attributed to a mixed mating system, where vegetative propagules drive local growth, and seeds distant colonisation (e.g. *Phragmites australis*: Albert et al., 2015; Kettenring and Whigham, 2018). In a study of introduced populations on islands, Rojas-Sandoval and Acevedo-Rodríguez (2015) found that sexual and asexual reproduction were associated with naturalisation and invasion, while small seeds better explained invasive spread.

The evolution of asexuality (apomixis) is often associated with polyploidy (Whitton et al., 2008). Apomictic (Hörandl et al., 2008) and polyploid (Lowry and Lester, 2006) taxa often occupy larger geographic areas compared to their sexual relatives, and it is plausible that a combination of apomixis and whole genome duplication can facilitate range expansion, and in the extreme case, invasion. Asexuality has been implicated in greater invasiveness (see Section 1.2). In a global study, Pandit et al. (2011) found that polyploid taxa were 20% more likely to become invasive compared to diploid congeners; however, it was difficult to isolate a causative link, as polyploids are usually more robust, and therefore more competitive. Moreover, polyploid formation can disrupt SI mechanisms, leading to a capacity for selfing (Miller and Venable, 2000; Barringer, 2007), but only in allopolyploids and not autopolyploids (Husband et al., 2008). There is also growing evidence that apomictic polyploid taxa—thought to be obligately asexual—in fact can undergo cryptic sexual reproduction (Thompson et al., 2008). The mating system in an apomictic polyploid group is likely to be the manifestation of the contextual interplay among polyploidy, hybridisation and asexuality (Hörandl, 2009).

In the present study, the mating systems of five Iridaceae taxa (*Gladiolus gueinzii*, *G. tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*) were investigated to ascertain whether any shift in reproductive mode has occurred, following their introduction from the Cape Floristic

Region (CFR) to Australia. While all three *Gladiolus spp.* outcross and produce cormels in their native range, only *G. gueinzii* is self-fertile. Both *G. tristis* and *G. undulatus* reportedly do set fruit in Australia (see Section 1.2). *W. meriana* var. *bulbillifera* is viviparous in its native range, but has been reported to set rare fruit with viable seeds in South Australia (Conran et al., 2003), while *W. meriana* var. *meriana* is allogamous but weakly SC in the CFR (Horn, 1962). Hand pollination trials were conducted in the field to identify mating system and pollen limitation, and pollinator visitation were recorded to assess the presence of natural pollen transfer. Pollen viability tests were run *ex situ* to compare the level of fruit and seed production against pollen viability. Next, reproductive output was measured, as a proxy for the capacity for potential spread. Finally, a glasshouse germination trial was conducted to estimate the germinability of propagules.

2.2 Materials and methods

2.2.1 Manual hand-pollination

Corms from all species were harvested during aestivation in 2011, returned to La Trobe University glasshouse facilities (Bundoora, Victoria, Australia, 37.7181 °S, 145.0467 ° E) where they were sown in pots in winter (June–August 2011). Only *G. tristis* flowered sufficiently *ex situ* during the following growing season. Consequently, mating system studies were primarily conducted *in situ* in Victoria in 2012–13, and intermittently thereafter from 2014–17. Prior to the commencement of hand pollination experiments, it was verified that the species exhibited protandry. In addition, pollen grains were verified to be viable for up to three days after dehiscence with Alexander's stain (Alexander, 1969), and stigmas were verified to be receptive 2–4 days following anther dehiscence with hydrogen peroxide (Kearns and Inouye, 1993).

The focal *Gladiolus* species typically produced 3–4 flowers on 2–3 spikes, and 3–4 flowers would be available for pollen manipulation concurrently, that is, having freshly-dehisced anthers and receptive stigmatic surfaces. On the other hand, both *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana* flowered profusely, such that sufficient flowers were available for manipulation. In Victoria, Australia, flowering begin in mid-September to early October for *G. tristis*, mid-October to early December for *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana*, and in December for *G. undulatus*. Along the New South Wales coast and in Mallacoota, Victoria, *G. gueinzii* flowers from early to late-October, with the more southern populations having a later onset of flowering (pers. obs.).

To maintain a comparable sample size across species, 10–20 plants were

randomly selected per population for each species, depending on the population size. A distance of at least 5 m between plants was maintained, to avoid the chance of selecting plants from the same ramet. Five buds per plant were tagged for the following treatments: (i) control—flowers were left undisturbed for natural pollination; (ii) self-pollination—flowers were pollinated with pollen from the same plant; (iii) within-population cross-pollination—flowers were pollinated with pollen collected from another randomly-selected individual at least 5 m away, and (iv) between-population cross-pollination—flowers were pollinated with pollen pooled from nearby populations. For *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana*, an additional treatment was applied: (v) inter-varietal cross-pollination—flowers of a variety were pollinated with pollen pooled from a nearby population of the other variety; for example, the population of *W. meriana* var. *meriana* at Casteron (WMAV03; Fig 2.3) was pollinated with pollen from the population of *W. meriana* var. *bulbillifera* at Chetwynd (WMAV09), approximately 36 km north of Casteron. A total of four populations were studied for *G. gueinzii*, seven for *G. tristis* (Fig. 2.1), nine for *G. undulatus* (Fig. 2.2), and nine for *W. meriana* s.l. (Fig. 2.3).

For hand pollination treatments (ii)–(v) above, buds were bagged with nylon tulle netting to exclude pollinators for the duration of the study. In addition, for treatments (iii), (iv) and (v), the buds were carefully emasculated to avoid self-pollination and re-bagged. For hand pollination, a fine painting brush was used to collect pollen from a source anther (or anthers), and transferred to the target stigma by gently brushing it against the stigmatic surface. Stigmas were visually checked for pollen adherence before the flower was re-bagged. A sterile brush was used for each pollen transfer. Pollen transfers were performed twice at different times (typically once in the morning and late afternoon), as stigma receptivity can differ over the day (Sedgley and Attanayake, 1988). After treatment, the test plants were observed fortnightly until pod dehiscence (for fruit set) or until die back set in.

Two indices were calculated based on the pollen supplementation experiments. The first, the index of self-incompatibility, is defined as:

$$ISI = \frac{S}{B} \tag{2.1}$$

where S signifies the mean reproductive units (number of fruits or seeds) from self-pollination (treatment [ii] above), and B the mean reproductive units from between-population cross-pollination (treatment [iv] above). A population is (i) self-incompatible if ISI $= 0$, (ii) partially self-incompatible if $0 <$ ISI < 1, (iii) self-compatible if ISI $= 1$, (iv) partially cross-incompatible if ISI > 1, and (v) cross-incompatible if ISI $\to \infty$ (Ramírez and Nassar, 2017). The second, the

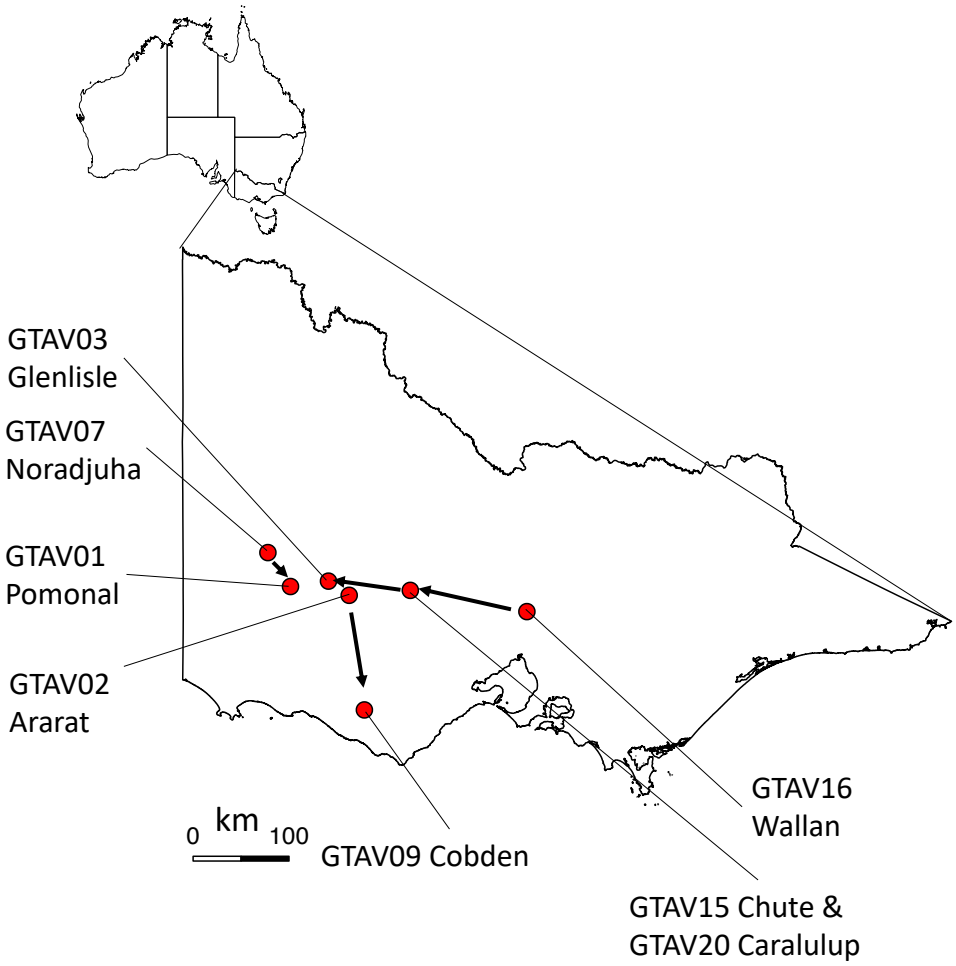

Figure 2.1: Study locations for *Gladiolus tristis* in Victoria, Australia. Each arrow shows the source population (base of arrow) and target population (tip of arrow) for pollen for cross-population manual hand-pollination trials (treatment [iv]; see the text for details).

pollen limitation index, is defined as:

$$PI = \frac{W - C}{C} \tag{2.2}$$

where W is the mean reproductive units from within-population cross-pollination, and C is the mean reproductive units from open pollination (the control; treatment [i] above). A population is pollen limited if PI > 0, and not under pollen limitation if PI $= 0$ (Eckert et al., 2010).

Hand pollination treatments were performed on one population for *G. gueinzii*, four populations for *G. tristis*, three populations for *G. undulatus*, and three populations for *W. meriana* var. *bulbillifera* and two populations for *W. meriana* var. *meriana* (Table 2.1). Pollen sources are illustrated for *G. tristis* on Figure 2.1, for *G. undulatus* on Figure 2.2, and for *W. meriana* var. *bulbillifera* and

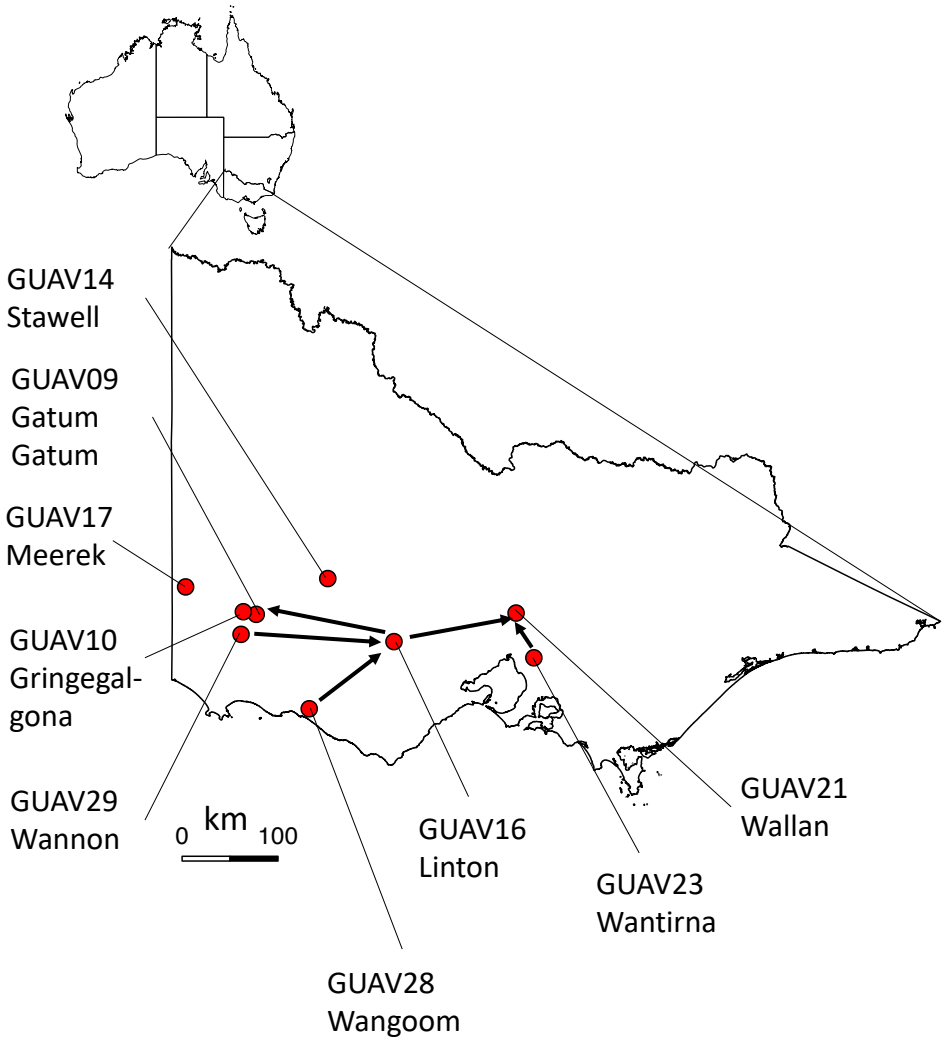

Figure 2.2: Study locations for *Gladiolus undulatus* in Victoria, Australia. Each arrow shows the source population (base of arrow) and target population (tip of arrow) for pollen for cross-population manual hand-pollination trials (treatment [iv]; see the text for details).

W. meriana var. *meriana* on Figure 2.3.

2.2.2 Pollen viability

A pollen viability test was conducted to identify possible pre-zygotic reproductive failure. Initially, Alexander's stain (Alexander, 1969) was used; however, the reliability of this test has been questioned (Pline et al., 2002). In 2015, it was replaced by Brewbaker–Kwack medium (Brewbaker and Kwack, 1963; Kearns and Inouye, 1993). As Iridaceae pollen are binucleate (Harley, 2004), the default medium was applied in Kearns and Inouye (1993). Pollen growth was inspected under a light microscope and photographed for counting in ImageJ (version 1.52a, National Institute of Health USA), using the built-in Analyze Particles function.

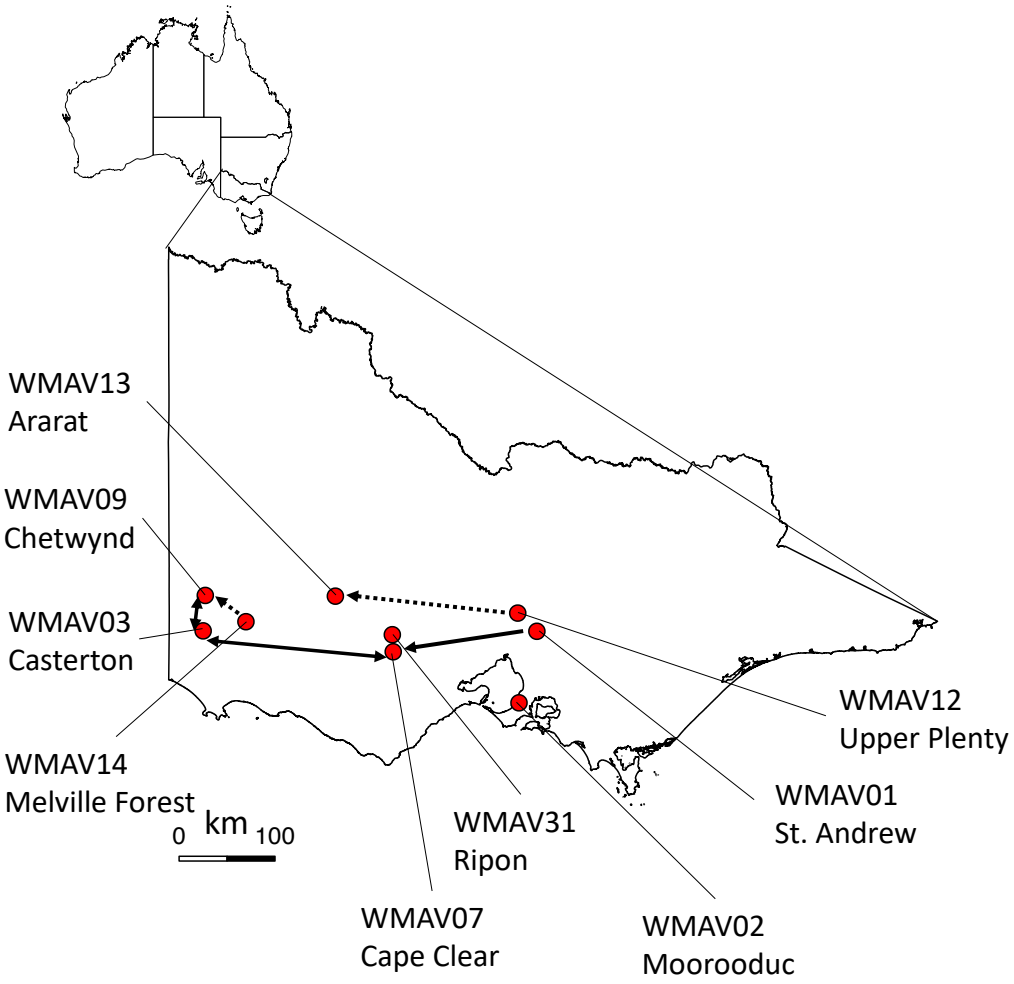

Figure 2.3: Study locations for *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana* in Victoria, Australia. Both varieties are sympatric at Cape Clear (WMAV07); only *W. meriana* var. *meriana* is found at Casterton (WMAV03). The populations at Chetwynd (WMAV09) and Melville Forest (WMAV14) produce aerial cormels and rarely seeds. Dashed arrows show the source population (base of arrow) and target population (tip of arrow) for pollen for cross-population manual hand-pollination trials (treatment [iv]), and solid arrows show the pollen movement for inter-varietal cross-pollination (treatment [v]; see the text for details).

Pollens were sourced from Ararat, Noradjuha, Chute and Wallan for *G. tristis* (Fig. 2.1); Linton, Wantirna, Wangoom and Wannon for *G. undulatus* (Fig. 2.2); Saint Andrew, Moorooduc and Upper Plenty for *W. meriana* var. *bulbillifera*; and Cape Clear for *W. meriana* var. *meriana* (Fig. 2.3).

2.2.3 Reproductive output

Three types of propagules were produced in the study species: cormels (all species), aerial cormels (*W. meriana* var. *bulbillifera*), and seeds (*W. meriana* var. *meriana*, and opportunistically, *W. meriana* var. *bulbillifera*). For each

taxon, two populations at least 50 km apart were sampled for propagule output quantity during 2015–16, when all taxa had begun to aestivate. Ten samples were collected from each population, with each sample as close to equidistant as possible along a transect spanning the population. For all populations and species, a root ball measuring 15 × 15 × 15 cm^3 was collected, soil removed and cormel production was determined. For *W. meriana*, a scape bearing aerial cormels or seeds was detached at the base with a pair of secateurs, and bagged. For aerial cormel forming populations, the scape was agitated by thrashing to remove fully-formed cormels for counting. In seed bearing populations, the number of viable seeds was determined by the imbibed seed crush test (Borza et al., 2007), where a seed was considered inviable if it collapsed under gentle pressure from a pair of forceps.

Populations tested for reproductive output were Ararat and Noradjuha for *G. tristis* (Fig. 2.1); Gringegalgona and Stawell for *G. undulatus* (Fig. 2.2); Chetwynd, Upper Plenty and Melville Forest for *W. meriana* var. *bulbillifera*; and Cape Clear and Casterton for *W. meriana* var. *meriana* (Fig. 2.3; see also Table 2.1).

2.2.4 Germination tests

For *G. tristis*, cormels were harvested from Noradjuha, and 100 were selected for germination testing (Fig. 2.1). Similar numbers were selected for *G. undulatus* from Gringegalgona and Stawell (Fig. 2.2, see also Table 2.1). Cormels were sown at 1 cm depth onto garden potting mix (1:1:1 clay:loam:sand) in a seed tray, and watered on alternate days, or when necessary to maintain moisture during winter in 2015. Growth was recorded every 7 days over 4 weeks. For each *W. meriana* var. *bulbillifera* population (Ripon, Melville Forest and Chetwynd; Fig. 2.3), 80 aerial cormels over 5 mm were randomly selected, and similarly sown and monitored for growth.

For *W. meriana* var. *meriana* (Casterton and Cape Clear) and *W. meriana* var. *bulbillifera* (Chetwynd) (Fig. 2.3) seeds were collected from ten plants in 2014, and stored in seed envelopes at room temperature. At the onset of winter in 2015, 48 seeds per population were randomly selected, and placed on filter paper (Whatman Type I) in a petri dish, and kept moist with distilled water over the duration of the experiment. No further treatment (e.g. sterilisation/scarification) was performed on the seeds. The petri dishes were housed in a growth cabinet at a constant 20 °C, with 12 hours' daylight simulation by fluorescent lighting. Dishes were randomly rearranged during re-watering on alternate days, and inspected for germination. The proportion of germinants (based on radical emergence) was recorded every 7 days over a four-week period.

2.2.5 Floral visitors

Floral visitor observations were conducted for *G. gueinzii* in 2011 during its flowering season, and between 2012–13 for other study species during their flowering season (*G. tristis*: mid-September–early October, *G. undulatus*: December, *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana*: October–November). Each observation activity lasted two hours on sunny and low wind days between 9 am to 1 pm. A cluster of five or more ramets was randomly selected, and floral visitors recorded for 20 minutes; and the process was iterated at a site at least 10 m away from the preceding site. Insects that actively foraged for nectar or pollen were captured and killed by placing them in a collecting jar containing a cotton ball infused with commercial insecticide (Mortein Powergard), and later identified in the laboratory.

In addition, two light traps (Model E700; Australian Entomological Supplies) were set up for capturing crepuscular pollinators to *G. tristis*, as this species is pollinated by nocturnal hawk moths in South Africa. On clear and low wind nights, the light traps were positioned among clusters of flowering ramets, with over 20 m separation between traps. Plants were observed from 6.30 pm to 10.30 pm, and trapped moths were collected the following morning, and identified using *Moths of Australia* (Common, 1990) and website *Australian Moths Online* (CSIRO, https://moths.csiro.au).

2.2.6 Statistical analyses

For fruit and seed set, exploratory data analyses showed that the data were not normally distributed, and the differences among treatments and populations for each species were inferred using a permutational multivariate analysis of variance (PERMANOVA) test (Anderson, 2001; McArdle and Anderson, 2001), with fruit/seed set as the random factor and treatment and population as the fixed factors. The PERMANOVA test was run with the function adonis in the R package VEGAN (Oksanen et al., 2019), with Gower distance method to calculate pairwise distances.

For pollen viability assays, summary statistics were calculated. Since these assays employed two media (Alexander's stain and Brewbaker–Kwack) that gave different results, a PERMANOVA test was performed, with the proportion of viable pollen as the random factor, and assay method and taxon as the fixed factors. In addition, for each species, a PERMANOVA test was run, with the proportion of viable pollen as the random factor, and population as the fixed factor.

For reproductive output and germination experiments, summary statistics were computed. To explain whether variation in reproductive output results

were explained by propagule type and/or population, PERMANOVA tests were run with propagule type and population as fixed factors, and propagule counts as the random factor. Likewise for germination experiments, variation in results were explained by running PERMANOVA tests with propagule type, population and taxon as fixed factors, and the proportion of growth/germination as the fixed factor.

All statistical analyses were performed with R (R Core Team, 2019).

Table 2.1: Study locations for *Gladiolus gueinzii*, *G. tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana* in Victoria and New South Wales between 2011–2017. Sample sizes are reported for manual hand-pollination trials (Section 2.2.1), pollen viability tests (Section 2.2.2), reproductive output assessments (Section 2.2.3) and germination tests (Section 2.2.4), and observation hours are reported for pollinator activity (Section 2.2.5). For *W. meriana* s.l., * = only *W. meriana* var. *meriana* was present at the site, ** = both *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana* were sympatric at the site, and *** = *W. meriana* var. *bulbillifera* with rare seed set. For pollinator activity observation on *G. tristis*, the figures in parentheses refer to the hours spent operating moth traps.

Site code	Site name	Hand-pollination (N)	Pollen viability (N)	Reproductive output (N)	Germination tests (N)	Pollinator activity (h)
G. gueinzii						
GGAN01	Shoalhaven	0	0	0	0	2
GGAN08	Pambula	0	0	0	0	2
GGAV01	Mallacoota	36	0	0	0	2
G. tristis						
GTAV01	Pomonal	74	0	0	0	4 (12)
GTAV02	Ararat	0	25	10	0	4 (12)
GTAV03	Glenlisle	61	0	0	0	0
GTAV05	Noradjuha	0	67	10	100	0
GTAV07	Portland	0	0	0	0	0
GTAV09	Cobden	63	3	0	0	0
GTAV15	Chute	39	74	0	0	4 (24)
GTAV16	Wallan	0	48	0	0	12 (48)
GTAV20	Caralulup	0	5	0	0	4 (12)
G. undulatus						
GUAV09	Gatum Gatum	32	4	0	0	5
GUAV10	Gringegalgona	0	0	6	100	0
GUAV14	Stawell	0	0	6	100	0
GUAV16	Linton	23	2	0	0	6
GUAV17	Meerek	0	0	0	0	0
GUAV21	Wallan	40	0	0	0	0
GUAV23	Wantirna	0	98	0	0	2
GUAV28	Wangoom	0	3	0	0	0
GUAV29	Wannon	0	6	0	0	0
W. meriana s.l.						
WMAV01	St. Andrew	0	186	0	0	2
WMAV02	Moorooduc	0	5	0	0	0
WMAV03*	Casteron	87	0	6	48	8
WMAV07**	Cape Clear	83	311	6	48	4
WMAV09***	Chetwynd	90	0	10	80	6
WMAV12	Upper Plenty	0	140	10	0	0
WMAV13	Ararat	32	0	0	0	0
WMAV14***	Melville Forest	0	206	10	80	0
WMAV31	Ripon	0	0	0	80	0

2.3 Results

2.3.1 Manual hand-pollination

For all hand–pollination treatments, *G. tristis* and *G. undulatus* did not set any fruit. By contrast, *G. gueinzii* was found to set fruit for all flowers tagged under the control treatment, with mean seed set and standard error of 31.78 ± 4.16 per pod. However, the mean seed set for the selfing treatment for *G. gueinzii* could not be computed, as pollinator exclusion bags were found to be removed prior to collection (possibly by strong coastal winds), and inadvertent pollinator visits could not be discounted (Table 2.2). The mean fruit and set seed per population across the study species are reported in Appendix B.1.

For *W. meriana* var. *bulbillifera*, only one population (Chetwynd) set low quantities of fruit and seeds. For cross-pollination treatment (iv), where the pollen was sourced from the Melville Forest population (see Fig. 2.3), mean fruit set was 0.05 ± 0.05 S.E. and mean seed set was 0.10 ± 0.10 S.E. Seed viability was low (8%). For inter-varietal treatment (v), where pollen was sourced from the *W. meriana* var. *meriana* population at Casteron, mean fruit set was 0.10 ± 0.10 S.E. and mean seed set was 0.40 ± 0.40 S.E. Again, seed viability was low (14.3%). There was no significant difference among treatments ($F = 1.121$, df = 4, $p = 0.240$), populations ($F = 1.287$, df = 2, $p = 0.260$) and interaction between treatments and populations ($F = 0.916$, df = 7, $p = 0.532$) for fruit set, and similar inferences were drawn for seed set (Table 2.3).

Both populations of *W. meriana* var. *meriana* readily set fruit and seeds for all hand-pollination treatments, except for self-pollination (treatment [ii]). Overall, all tagged flowers for the control treatment (i) and within-population cross-fertilisation treatment (iii) developed into pods. Seed set for the control treatment was higher (mean = 36.50 ± 1.21 S.E.) compared to the within-population cross-fertilisation treatment (mean = 32.23 ± 1.42 S.E.). Between-population fruit set (mean = 0.70 ± 0.07 S.E.) and seed set (mean = 23.42 ± 2.63 S.E.) was lower than control and within-population treatments. Seed viability ranged from 42.55 % to 50.05 % across control, within- and between-population treatments.

For the inter-varietal cross-pollination (treatment [v]), fruit set (mean = 0.11 ± 0.08 S.E.) and seed set (mean = 3.61 ± 2.53 S.E.) were low but present. For the self-pollen treatment, only one plant in Cape Clear was observed to set fruit (mean = 0.02 ± 0.02 S.E.) and seeds (mean = 0.48 ± 0.48 S.E.), with low seed viability (0.23 %).

There were significant differences in fruit set ($F = 114.000$, df = 4, $p = 0.001$) and seed set ($F = 87.507$, df = 4, $p = 0.001$) among treatments for *W. meriana* var. *meriana*, while population was only a significant factor for seed set

Table 2.2: Results of manual hand-pollination for textitGladiolus gueinzii, *G. tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*. **Fruit** and **Seed** refer to the mean fruit and seed set ± S.E. **Viable** refers to the number of viable seeds, inferred from crush testing. For the treatments, **Control** means no pollinator exclusion, **Self** means pollination with self-pollen, **Within** means cross-pollination with pollen sourced within a population, **Between** means cross-pollination with pollen sourced from other populations, and **Intervariety** refers to cross-pollination between *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana*.

	N	Fruit	Seed	Viable
G. gueinzii				
Control	18	1.00 ± 0.00	31.78 ± 4.16	16.97 ± 0.56
G. tristis				
Control	67	0.00 ± 0.00	0.00 ± 0.00	—
Self	48	0.00 ± 0.00	0.00 ± 0.00	—
Within	53	0.00 ± 0.00	0.00 ± 0.00	—
Between	69	0.00 ± 0.00	0.00 ± 0.00	—
G. undulatus				
Control	24	0.00 ± 0.00	0.00 ± 0.00	—
Self	23	0.00 ± 0.00	0.00 ± 0.00	—
Within	24	0.00 ± 0.00	0.00 ± 0.00	—
Between	24	0.00 ± 0.00	0.00 ± 0.00	—
W. meriana var. bubillifera				
Control	43	0.00 ± 0.00	0.00 ± 0.00	—
Self	45	0.00 ± 0.00	0.00 ± 0.00	—
Within	43	0.00 ± 0.00	0.00 ± 0.00	—
Between	46	0.02 ± 0.02	0.54 ± 0.54	0.04 ± 0.04
Intervariety	28	0.04 ± 0.04	1.00 ± 1.00	0.14 ± 0.14
W. meriana var. meriana				
Control	36	1.00 ± 0.00	36.50 ± 1.21	15.53 ± 1.51
Self	48	0.02 ± 0.02	0.48 ± 0.48	0.23 ± 0.23
Within	31	1.00 ± 0.00	32.23 ± 1.42	16.13 ± 1.44
Between	43	0.70 ± 0.07	23.42 ± 2.63	11.40 ± 1.51
Intervariety	18	0.11 ± 0.08	3.61 ± 2.53	1.28 ± 0.88

($F = 13.202$, df = 1, $p = 0.001$). There was no significant interaction between treatments and populations.

The ISI values for *G. tristis, G. undulatus* and *W. meriana* var. *bulbillifera* showed them to be cross-incompatible (Table 2.3). On the other hand, *W. meriana* var. *meriana* is partially self-incompatible, with ISI value of 0.029 (fruit) and 0.020 (seed). In addition, *W. meriana* var. *meriana* did not experience pollen limitation for fruit (PI = 0.000) and seed (PI = -0.117).

Table 2.3: Permutational ANOVA (PERMANOVA) results for *Gladiolus tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana* for fruit (top) and seed (bottom) set and explanatory factors (hand-pollination treatment, treated population and treatment × population). For each species, the degree of freedom (df), F statistics (F) and p values are reported, as well as the index of self-incompatibility (ISI) and pollen limitation index (PI).

Fruit set	Treatment			Population			Treatment x Population				
	df	F	p	df	F	p	df	F	p	ISI	PI
G. tristis	3	—	—	3	—	—	9	—	—	Infinity	Infinity
G. undulatus	3	—	—	2	—	—	6	—	—	Infinity	Infinity
W. meriana var. bulbillifera	4	1.121	0.240	2	1.287	0.260	7	0.916	0.523	Infinity	Infinity
W. meriana var. meriana	4	114.000	0.001	1	0.811	0.362	3	0.851	0.482	0.029	0.000

Seed set	Treatment			Population			Treatment x Population				
	df	F	p	df	F	p	df	F	p	ISI	PI
G. tristis	3	—	—	3	—	—	9	—	—	Infinity	Infinity
G. undulatus	3	—	—	2	—	—	6	—	—	Infinity	Infinity
W. meriana var. bulbillifera	4	1.177	0.234	2	1.287	0.371	7	0.975	0.441	Infinity	Infinity
W. meriana var. meriana	4	87.507	0.001	1	13.202	0.001	3	2.590	0.066	0.020	-0.117

2.3.2 Pollen viability

Pollen viability assays using Alexander's stain (AS) consistently gave a higher measure of viability, compared to Brewbaker–Kwack (BK) solution (Table 2.4), with viability proportions ranging from 4 times greater for *G. tristis* to over 350 times for *G. undulatus*. The variation in pollen viability were explained by differences in assay ($F = 1030.803$, df = 1, $p = 0.001$), species ($F = 13.213$, df = 2, $p = 0.001$) and their interaction ($F = 20.578$, df = 2, $p = 0.001$) with a PERMANOVA test. The viability assay results for each medium and population are reported in Appendix B.2.

Table 2.4: Pollen viability results based on Alexander's staining and Brewbaker–Kwack methods for *Gladiolus tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*. The sample size, the mean proportion of viable pollen ± S.E. are reported. For each species, a PERMANOVA was used to explain the variation in proportional pollen viability by population; df = degrees of freedom, $F = F$ statistic and $p = P$ value.

	Alexander's stain				
	N	Proportion viable	df	*F*	*p*
G. tristis	15	0.032 ± 0.021	1	3.063	0.177
G. undulatus	15	0.354 ± 0.043	3	14.600	0.002
W. meriana var. bubillifera	527	0.347 ± 0.008	3	189.126	0.001
W. meriana var. meriana	311	0.640 ± 0.012			—
	Brewbaker–Kwack				
	N	Proportion viable	df	*F*	*p*
G. tristis	207	0.008 ± 0.002	4	13.987	0.001
G. undulatus	98	0.001 ± 0.000			—
W. meriana var. bubillifera	10	0.019 ± 0.006			—
W. meriana var. meriana		—			—

Both assays showed low pollen viability levels for *G. tristis* (proportion viable: AS = 3.2 %, BK: 0.8 %). For *G. undulatus*, pollen viability was higher with Alexander's staining (35.4 %) but lower with Brewbaker–Kwack (0.1 %). For *W. meriana* var. *bulbillifera*, pollen viability was 34.7 % (AS) and 1.9 % (BK). *W. meriana* var. *meriana* demonstrated the highest level of pollen viability (AS: 64.0 %).

At the specific level, population was not a significant factor for Alexander's staining for *G. tristis* ($F = 3.063$, df = 1, $p = 0.177$), while it was significant for Brewbaker–Kwack method ($F = 13.987$, df = 4, $p = 0.001$). For *G. undulatus*, population was significant as a fixed factor for the AS assay ($F = 14.600$, df = 3, $p < 0.010$). Similarly for *W. meriana* var. *bulbillifera*, population was an important factor (AS: $F = 189.126$, df = 3, $p = 0.001$).

2.3.3 Reproductive output

Overall, reproductive output were variable among species and propagule type. As the intra-population samples were inadvertently mixed for both populations of *G. tristis*, the total number of cormels were pooled for each sample, giving a sample size of two. Likewise for *G. undulatus* the samples for Stawell (GUAV14) were inadvertently mixed, and pooled as one sample.

G. undulatus registered the lowest output of propagules in the form of cormels (25.08 ± 18.28 S.E.; Table 2.5); on the other hand, *G. undulatus* produced over 14 times as many cormels per plant on average (352.60 ± 26.94 S.E.). For *Watsonia meriana* s.l., *W. meriana* var. *meriana* overall produced almost three times as many propagules (seeds: 418.58 ± 56.62 S.E.) compared to *W. meriana* var. *bulbillifera* (seeds: 30.90 ± 7.98 S.E., aerial cormels: 112.70 ± 11.62 S.E.).

Both propagule type ($F = 14.048$, df = 2, $p = 0.001$) and population ($F = 20.974$, df = 3, $p = 0.001$), and their interaction ($F = 77.235$, df = 6, $p = 0.001$) were important factors explaining the differences in reproductive output. For the additional PERMANOVA carried out on *W. meriana* var. *bulbillifera* with aerial cormels and seed set, propagule type ($F = 99.755$, df = 2, $p = 0.001$), population ($F = 16.982$, df = 2, $p = 0.001$) and their interaction ($F = 13.644$, df = 4, $p = 0.001$) were significant at $\alpha = 0.05$.

Reproductive output by propagule type, species and population are reported in Appendix B.3.

Table 2.5: Summary of propagule type, sample size, mean number of propagules produced per sample (with standard error) for *Gladiolus tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*.

	N	Cormel	Aerial cormel	Seed
G. tristis	2	25.08 ± 18.28	0	0
G. undulatus	7	352.60 ± 26.94	0	0
W. meriana var. bulbillifera	30	0	112.70 ± 11.62	30.90 ± 7.98
W. meriana var. meriana	12	0	0	418.58 ± 56.62

2.3.4 Germination

There was very low rate of growth from cormels over the duration of the experiment (Fig. 2.4). *G. tristis* registered the highest proportion of growth (20 %), while cormels for *G. undulatus* showed a lower growth rate (1 %, Gringegalgona) or had no growth at all.

For *W. meriana* var. *bulbillifera*, aerial cormels collected from Ripon (producing only aerial cormels) showed a high germination rate of 94 % (Fig. 2.5), while those from Chetwynd (producing both seeds and aerial cormels) were 4 times less likely to germinate (22 %). Half of the aerial cormels from Meville

Forest (also observed to produce seeds occasionally) had grown by the end of the experiment.

Seeds from *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana* showed similar dormancy, with both varieties breaking dormancy within 7 days (Fig 2.6). However, germination rate for the mixed-propagule *W. meriana* var. *bulbillifera* population at Chetwynd was slightly lower (95.8 %), compared to seed-only *W. meriana* var. *meriana* populations at Cape Clear and Casterton (both 93.8 %).

PERMANOVA tests showed that propagule type (cormel, aerial cormel and seed) was a significant factor in explaining the variation in proportion germination or growth ($F = 11.944$, df $= 2$, $p < 0.05$), as well as taxon ($F = 4.544$, df $= 3$, $p < 0.05$). However, population was not inferred to be an important factor ($F = 0.612$, df $= 7$, $p = 0.694$).

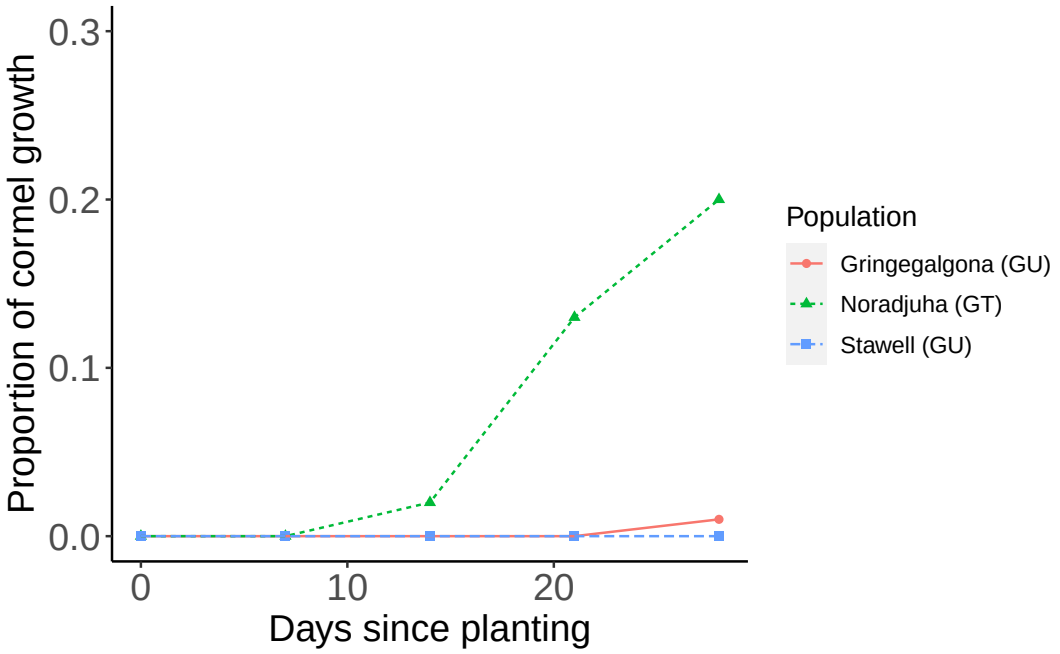

Figure 2.4: Results from growing trials with cormels for *Gladiolus tristis* and *G. undulatus*. For each population, 100 cormels were sown, and growth rates were checked every seven days for four weeks (28 days). On the legend, GT = *G. tristis* and GU = *G. undulatus*. Refer to Figs. 2.1 and 2.2 for the location of the populations.

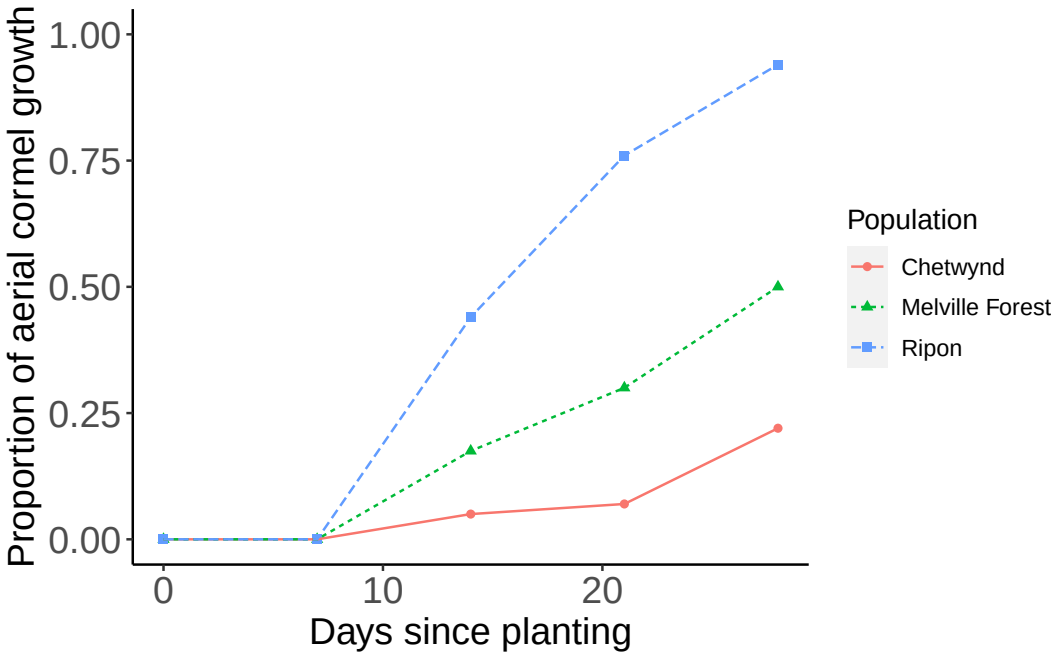

Figure 2.5: Results from growing trials with aerial cormels for *Watsonia meriana* var. *bulbillifera*. For each population, 80 aerial cormels were sown, and growth rates were checked every seven days for four weeks (28 days). The populations at Chetwynd and Melville Forest occasionally set seeds. Refer to Fig. 2.3 for the location of the populations.

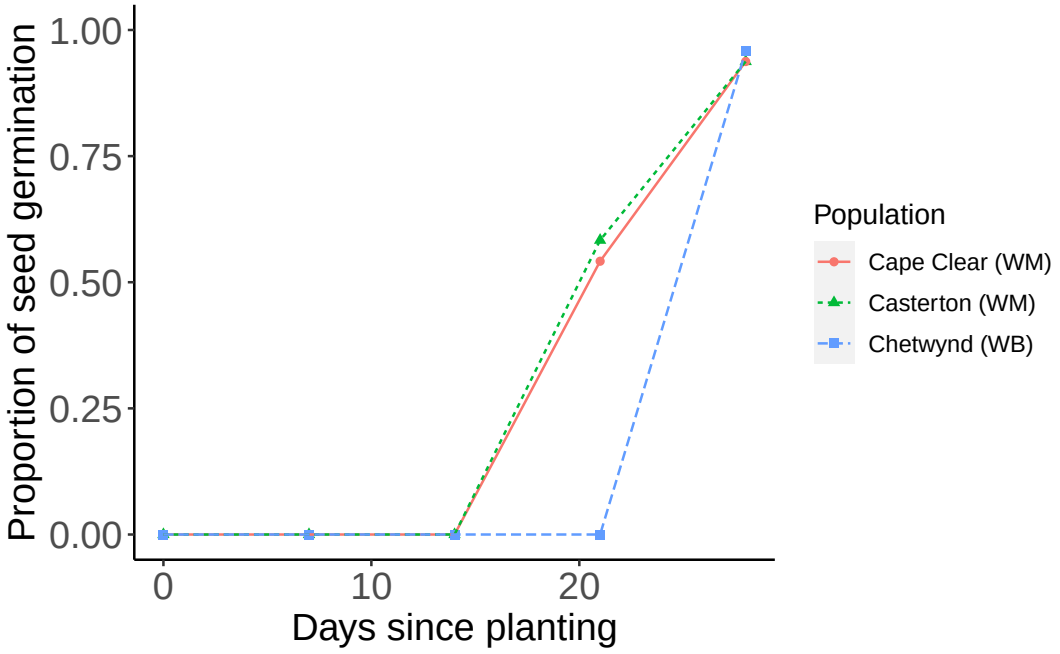

Figure 2.6: Results from germination trials with seeds for *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*. For each population, 48 seeds were sown, and germination rates were checked every seven days for four weeks (28 days). Note that the Chetwynd population predominantly produced aerial cormels rather than seeds. On the legend, WB = *W. meriana* var. *bulbillifera* and WM = *W. meriana* var. *meriana*. Refer to Fig. 2.3 for the location of the populations.

Table 2.6: Results from pollinator visitation observations and moth light trapping on *Gladiolus gueinzii*, *G. tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*. No visitors were recorded for *G. gueinzii*, *G. tristis* and *G. undulatus*. For species visited, WB means *W. meriana* var. *bulbillifera* and WM means *W. meriana* var. *meriana*. Refer to the text for visitor species details.

Species	Order	Family	Species visted	Location	No. days sampled	Total hours sampled	No. of individuals observed	No. trapped
A. mellifera	Hymenoptera	Apidae	WB	Cape Clear	2	4	63	—
			WM	Cape Clear	2	4	48	—
P. novaehollandiae	Passeriformes	Meliphagidae	WB	Chetwynd	1	2	2	—
			WM	Cape Clear	2	4	2	—
			WM	Casterton	3	8	2	—
B. cordelia	Lepidoptera	Oecophoridae	—	Ararat	1	12	—	3
P. antidoxa	Lepidoptera	Oecophoridae	—	Ararat	1	12	—	2

2.3.5 Floral visitors

Both *W. meriana* var. *meriana* and *W. meriana* var. *bulbillifera* attracted similar floral visitors, although their activity varied among populations. In Casterton and Cape Clear, *W. meriana* var. *meriana* was mainly visited by the European honey bee *Apis mellifera*, with bees alighting on anthers and entering the floral tube. An avian visitor, the New Holland honeyeater *Phylidonyris novaehollandiae* was also observed clasping the scape, and dipping its beak into floral tubes in Cape Clear and Casterton. In Cape Clear, the honeyeaters visited only *W. meriana* var. *meriana*, and were not observed to visit neighbouring *W. meriana* var. *bulbillifera* plants. However, New Holland honeyeaters were observed feeding on *W. meriana* var. *bulbillifera* flowers at Chetwynd. No visitors were recorded for other populations of *W. meriana* var. *bulbillifera*.

Neither *G. undulatus* nor *G. gueinzii* were observed to receive visitors. Similarly, noctural observation of *G. tristis* did not reveal any pollinator visitation. Only short-proboscid moths (Oecophoridae: *Barea cordelia* and Oecophoridae: *Piloprepes antidoxa*) were recovered from the moth trap.

2.4 Discussion

The aim of this study was to ascertain the reproductive ecology of introduced Iridaceae in Australia. I found that *G. tristis* and *G. undulatus* only reproduced asexually in Victoria, Australia, and that there was no evidence of pollinator visitation to their flowers. Similarly, *W. meriana* var. *bulbillifera* regenerated primarily via aerial cormels ('bulbils' in some literature), but some populations were recorded setting seeds occasionally. In contrast, *W. meriana* var. *meriana* was found to be predominantly xenogamous, with pollinator service provided by the introduced honeybee (*Apis mellifera*) and the indigenous New Holland honeyeater (*Phylidonyris novaehollandiae*). While the coastal *G. gueinzii* was observed to set seed when treated with self-pollen, the level of reproductive output could not be ascertained for this study.

Both *Gladiolus tristis* and *G. undulatus* are allogamous in the Cape region, with regeneration supplemented by cormel production; in Victoria, sexuality appeared to have been lost because of poor pollen viability and/or a lack of pollinator attraction, and propagation was via cormel production and dispersal. Although an assessment of allogamy was not undertaken, *G. gueinzii* maintained autogamy along the eastern coast of Australia, and was capable of producing myriad cormels that were dispersed by ocean currents (Heyligers, 1999), which compensated for the lack of observed pollinator visitation. *W. meriana* var. *meriana* has retained its ancestral allogamous mating system, and has managed to co-opt local pollinators. Although one instance of self-fertilisation was

recorded, this was insufficient grounds to believe that a mating system transition had occurred. While *Watsonia meriana* var. *bulbillifera* primarily regenerated via axillary cormels, some populations were noted to set fruit and seed, but of low quantity and fertility. There was no fruit set in *G. tristis* and *G. undulatus*, for all experimental hand pollination trials. My observations contradicted van Kleunen and Johnson's (2007b) assumption that high reproductive output and rapid germination in Iridaceae contributed to their naturalisation elsewhere. Specifically, the authors observed seed set in *G. tristis*; however, their seeds were sourced from South Africa.

There are several plausible reasons for the absence of fruit set in *G. tristis* and *G. undulatus* following artificial pollination experiments. Extant plants might have descended from one founding member, or from the same lineage, such that they are all clones of the parental stock (Zohary, 2004), and therefore lack compatible mates. Second, pollen viability was low, which presented a prezygotic barrier to successful fertilisation. Pollen inviability might have arisen during horticulture from hybridisation (Rameau and Gouyon, 1991; Ohri, 2013) or polyploidisation (Kutlunina et al., 2017). Furthermore, pollinator decoupling was evident, with no pollinator activity observed for either species. Thus, the spread of these species in Australia likely stemmed from cormel or human dispersal (e.g. grading of roadsides and/or dumping of garden waste: Hodkinson and Thompson, 1997).

Abundant fruit production was observed for *W. meriana* var. *meriana* all treatments, except for self-pollen, but a single plant in one population (Cape Clear) produced seed when self-pollinated. Although inadvertent contamination with allogamous pollen could not be ruled out, this might corroborate the weak self-incompatibility system in *Watsonia* (Horn, 1962). Moreover, there was no significant pollen limitation in *W. meriana* var. *meriana*. The availability of pollen constrains reproductive output in many species (Knight et al., 2005; Harmon-Threatt et al., 2009), with the risk of failure greater in self-incompatible species (Larson and Barrett, 2000), when a specialist pollinator is lost, or a population is fragmented (Wilcock and Neiland, 2002). As an introduced species with decoupled mutualism, self-incompatibility system, and fragmented populations, *W. meriana* var. *meriana* seemed to exhibit the full gamut of options that should invoke pollen limitation. In contrast to expectations, seed set for the control treatment (where flowers were tagged but not manipulated) was greater than for other manipulative treatments. This may be due to the high number of visitations from pollinators, compared to two applications of pollen during hand-pollination.

In contrast to Conran et al. (2003), who found inviable pollen in four out of five populations for *W. meriana* var. *bulbillifera* in South Australia, all pop-

ulations surveyed in Victoria had some level of pollen viability. However, the differences might arise owing to different methods for the assessment of pollen viability. Adopting the more conservative results based on the Brewbaker–Kwack assay, *G. tristis, G. undulatus* and *W. meriana* var. *bulbillifera* all had low mean values of pollen viability (under 2%). Where Conran et al. (2003) also reported an average of 1.8–3.9 seeds per pod across his *W. meriana* var. *bulbillifera* populations (without pollen supplementation), the Victorian populations only set seed intermittently. At present, the mechanism of seed production in *W. meriana* var. *bulbillifera* is not clear. The fact that seed production occurred (albeit at low levels) in *W. meriana* var. *meriana* when treated with *W. meriana* var. *bulbillifera* pollen, coupled with rare seed production in *W. meriana* var. *bulbillifera* indicated that both pre- and post-zygotic barriers operate in the latter. Although sterility is associated with triploidy, there is a possibility that triploid spores can form bivalent cells during meiosis, and undergo meiotic recombination with diploid cytotypes; such intraspecific cytotype hybridisation has been recorded in *Taraxacum officinale* (van Baarlen et al., 2000).

A review of the number of geographical records for *G. tristis* and *G. undulatus* against *W. meriana* var. *meriana* (see Chapter 1) could not explain their prevalence, given the low growth rates of their cormels shown by the germination tests here. Additional work is required to extricate the reasons why. For one, the study period (28 days) might have been too short, such that dormancy was still arrested at the end of that period (towards the end of winter). In a study on the Chilean geophyte *Zephyra elegans* (Tecophilaeaceae), Yañez et al. (2005) found that while corms resprouted 19–38 days after planting regardless of weight, day and night temperatures affected shoot emergence. However, temperature as a factor seemed unlikely, given the high germination rate of *W. meriana* var. *meriana* seeds. In another study on the Chilean congener, *Z. compacta*, De la Cuadra et al. (2017) determined that the optimum germination temperature for the species was 10–20 °C, which aligned with the optimum day/night temperature of 15/10 °C (Yañez et al., 2005) for *Z. elegans*.

In the Greater Cape Floristic Region, *G. tristis* is pollinated by large moths from order Noctuidae and possibly Sphingidae; Goldblatt and Manning (2002) observed the noctuid moth *Syngrapha circumflexa* as a pollen carrier for *G. tristis*. Another cosmopolitan sphingid moth, the convolvulus hawk moth *Agrius convolvuli* pollinates a close relative, *G. longicollis* (Goldblatt and Manning, 2002). This hawk moth is common in tropical and sub-tropical Australia, where its larvae feed on Convolvulaceae species (Common, 1990), and was expected to co-opt *G. tristis* as a food source. However, it was not observed to do so, possibly arising from phenological mismatch. (*A. convolvuli* is migratory in the Seychelles, where it was recorded from November to February (Matyot, 2005)).

More observations, especially on the northern populations of *G. tristis* and on other study species would be recommended for future work.

Despite the advantage of seeds as specialised dispersal structures, which can propel invasive spread (Rejmanek and Richardson, 1996; Albert et al., 2015), it is unclear why *W. meriana* var. *meriana* is presently more geographically restricted in Victoria, compared to other focal species. Compared to vegetative propagules, seeds may be more susceptible to herbivory (Ronsheim, 1994), or have more stringent germination conditions (Kettenring and Whigham, 2018). Despite the absence of a protective coat, bulbils germinate more readily than seeds in stressful environments (Alsos et al., 2013), and plants derived from seeds may take longer to reach maturity compared to those resprouting from offsets (Fortanier, 1973). In general, vegetative fragments lack dormancy, and are considered more competitive than seeds based on size (Klimeš et al., 1997); in disturbed environments, the robustness of vegetative fragments may be more advantageous than seeds (Dong et al., 2006).

2.4.1 Conclusions and a look ahead

Contrary to my hypothesis that sexual reproduction engenders potential adaptability via genetic recombination (Chapter 1), I have shown that the level of invasiveness, as measured by the prevalence of an introduced species regionally, cannot be solely explained by its reproductive ecology in its adventive range, nor its reproductive ecology in its native range. The xenogamy of *W. meriana* var. *meriana*, facilitated by co-opting local pollinators, and coupled with its high seed output and high germinability did not result in a remarkable presence in Victoria in south-eastern Australia. While the variety is more abundant in Western Australia, current records showed that the predominantly asexual *W. meriana* var. *bulbillifera* was more prevalent across the continent. On the flip side, this study has demonstrated that asexuality (and partial asexuality) does not necessarily retard invasive spread. Future studies should focus on the reproductive ecology of the Iridaceae in their native range, and in particular, the contribution of asexual (cormels) and sexual (seeds) components to population structure. This will form a basis for comparison between their native range in southern Africa and Australia (and other adventive regions). In addition, the present work should be extended to the entire continent, where the answer to the differential invasiveness of the introduced Iridaceae may lie.

Chapter 3

Genetic patterns in introduced *Gladiolus* populations in southeastern Australia

3.1 Introduction

Invasive species have become a global issue, and show no signs of abating (Seebens et al., 2017). Where there is progressive understanding of how biological traits can propel invasiveness (Baker and Stebbins, 1965; Rejmanek and Richardson, 1996; Sakai et al., 2001; Pyšek and Richardson, 2007; van Kleunen et al., 2010b; Hulme and Bernard-Verdier, 2018), fundamental questions continue to plague invasion genetics, such as the contribution of genetic variation to invasiveness (Lee, 2002; Bock et al., 2015; Estoup et al., 2016; Chu et al., 2019). An invasion event comprises four stages: transport, introduction, establishment and spread (Blackburn et al., 2011). Although some species are deliberately introduced in large numbers for human use (Robbins, 2004; Cook and Dias, 2006) or biological control (Vink et al., 2003), other introduction events may involve small numbers of individuals transported over long distances, and little chance of immediate gene flow from their source populations. Frequent demographic and genetic bottlenecks result in low allelic richness and heterozygosity in nascent populations (Dlugosch and Parker, 2008a). Despite adaptive evolution being contingent on additive genetic variance, which may be scarce in introduced populations, rapid adaptation appears to be common in colonising species (Maron et al., 2004; Dlugosch and Parker, 2008b; Buswell et al., 2011; van Boheemen et al., 2019; van Kleunen et al., 2018). It is presently unclear how pre-introduction and post-introduction adaptation determine the outcome of invasion, given that abiotic and biotic contexts may differ between ranges (Bock et al., 2015), or how genetic variation leading to adaptation in invasive species is partitioned between additive and non-additive components (Gilchrist and Lee,

2007). Thus, while invasive species are perceived to have negative effects across biological hierarchies and are economically costly (Leung et al., 2002; Gurevitch and Padilla, 2004; Cook et al., 2007; Gaertner et al., 2009; Pejchar and Mooney, 2009), successful invaders provide "natural" lessons in evolutionary ecology (Sax et al., 2005).

To attain invasive status, a colony needs to spread and establish viable populations elsewhere (Richardson et al., 2000b). However, as invasive spread proceeds, the population may lose allelic richness and heterozygosity, as each naturalisation event during range expansion acts to sample the genetic variability present in the source subpopulation (Carroll and Dingle, 1996). Therefore, the spread of an invasive population leaves a genetic signature of gradual erosion. While naturalisation success is more likely if an introduction is made to a matching environment, other potentially limiting factors determine invasion success (Nuñez and Medley, 2011), such as biotic resistance (Levine et al., 2004), propagule pressure (Lockwood et al., 2005), dispersal limitation (Yakimowski et al., 2005) and environmental disequilibrium (de Andrade et al., 2019). These factors may require adaptive genetic evolution before invasive spread can resume, which depends on the level of additive genetic variation in the population (Lande and Shannon, 1996; Kawecki, 2008).

The reproductive strategy and traits in introduced species are important determinants of genetic diversity (Barrett et al., 2008; Colautti et al., 2010a; Barrett, 2011). Baker (Baker, 1955; Baker and Stebbins, 1965) postulated that species capable of uniparental reproduction would show greater success during colonisation, as they would enjoy reproductive assurance in the absence of mates. While there is evidence for this hypothesis ("Baker's rule": Stebbins, 1957) (Rambuda and Johnson, 2004; van Kleunen and Johnson, 2007a; Burns et al., 2011; Hao et al., 2011; Ward et al., 2012), the question remains whether the benefits of uniparental reproduction extend beyond the incipient stages of an invasion event. Uniparental reproduction, such as asexuality and selfing limit opportunities for recombination and genetic admixture. In small plant populations with self-incompatibility and partial asexuality, asexual reproduction can be more beneficial than sexual reproduction, as the former maintains an excess of heterozygosity, while cross-compatibility is eroded by a decrease in S-allele richness through genetic drift (Navascués et al., 2009). Some invasive asexual populations were found to be genetically uniform or near-uniform, such as the riparian *Arundo donax* (Ahmad et al., 2008), orange hawkweed *Hieracium aurantiacum* (Loomis and Fishman, 2009) and water hyacinth *Eichhornia crassipes* (Zhang et al., 2010). Despite its invasiveness, the capacity for adaptive evolution may be reduced in *E. crassipes*, as seedlings were unable to consistently germinate in temperatures below 30°C despite rare outcrossing (Zhang et al., 2010).

By contrast, obligate self-fertilisation results in elevated levels of homozygosity, and expression of potentially deleterious recessive mutations, particularly for populations transitioning from obligate or near-obligate outcrossing (Bernstein et al., 1985). However, with time these deleterious mutations may be purged by inbreeding depression (Byers and Waller, 1999).

There is ample evidence that invasive species are not genetically depauperate (Dlugosch et al., 2015), and in fact, can boast greater genetic variation compared to native-range populations (Kolbe et al., 2007). Genetic diversity in introduced species can be augmented by multiple introductions (Ellstrand and Schierenbeck, 2000; Keller and Taylor, 2010; Ferrero et al., 2015), and is likely to occur more in cultivated species (Culley and Hardiman, 2009). Admixture can create novel genotypes that give higher fitness in their new range (Lavergne and Molofsky, 2007), but can also give rise to negative epistasis when there is high divergence among source populations, thereby affecting performance (Barker et al., 2019). In addition, diversity may remain low even with admixture (Hagenblad et al., 2015), and a lengthy time lag may act before the benefits of admixture are reaped (Kolbe et al., 2007).

Another process that can enrich additive genetic diversity and variation is polyploidisation. For allopolyploids, where chromosomal content is multiplied through syngamy by different species, the benefits of admixture via hybridisation is automatic (Ellstrand and Schierenbeck, 2000), such as the fixation of heterozygosity for homeologous alleles, masking of recessive alleles via gene redundancy, and transitioning to uniparental reproduction (Comai, 2005). While autopolyploids (where multiple chromosome sets are derived intraspecifically) also benefit from polyploidisation, the odd number of chromosomes in autotriploids and autopentaploids cannot segregate into balanced products during meiosis, resulting in aneuploid gametes and potentially inviable zygotes (Comai, 2005). Invasiveness can be conferred in polyploid taxa if the benefits of polyploidy outweigh the drawbacks; indeed, polyploidy is associated with invasiveness (Pandit, 2006; Pandit et al., 2011; te Beest et al., 2012), although polyploidy may be a short-term advantage (Van de Peer et al., 2017). The enhancement of invasiveness by polyploidisation was illustrated in *Centaurea maculosa*, where the tetraploid dominated the diploid cytotype in their introduced range (Treier et al., 2009). By contrast, the infertile triploid tiger lily *Lilium lancifolium* attained invasive global spread, whereas the diploid cytotype was restricted to its ancestral range; however, its greater range was likely to be brought about by its use as an ornamental and medicinal plant (Herrando-Moraira et al., 2019).

Here, the level of genetic variation and structure in Iridaceae introduced to south-eastern Australia from southern Africa was addressed with microsatellites. Microsatellites are a class of neutral, co-dominant and highly variable molecular

markers, and are well placed to answer questions in evolutionary ecology (Selkoe and Toonen, 2006). Previously (Chapter 2), it has been shown that *Gladiolus gueinzii* was capable of self-fertilisation, while *G. tristis* and *G. undulatus* did not set fruit following manual pollen supplementation, including pollen sourced from mixed populations in Victoria, suggesting that invasive spread in this region was via vegetative fragments (cormels). *Watsonia meriana* var. *bulbillifera* was likewise found to be predominantly asexual, although at least two populations in Victoria demonstrated low seed set. By contrast, *W. meriana* var. *meriana* was primarily outcrossing, although its self-incompatibility system was suspected to be compromised in an instance.

Selfing populations are expected to show reduced genetic variation within populations in the form of low heterozygosity, and increased genetic divergence among populations, due to drift and reduced gene flow (Loveless and Hamrick, 1984). Genetic diversity is also likely to be reduced (Hamrick and Godt, 1996; Nybom, 2004; Glémin et al., 2006). While high levels of genetic diversity have been detected in asexual plant species, such species are expected to demonstrate higher spatial genetic structure compared to seed-setting species, as dispersal is hampered by the relatively larger propagule size (Vallejo-Marín et al., 2010). Thus, genetic variation within populations will be high in clonal species due to persistent genotypes, as genetic equilibrium cannot be achieved in the absence of recombination (Heywood, 1991), but such diversity depends on the number of genets in the population (Loveless and Hamrick, 1984). Moreover, spatial genetic structure also depends on propagule type, as smaller structures such as bulbils experience secondary dispersal from the parent plant (Vallejo-Marín et al., 2010). While biological invasions are no different to natural colonisations (Hoffmann and Courchamp, 2016a,b, but see Wilson et al., 2016), human agency during and after introduction events can confound natural spread patterns (Gravuer et al., 2008). Therefore, the aims here are to evaluate spatial genetic variation and structure in introduced Iridaceae, specifically three *Gladiolus* species, in relation to (1) their reproductive modes, and (2) to the patterns expected from natural populations.

3.2 Materials and methods

3.2.1 Sampling

In 2011–12, leaf samples were collected for all *Gladiolus* species during the vegetative growing period in spring for DNA isolation. Seven populations were sampled each for *G. gueinzii* (3.1), *G. tristis* (3.2) and *G. undulatus* 3.3. For large populations of over 30 ramets, a transect was established across the broadest extent, and 30 ramets were sampled at roughly equidistant points along the

transect, while keeping a minimum distance of 5 m between each sample. For smaller populations, ramets were sampled every 5 m along the transect until the end of the transect. For each plant, around 2 cm of leaf material was collected, and immediately placed in a sealed, labelled bag and rapid dried in self indicating, silica gel orange (Chem-Supply, Gillman, South Australia). Leaf samples were also collected from South African accessions germinated in a glasshouse (seeds sourced from Silverhill Seeds, Cape Town, South Africa; *G. gueinzii*: Plettenberg Bay, Western Cape; *G. tristis*: Swellendam, Western Cape; *G. undulatus*: Gifburg, Western Cape). These South African accessions were included during multiplexing (see below).

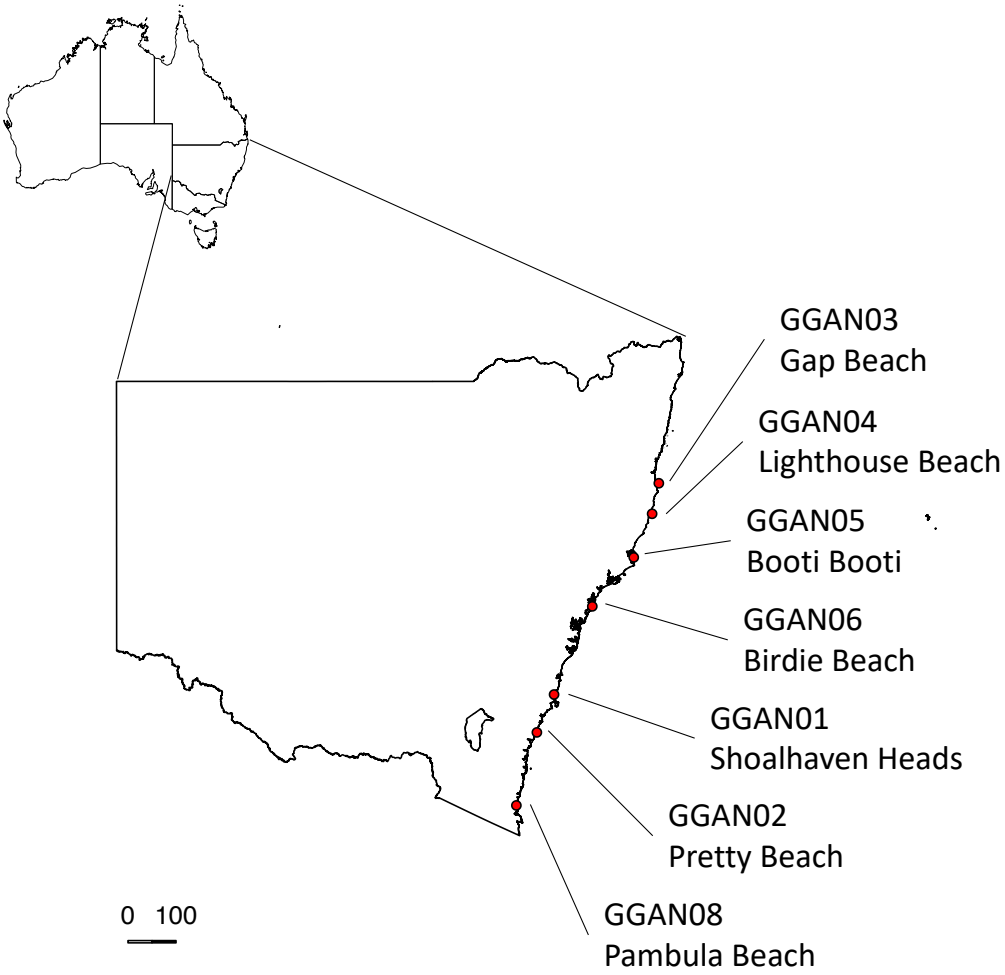

Figure 3.1: Sampling locations for *Gladiolus gueinzii* in New South Wales, Australia. Population identifiers and locations are labelled. Scale bar is in km.

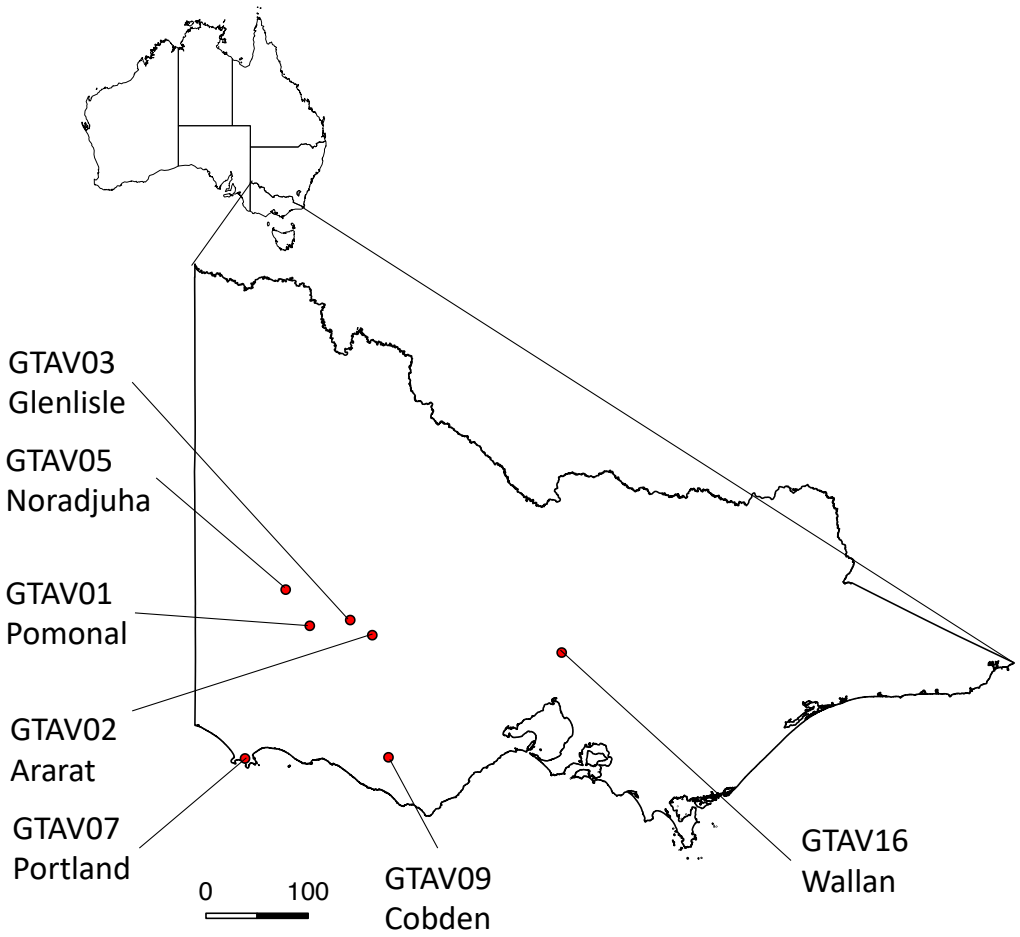

Figure 3.2: Sampling locations for *Gladiolus tristis* in Victoria, Australia. Population identifiers and locations are labelled. Scale bar is in km.

3.2.2 DNA isolation and genotyping

For each specimen, about 20 mg of dessicated leaf material was ruptured using a TissueLyser II (Qiagen, Venlo, The Netherlands). Genomic DNA was isolated using Qiagen DNeasy Plant Mini kit, following the manufacturer's protocol. Purified DNA was eluted with 100 μL of supplied buffer, and stored at -20°C until amplification. DNA quality was assessed by electrophoresis on 1% agarose gel and staining with Biotium Gel Red, and nucleic acid concentration was quantified with NanoDrop Lite (Thermo Fisher Scientific, Waltham, Massachusetts) following the manufacturer's instructions.

Forty-three sets of primers for microsatellites markers developed for *Gladiolus longicollis* (Paul Rymer, unpublished) were assessed for transferability to

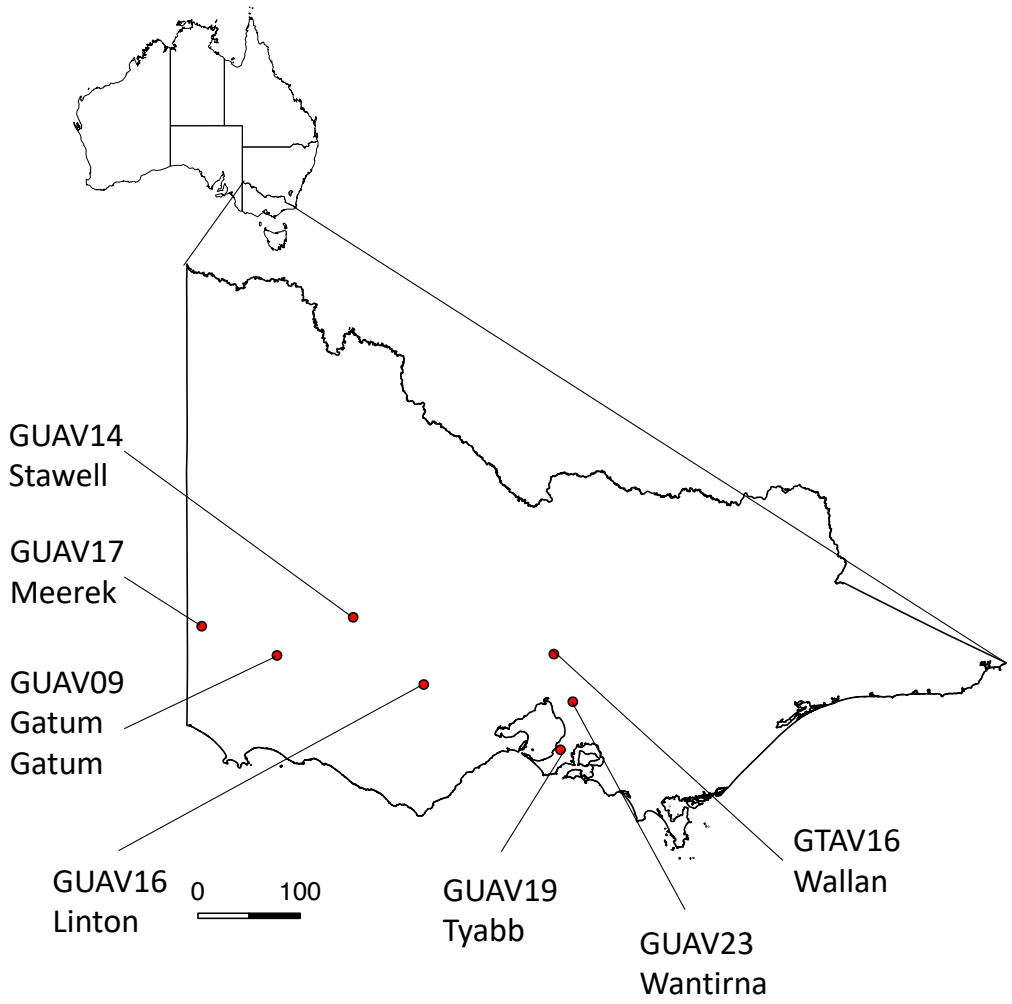

Figure 3.3: Sampling locations for *Gladiolus undulatus* in Victoria, Australia. Population identifiers and locations are labelled. Scale bar is in km.

the focal *Gladiolus* species. Gel electrophoresis was initially used to visualise DNA amplification products, and primer pairs were subsequently screened for polymorphic variation by capillary electrophoresis on the AB3730 platform (provided by AGRF, Melbourne, Australia) on nine accessions (three per *Gladiolus* species). Nine microsatellite loci (GL07, GL13, GL21, GL35, GL40, GL41, GL45, GL63 and GL84) were found to amplify well, with amplicons in the expected fragment size range, and were used to genotype 105 samples (3 species $\times$ 7 populations per species $\times$ 5 individuals per population) by multiplex PCR.

The forward primer of each locus was labelled with a fluorochrome tail (FAM, NED, PET or VIC), with dyes chosen to avoid overlapping between predicted amplicon ranges among loci. Based on size segregation, the nine microsatellite

loci were split into two sets for multiplexing, with five in Set 1 and four in Set 2. The multiplex PCRs were conducted with 25 μL reaction volume, containing Type-it PCR Master Mix (1×), 0.4 μL of each primer, 1 μL of genomic DNA, and RNAse-free water. Both multiplexes were simultaneously amplified on a gradient thermal cycler using a Type-it Microsatellite PCR Kit (Qiagen). Following trials for gradient and touchdown procedures, each PCR was run with an initial activation step at 95°C for 5 min, followed by 28 cycles of denaturation (95°C for 30 s), annealing (60°C for 90 s) and extension (72°C for 30 s), and a final 30 min extension at 60°C. Resultant PCR products were separated by capillary electrophoresis on the AB3730 platform with a 500 LIZ size standard (provided by AGRF, Melbourne, Australia).

Electropherogram profiling was performed in Geneious v9.1.8 (Biomatters, New Zealand). Peak calling was done automatically by the software, and checked manually.

Table 3.1: Details of microsatellite loci used in this study, including the repeat motif size, repeat motif, expected fragment size for *Gladiolus longicollis* (provided by P. Rymer), fragment size range for test species (*G. gueinzii*, *G. tristis* and *G. undulatus*), forward primer labelled fluorophore and PCR mutiplex set.

Locus	Repeat	Repeat motif	Fragment size (bp)	Test species (bp)	Flurophore	Multiplex
GL07	Dinucleotide	$(CT)_{17}$	210	150–240	PET	1
GL13	Trinucleotide	$(GTA)_{17}$	337	300–360	FAM	1
GL21	Dinucleotide	$(GT)_{16}$	166	170–250	NED	1
GL35	Dinucleotide	$(GA)_{30}$	160	120–280	PET	2
GL40	Tetranucleotide	$(GAAA)_{7}$	163	150–190	VIC	2
GL41	Trinucleotide	$(AAC)_{17}$	298	190–330	FAM	2
GL45	Dinucleotide	$(GA)_{19}$	174	150–220	FAM	1
GL63	Dinucleotide	$(CT)_{22}$	215	220–260	NED	2
GL84	Trinucleotide	$(TCT)_{14}$	326	50–200	NED	1

3.2.3 Data analysis

During the screening for microsatellite loci, more than two peaks per locus were observed on the electropherograms at two loci in *G. tristis*, and at one locus in *G. undulatus*. Various mechanisms can give rise to multiple peaks per locus, such as polyploidy, artefacts from PCR or duplication of DNA sequence containing a microsatellite locus (Flores-Rentería and Krohn, 2013). As it was not ascertained that the affected loci were homologous to *G. longicollis*, electropherogram peaks for each species were analysed as dominant presence–absence data. As such, the microsatellite patterns were analysed as multilocus genotypes, in the sense of "allele phenotypes" for dominant markers(Clark and Jasieniuk, 2011), and amplicon richness as "allele phenotype diversity" rather than genetic diversity. Amplicon sizes were converted to a binary matrix with the POLYSAT package (Clark and Jasieniuk, 2011) on the R platform. The binary matrix was further

exported to the ADEGENET package (Jombart, 2008), and a principal component analysis (PCA) was performed to ascertain the variability among populations. All statistical analyses were performed with R (R Core Team, 2019).

3.2.4 Chromosome analysis

Root preparations for assessment of ploidy level were undertaken, given a suspicion of chromosome duplication in *G. tristis* and *G. undulatus*, as more than two amplicons were observed at some loci. Following aestivation, corms were harvested in the summer of 2017 from two populations for each species (*G. tristis*: Ararat and Noradjuha; *G. undulatus*: Gringegalgona and Stawell), and stored at ambient temperature. During mid-winter, the corms were placed in trays containing liquid seaweed solution (Seasol, Bayswater, Australia), and diluted to the manufacturer's recommendation. The solution was changed every 2–3 days. Root development took place at seven days, and root tips were sampled when roots were at least 2 cm in length (approximately 5–7 days after initial growth). The root tips were prepared using a modified procedure in Murray and Young (2001) with an incubation in 0.1% colchicine at 4°C for ten hours, and fixation in acetic–alcohol for 24 hours. They were then transferred to 70% ethanol for storage at -20°C. Before chromosome counting, the tips were hydrolysed in 1M HCl at 60°C for 10 minutes. The terminal tip was excised (1–2 mm) and squashed in FLP orcein on a microscope slide. The slide was warmed briefly over a flame, and photographed with a Toupcam microscope CCD camera (ToupTek, Hangzhou, P. R. China). In total, 30 samples were assessed for each species, with 4–5 root tips per sample.

3.3 Results

3.3.1 Amplicon analysis

The locus GL13 was found to be invariant across all three *Gladiolus* species, and discarded from further analyses. Although some loci amplified well (for example, GL45 and GL84 for *G. gueinzii*), loci generally showed inconsistent amplification across species. In particular, "rooster-comb" patterns were observed for some loci, such as GL35 for *G. tristis* (Fig. 3.4C). For such cases, the rightmost peak was called. Some markers showed confounding peaks that were isolated to one or few accessions; these peaks were either weakly amplified, or had sizes highly different to expected values, and were thus not considered during amplicon calling. Detailed results for amplicon calling are listed in Appendix C.

Generally, there was fidelity in amplicon sizes between African and Australian

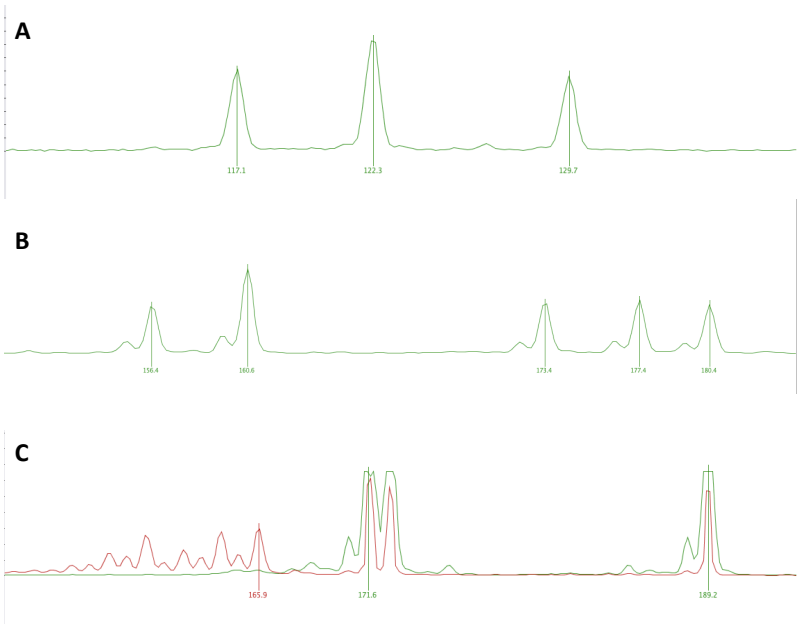

Figure 3.4: Electropherograms giving rise to ambiguity in peak calling. **A** *G. tristis*, locus GL84. Here, the distances between peaks (in bp) are not multiples of the locus repeat size (3 bp). **B** *G. undulatus*, GL40. While the leftmost peak (156.4 bp) is likely to be a stutter peak for the amplicon at 160.6 bp, and the third peak (173.4 bp) is likely to be a stutter peak for the fourth peak (177.4 bp), there is only a 3 bp difference between the fourth and fifth peak (180.4 bp), against the repeat size of 4 bp for this locus. **C** Rooster-comb pattern at GL35 for *G. tristis*. The rightmost peak is called (165.9 bp). The peaks around 172 bp and 190 bp are pulled up by VIC.

accessions (Tables 3.2, 3.3 and 3.4), but notable differences were observed in one locus. For *G. undulatus*, the amplicon size for GL35 was 161 bp in an African accession, but they were larger in Australian accessions (225/255/261 bp).

Australian populations of *G. gueinzii* were invariant for GL21, GL45 and GL84 (Table 3.2). For these invariant loci, all consisted of only one amplicon. Overall, there were high levels of "homozygosity" among Australian populations, with only one locus (GL41) showing more than one amplicon per accession. Overall, amplicon diversity was low. Four loci were monomorphic, and multiple allele phenotypes were observed for GL40.

G. tristis demonstrated the greatest amplicon and allelic phenotypic diversity among the three focal species. All loci had two or more distinctive amplicons, with two loci (GL35 and GL45) scoring six amplicon types (Table 3.3). In addition, there were "fixed heterozygosities" for GL07, GL40, GL41 and GL84, where a single allele phenotype was found in all assessed individuals in a population. Generally, two populations (GTAV02 and GTAV05) bore different allele genotypes at four loci (GL07, GL40, GL41 and GL84) compared to other populations, which had common phenotypes (for example, 181/181 bp for GL07). On the other hand, there was no common amplicon type in GTAV02 and GTAV05 for GL45, with the former population homozygous (215/215 bp) and the latter heterozygous (211/213, 213/230 and 213/231 bp). Multiple amplicons were

Table 3.2: Amplicon sizes for *G. gueinzii* by loci and population, including a South African accession (SA). N = the number of distinctive amplicons observed in Australian populations for that particular locus.

	GL07	GL21	GL40	GL41	GL45	GL63	GL84
SA	209/209	—	176/180	279/282	167/167	230/230	—
GGAN01	207/207 209/209	184/184	168/168 176/176 180/180	278/281	167/167	238/238	95/95
GGAN02	207/207 209/209	184/184	168/168 176/176 180/180	—	167/167	238/238	95/95
GGAN03	207/207 209/209	184/184	168/168 176/176	278/278 278/281	167/167	238/238	95/95
GGAN04	207/207 209/209	184/184	168/168	278/278 278/281	167/167	238/238	95/95
GGAN05	207/207 209/209	184/184	168/168 176/176 180/180	278/278 278/281	167/167	238/238	95/95
GGAN06	207/207 209/209	184/184	168/168 176/176 176/180	278/278 278/281	167/167	238/238	95/95
GGAN07	207/207	184/184	176/176 180/180	278/278 278/281	167/167	238/238	95/95
N	2	1	3	2	1	1	1

registered at GL84 for all populations, which could not be resolved as stutter products, as the distances between peaks were not multiples of the repeat size (3 bp) for this locus. In addition, triplets were observed for the South African accession at GL40 (176/180/184 bp), and for Australian populations at GL41 (278/281/309 and 275/278/297 bp). The preceding peaks were likely to be stutter products, and considered as such.

Allele phenotype diversity in *G. undulatus* was greater than *G. gueinzii*, but less than *G. tristis* (Table 3.4). Individuals were all homozygous at GL07 and GL84. Fixed heterozygosity was detected in two loci (GL45 and GL84), and populations were invariant for these loci. A three-amplicon pattern was observed at GL40, which could not be explained by peak stutter. In contrast to *G. tristis*, where all individuals displayed amplicon triplets, amplicon pairs were present in two populations (GUAV19 and GUAV23). However, the amplicon sizes for these doublets were subsets of triplet sizes within the same population (161/180 bp versus 161/177/180 bp).

The first and second axes of the PCA for *G. gueinzii* explained 36.7% and 16.8% of the variation among populations, respectively (Fig. 3.5A). No distinc-

Table 3.3: Amplicon sizes for *G. tristis* by loci and population, including a South African accession (SA). N = the number of distinctive amplicons observed in Australian populations for that particular locus.

	GL07	GL21	GL35	GL40	GL41	GL45	GL63	GL84
SA	—	175/183	164/164	176/184	281/297	221/231	226/226	127/152
GTAV01	181/181	183/183 183/195	163/163	176/178	281/310	209/209	229/229 229/250	117/122/130
GTAV02	200/204	183/195	163/166 166/166	172/190	278/297	215/215	239/245	126/130/133
GTAV03	181/181	183/183 183/195	161/173 163/173	176/180	281/310	209/209	229/250 239/250	117/122/130
GTAV05	200/204	183/195	166/166	172/190	278/297	211/213 213/230 213/231	245/245	126/130/133
GTAV07	181/181	183/183 183/195	163/163 163/173	176/180	281/310	209/209	239/250	117/122/130
GTAV09	181/181	183/183 183/195	163/163 163/173	176/180	281/310	209/209	239/239 239/250 239/252	117/122/130
GTAV16	181/181	183/183 183/195	163/173 166/173 176/180	176/180	281/310	209/209	239/239 239/250	117/122/130
N	3	2	6	5	4	6	5	5

tive population clustering was observed for this species. On the other hand, there were two distinctive clusters in *G. tristis*, where GTAV02 (orange circles) and GTAV05 (red crosses) were segregated from other populations (Fig. 3.5B). Here, the first and second axes of the PCA explained 66.4% and 8.0% of the variation among populations, respectively. For *G. undulatus*, the first two axes of the PCA explained 46.5% and 11.7% of the total variation among populations (Fig. 3.5C). While the individuals were distributed fairly evenly over PCA space, GUAV14 (mauve triangles) and GUAV09 (orange circles) were more clustered compared to other populations.

3.3.2 Chromosome analysis

Few root tips presented condensed chromosomes, except for one accession of *G. tristis* from Noradjuha, and one sample of *G. undulatus* from Stawell. For *G. tristis*, the chromosome numbers were close to $2n = 2x = 30$ (Fig. 3.6A). However, three root tips of *G. undulatus* presented chromosome numbers of $2n = 31–35$ (Fig. 3.6B).

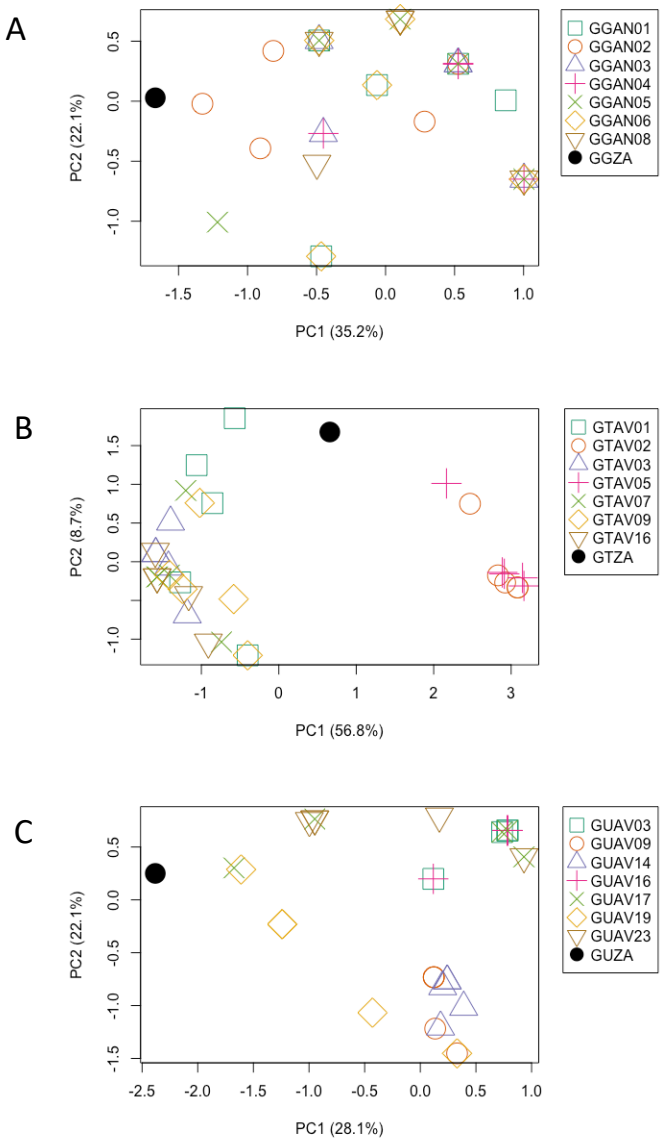

Figure 3.5: Principal component analyses of allelic phenotypic diversity within and among populations. **A**: *G. gueinzii*, **B**: *G. tristis*, and **C**: *G. undulatus*. The first two principal components are shown.

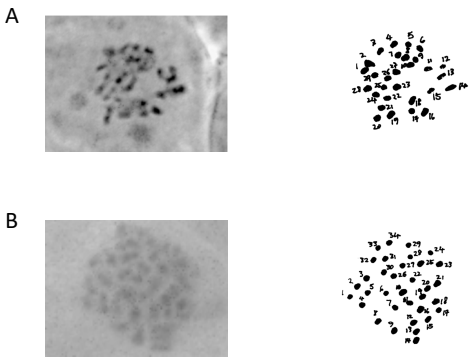

Figure 3.6: Root tip squashes: **A** *G. tristis* sample from Noradjuha (GTAV07), with 29 chromosomes observed, close to the expected $2n = 30$ chromosome number, (B) *G. undulatus* accession from Stawell (GUAV14), showing 34 rather than the expected $2n = 30$ chromosomes. Additional chromosomes may be have obscured by overlapping.

Table 3.4: Amplicon sizes for *G. undulatus* by loci and population, including a South African accession (SA). N = the number of distinctive amplicons observed in Australian populations for a locus.

	GL07	GL35	GL40	GL41	GL45	GL63	GL84
SA	175/175	161/161	176/180	279/282	193/208	242/242	122/125
GUAV03	172/172 174/174	225/261	161/177/180	279/285	184/193	229/241	122/122
GUAV09	172/172 174/174	225/225 225/255	161/177/180	279/285	184/193	229/243	122/122
GUAV14	172/172 174/174	225/255	161/177/180	279/285	184/193	229/229 229/233 229/243	122/122
GUAV16	172/172	225/261	161/177/180	279/285	184/193	229/241	122/122
GUAV17	172/172 174/174	225/261	161/177/180	279/285	184/193	229/241	122/122
GUAV19	172/172 174/174	225/255	161/180 161/177/180	279/279 279/285	184/193	229/243	122/122
GUAV23	172/172 174/174	225/261	161/180 161/177/180	279/285	184/193	229/241	122/122
N	2	3	3	2	2	4	1

3.4 Discussion

On the surface, microsatellite results suggest a lack of population genetic structure in *Gladiolus gueinzii* and *G. undulatus* in south-eastern Australia, while molecular phenotypic structure was partitioned in *G. tristis*. There was also evidence that *G. tristis* and *G. undulatus* were triploid. At the same time, a three-amplicon pattern was detected on one locus of a Southern African accession of *G. tristis*, raising the suspicion of polyploidy in the species' native range, although this might be an artefact (see below).

However, these results must be interpreted with great caution, as the amplicons used in the analyses were not unambiguously identifiable as originating from microsatellite loci homologous with *G. longicollis*. While cross-species transferability of microsatellite markers promises a more cost-effective way to population genetic studies, cross-transferability decreases with phylogenetic distance, as primer sequences may not be conserved across species. The degree of marker transferability depends on the taxonomic group and range: in plants, likelihood is higher within genera, with 60% successful transfers in eudicots, 40% in monocots, and decreasing to 10% across eudicot genera (Barbará et al., 2007). This observation may explain the lack of PCR amplification in *W. meriana* var. *bulbillifera*. For *Gladiolus*, phylogenetic analyses based on derived features such

as corm tunics, cataphylls, and floral and seed characters placed *G. longicollis* and *G. tristis* close together (Section *Homoglossum*, Series *Tristis*), but positioned *G. gueinzii* and *G. undulatus* in a distinct monophylectic group (Section *Blandus*, Series *Sabulosus* and *Blandus*, respectively) (Goldblatt and Manning, 1998). Therefore, the unexpected detection of multiple amplicons on a single locus demands that the underlying fidelity of the markers be questioned.

Multiple amplicons can occur during cross-specific microsatellite marker screening (Chagné et al., 2004; Rutkowski et al., 2011; Kang et al., 2017), which can arise from mosaicism, chimerism or locus duplication (Zamir et al., 2002). Loci with multiple amplification products can be neglected during population genetic analyses, but only with the certainty that the test subject is diploid (Flores-Rentería and Krohn, 2013). However, diploidy could not be assumed for the focal *Gladiolus* taxa, and in particular, *G. tristis* and *G. undulatus*, which were very likely introduced to Australia as ornamental plants. As polyploid ornamentals bear larger flowers over a longer period, induced polyploidy is practised in floriculture (Emsweller and Ruttle, 1941), and colchicine as a polyploidising agent was reported from the late 1930s (Eigsti, 1938; Emsweller and Ruttle, 1941). Moreover, polyploid *Gladiolus* species are self-compatible, as gametophytic self-incompatibility breaks down due to *S*-allele interaction in the pollen (Ohri, 2013); however, this probably applies to tetraploids and even-level polyploids that can evade infertility by polyploidisation (Comai, 2005). Suzuki et al. (2005) reported chromosome doubling in *G. tristis* and other wild congeners by colchicine treatment of callus-induced corms; while no literature on polyploidisation of *G. undulatus* has been uncovered, there is reason to believe that polyploidy can be induced in the latter species, due to the plasticity of the *Gladiolus* genome (Ohri, 2013). Although polyploidy might be artificially induced, tetraploids (rather than odd-level ploidies) would have been produced. Interspecific hybridisation was also unlikely for *G. tristis*, as hybrids do not produce fragrance (Cantor and Tolety, 2011), the latter of which has been noted in all surveyed populations in Victoria (pers. obs.) An alternative explanation is that polyploidisation was triggered by environmental stress (De Storme et al., 2012), such as a climatic shift during introduction to Australia. In addition, the high levels of pollen inviability observed for *G. tristis* and *G. undulatus* agreed with odd-level ploidy. In other species, female fertility was reduced in the triploid grass *Miscanthus sinensis* (Rounsaville et al., 2011), while male sterility was observed in the asexual dandelion *Taraxacum* sect. *Ruderalia* (Meirmans et al., 2006).

At the same time, it is difficult to reconcile polyploidy in *G. tristis* and *G. undulatus* with the results of chromosome counting. While the exact numbers of chromosomes in both species were not definite for want of optical resolution, it

was unlikely that *G. tristis* and *G. undulatus* had chromosome counts exceeding 30 and 35, respectively. By comparison, following the detection of multiple amplicons, polyploidy has been confirmed by chromosome counting in *Grevillea renwickiana* (James and McDougall, 2014) and by flow cytometry in Laperrine's olive *Olea europaea* subsp. *laperrinei* (Besnard and Baali-Cherif, 2009). While the number of chromosomes in *G. tristis* was reasonably close to the diploid number ($x = 15$) (Goldblatt et al., 1993), the excessive chromosomes observed in *G. undulatus* were more difficult to account for.

The contrasting results from PCR amplification and chromosome counting could be explained by cytogeography, the distribution of cytotypes in geographic space. While cytotypes may be spatially segregated (for example, in *Lilium lancifolium*, where diploids and triploids were found in distinctive habitats; Chung et al., 2015), sympatry and perapatry in cytotypes appears to be fairly common. Cytotype admixture was reported in the daisy *Centaurea seridis* (Ferriol et al., 2014), grass *Paspalum intermedium* (Karunarathne et al., 2018) and wattle *Acacia dealbata* (Nghiem et al., 2018), and hybrid zones were detected for the mustard plants *Rorippa austriaca* and *R. sylvestris*, which engendered triploid and pentaploid hybrids (Bleeker and Matthies, 2005). Therefore, it would not be unreasonable to assume that chromosome counting was performed on diploid (or near-diploid) individuals, while PCR amplification was conducted on polyploid individuals in an admixed population, given that chromosome counting was only successful on one sample for each species. For example, rare triploids were detected in diploid populations of *Lilium lancifolium* after intensive surveying (Lee et al., 2016).

The molecular evidence for polyploidy was however by no means forthright. A criterium for marker cross-species transferability is the successful amplification of DNA fragments of expected size, indicating a high degree of sequence homology (King et al., 2001). When such a condition is met, the presence of multiple alleles has been reported as apparent ploidy (for example, McEwen et al., 2011). In general, most markers tested on *Gladiolus* showed reasonable amplicon size fidelity, except for GL84 (expected: 326 bp; observed: 80–137 bp with fluorophore tail accounted for: Blacket et al., 2012). However, three amplicons were detected on a South African *G. tristis* accession for one of the loci (GL40); Australian accessions of *G. undulatus* also demonstrated multiple peaks for this locus. On the other hand, multiple peaks were detected at another locus (GL41) for *G. tristis* individuals in Australia. The extraneous peak reported for the South African accession might be an artefact of PCR, or could indicate true polyploidy in South African populations.

The interpretation of polyploidy from electropherogram profiles alone is obfuscated by stutter bands, which are extraneous PCR products from an allele

that in size by multiples of the repeat unit size, caused typically by slippage of *Taq* polymerase during PCR (Walsh et al., 1996). Thus, the pattern observed on the South African accession of *G. tristis* at the locus GL40—176/180/184 bp—might have arisen from stutter bands of 176 bp and 180 bp from the main fragment of 184 bp. The same argument could be applied to the triplet 126/130/133 bp on GL84 (with a trinucleotide repeat) for the same species: that the 130 bp fragment was a stutter product from the 133 bp allele, and that these accession were in reality biallelic. In a study of triploidy in *Quercus*, a triallelic pattern synonymous with stuttering was detected (Table 1 in Dzialuk et al., 2007), based on a microsatellite with dinucleotide repeat (Steinkellner et al., 1997); here, triploidy was verified by flow cytometry. While stuttering is common for microsatellites with dinucleotide repeats, it is much rarer for tri- and tetranucleotide repeats (Goldstein and Schlötterer, 1999). Furthermore, stutter peaks were found not to exceed 10% of peak heights of main alleles in a tetranuclotide-repeat microsatellite (Walsh et al., 1996), in contrast to the electropherograms observed in the focal *Gladiolus* species. PCR multiplexing requires the careful calibration of steps, without which artefacts such as stuttering and/or incomplete adenylation may occur (Guichoux et al., 2011). In effect, loci with multiple banding or erratic amplification should be discarded during marker screening (Grusz and Pryer, 2015).

Further evidence against the polyploid nature of *G. tristis* and *G. undulatus* arose from studies in cytology, which revealed that the Cape is poor ground for polyploidisation; in particular, polyploidy in Cape Iridaceae is significantly lower than elsewhere (Oberlander et al., 2016). Moreover, Goldblatt et al. (1993) has confirmed the diploid nature of *G. tristis* and *G. undulatus*. Therefore, the suspected case for triploidy in the South African accession of *G. tristis* is puzzling. The presence of more loci (in the broad sense, and not merely microsatellites) could have arisen through differential polysomy between Cape and Australian accessions, which might account for the presence of unexpected amplicons in *G. tristis*. Conversely, Goldblatt and Takei (1993) posited that the chromosome number variability in the African Iridaceae genus *Lapeirousia* was due to cryptic polyploidy, where polyploidisation followed (or was preceded by) descending dysploidy; such a mechanism might apply to *Gladiolus*. Resolution of the discrepancy between molecular and cytological evidence can be brought about by sequencing the affected loci for homology (Peakall et al., 1998), a wider survey of cytology in both regions, optimising the PCR process, and re-amplification of samples (Flores-Rentería and Krohn, 2013). Based on the detection of infertility in *G. tristis* and *G. undulatus*, amplicon size distribution patterns and reasonable amplicon size fidelity, it will be assumed that both species are indeed polyploid in sampled populations.

For *G. gueinzii*, although most populations had more than one amplicon type for four loci, allele phenotypes were highly homozygous. On the other hand, the lack of molecular structure among populations in *G. gueinzii* suggested that the species was predominantly outcrossing, as pollen flow reduces divergence (Loveless and Hamrick, 1984). Moreover, opportunities for outcrossing were likely to be limited by a lack of pollinator visitation observed in the species. Therefore, it was possible that the molecular homogeneity among populations was caused by dispersal of cormels by ocean currents, despite the production of winged seeds adapted for wind dispersal. Cormels were found to remain buoyant for up to six months, and to remain viable for up to 16 months in seawater (Heyligers, 1999). The profuse seed set likely attested to the high degree of autogamy. In contrast to the common reed *Phragmites australis*, where long-distance dispersal by seed assured invasive spread, and vegetative fragmentation to local dominance (Albert et al., 2015), *G. gueinzii* was more likely to achieve long-distance spread through vegetative reproduction, and local dominance through seed production. This hypothesis was supported by the geography of the coastline along south-eastern Australia. The dunes (as habitats for the species) are not contiguous, but are instead nested in bays flanked by headlands, which can be craggy or forested, thus presenting a barrier to seed dispersal by wind. Indeed, surveys around Kiama (34.679°S, 150.854°E) and Tuross Head (36.046°S, 150.141°E) in 2011–12 failed to uncover the species, despite the presence of reproductive populations flanking these locations. The question of how population genetic variation and structure are partitioned between sexual and asexual components will require *bona fide* molecular markers (for example, Koelling et al., 2011).

With respect to spatial molecular structure, *G. tristis* and *G. undulatus* delivered contrasting results, with *G. tristis* in agreement with the expectation of greater structuring in asexual populations from principal component analyses. On the other hand, *G undulatus* had greater molecular phenotypic diversity within populations than *G. tristis* and *G. gueinzii*. Therefore, neither species demonstrated spatial molecular structure consistent with clonal populations, although the presence of prevalent heterozygotic amplicons across the majority of loci suggested a high degree of fixed heterozygosity, in agreement with the genetics variation expected of clonal organisms (Balloux et al., 2003). Instead, the inter-population molecular homogeneity of *G. undulatus*, as demonstrated by PCA, suggested the dispersal of one or few individuals from an original source population across Victoria, with little mutation occurring during invasive spread. By contrast, PCA results indicated that there were two putative source populations of *G. tristis* with distinctive genetic variation in Victoria. A situation similar to *G. undulatus* might apply to *G. tristis*, with one or more propagules dispersing to form a new population, and with little or no mutation

during invasive spread. However, the inference of invasion route from genetic data requires the derivation of robust genetic and/or demographic parameters (Estoup and Guillemaud, 2010), which is beyond the capacity of the present study. However, the geographic locations of *G. tristis* gives an indication of spread vector. PCA demonstrated molecular similarity between GTAV02 and GTAV05 in western Victoria, however, GTAV03 (Glenlisle) was substantially closer to GTAV05. The roads linking GTAV03 and GTAV05 were surveyed during the flowering season (when the species was most conspicuous) from 2011 to 2014, but no intervening populations have been found. Therefore, population spread in *G. tristis* was likely by jump dispersal (Wilson et al., 2009) through human agency. Likewise, the isolated nature of *G. undulatus* populations indicated a similar mode of dispersal.

While molecular data suggested that *G. gueinzii* was predominantly autogamous, and that *G. tristis* and *G. undulatus* were asexual, facultative sexual reproduction in Australia overall could not be ruled out for *G. tristis* and *G. undulatus*, although no fruit or seed set was observed during pollen supplementation experiments (Chapter 2). Primarily, the lack of confidence that the markers used in this study were homologous microsatellite loci, coupled with the low number of samples per population (5), precluded the calculation of population genetic indices. In addition, the confirmation of clonality in a lineage requires the demonstration of identical genotypes between parent and offspring to distinguish similar genetic patterns from other mating systems, as apparent clonality can surface when an ancestral progenitor with low genetic variation bear autogamous offspring (Ahrens and James, 2015). In particular, the multilocus genotypic signature from clonal propagation is difficult to distinguish from sexual reproduction, and cannot be inferred based on the detection of identical genotypes in a population sample (Gregorius, 2005). Although the genetic diversity and variation in focal *Gladiolus* species were not quantified in this study, it is reasonable to expect that substantial levels of genetic diversity exist in their native range, as these species outcross in the Cape region (Goldblatt and Manning, 1998). More comprehensive studies of the genetics and cytology of introduced Iridaceae globally would be illuminating.

Chapter 4

Multiply, and go forth: Potential distribution and niche dynamics of invasive Iridaceae in Australia

4.1 Introduction

The fundamental niche of a species is the multidimensional abiotic space within which the species can maintain a positive growth rate (Hutchinson, 1957). Despite the spatial contiguity of environments, a biological population will inevitably encounter new conditions upon movement (or for plants, propagule dispersal), to which they must adapt to, or perish. The mechanisms and rates of evolution at range margins have long been a pressing query (Sexton et al., 2009; Alexander and Edwards, 2010). Biological invasions—the long-distance transport of biota by human agency—present extreme cases in saltatory population movement, and the question of how successful invaders are able to adapt rapidly to novel environments can enhance the understanding of eco-evolutionary processes (Sax et al., 2005).

Apart from the likelihood of novel environments, introduced populations may also face demographic challenges. Introduction events can invoke negative Allee effects if they involve one or few individuals, which hinder reproductive success and population persistence in the new range (Taylor and Hastings, 2005). In turn, the reproductive system of a nascent population plays a prominent role in its invasiveness, as it dictates the population genetic structure and genetic variability, and capacity for adaptation and spread (Barrett et al., 2008; Barrett, 2011). Therefore, the reproduction strategies of an introduced population determine its demography and niche dynamics (Pulliam, 2000). In particular, genetic recombination via outcrossing generates new genotypes with high fitness for novel environments (McDonald et al., 2016). While self-fertility and asexual forms of reproduction provide reproductive assurance, genetic diversity

is expected to diminish in the long term, and reduce invasiveness (Sakai et al., 2001).

Despite the crucial role of reproduction success in biological invasions, there are currently few studies relating niche behaviour to plant reproduction for introduced populations (Dellinger et al., 2016). It is widely held that most species occupy only a portion of their fundamental niche (that is, their realised niche), due to biotic interactions, dispersal limitations or ongoing range infilling that constrain expansion into novel but tolerable environments (Svenning and Skov, 2004; Soberón and Peterson, 2005). Previous studies have addressed this issue by relating habitat heterogeneity to reproductive traits. For example, Lambrinos (2001) showed that the sexual *Cortaderia selloana* expanded more rapidly into a greater set of environments compared to its asexual congener, *C. jubata*. Common-garden experiments and reciprocal transplants have also been used to demonstrate evolution in invasive species, such as a shift to earlier flowering in *Xanthium strumarium* (Griffith and Watson, 2006) and *Lythrum salicaria* (Colautti and Barrett, 2013) towards higher latitudes.

Although a direct manipulation of species traits may serve to uncover instances of local adaptation, this approach is usually unable to discern whether such evolutionary processes are indeed triggered by environmental change (fundamental niche evolution), or by other processes operating (or not) in the new range (Pearman et al., 2008). For example, an introduced plant population may evolve to allocate more resources to growth than defence after escaping from specialist herbivores in their native range (evolution of increased competitive ability (EICA): Blossey and Notzold, 1995), but this represents an evolution of realised, rather than fundamental niche. Specifically, the elucidation of niche properties for biological invasions needs to account for environmental variability between native and introduced ranges.

Until recently, there was no consistent approach to niche quantification between geographical ranges, leading to contrasting results in plants (Gallagher et al., 2010; Petitpierre et al., 2012; Early and Sax, 2014). However, the combination of randomisation tests (Warren et al., 2008) and ordination (Broennimann et al., 2012) provided a way to quantify niche dynamics in an ecological niche model (ENM) between two species or between two geographical locations of a species. In particular, comparison of results from randomisation tests for species occurrence patterns and environment prevalence permitted the inference of niche conservatism or divergence (Di Cola et al., 2017). Using this approach, Datta et al. (2019) showed that the invasive apomictic plant *Ageratina adenophora* has undergone niche expansion globally, although the shift likely acted on the realised rather than the fundamental niche. In another study, Ahmad et al. (2019) found different niche overlap levels globally in the invasive *Leucanthemum vul-*

gare, a highly variable cosmopolitan species (Clements et al., 2004).

Species distribution modelling identifies species–environment correlative patterns in their native range (the training region), which can then be projected to another region (the target region) for distribution mapping (Elith et al., 2010). As a species distribution model (SDM) is typically built upon the biology of a species, it inherits the concept of the ecological niche as its conceptual foundation. Although some authors use the terms ENM and SDM interchangeably, here I distinguish them by their output: ENMs report on a species' niche characteristics (niche positions, widths and shifts), whereas SDMs predict its geographic distribution patterns, whether projected onto its native range or elsewhere (but see Peterson and Soberón, 2012). Beyond predicting species distributions, SDMs serve two additional goals. First, species occurrence locations falling outside of the projected space can signify the presence of processes that disrupt the equilibrium between regions (Albright et al., 2010), which can corroborate ENM findings. Second, as a statistical method, SDMs can identify key variables (predictors) that drive species distribution (Araújo and Guisan, 2006). However, there is little consensus currently on predictor selection (Petitpierre et al., 2017; Gardner et al., 2019) and algorithm choice (Feng et al., 2019; Qiao et al., 2019).

The use of closely-related species with different focal traits provides a powerful method for investigating invasiveness, which may otherwise be obscured by phylogenetic bias (Grotkopp et al., 2010). Previously, van Kleunen and Johnson (2007b) has shown that Iridaceae with rapid and profuse seedling emergence had higher rates of naturalisation, and further work (van Kleunen et al., 2008) demonstrated that naturalisation was linked to autonomous fruit set and seed production. Here, I extend their research by investigating the potential versus current distributions and the ecological niche dynamics of five Iridaceae introduced from southern Africa to Australia, in relation to their reproductive modes. It was shown previously (Chapter 2) that *Gladiolus tristis* and *G. undulatus* were obligately clonal in Victoria, Australia, and that the coastal species *G. gueinzii* in New South Wales was self-fertile. *Watsonia meriana* var. *bulbillifera* was primarily clonal, but some populations set low amounts of seed. By contrast, *W. meriana* var. *meriana* was found to be predominantly xenogamous, although weak self-compatibility was suspected. At present, the species' potential distribution and niche dynamics in Australia are unknown.

There are three overarching aims in this chapter. First, I estimate model performance among the study species; in particular, I ascertain whether there is any consensus in algorithm and predictor selection across the species. Second, I investigate the degree of actual versus potential geographical occupancy by species distribution modelling. Third, I hypothesise that xenogamy engenders adaptive potential through genetic recombination, which leads to a more

pronounced niche evolution, compared to self-fertile and asexual study species.

4.2 Methods

4.2.1 Species distribution modelling

Occurrence data

Species occurrence data for Africa and Australia were collated from the Global Biodiversity Information Facility (GBIF: www.gbif.org; see Chapter 1), and supplemented by field surveys in Australia (New South Wales, South Australia and Victoria) between 2011–2015. Field survey data were recorded with a handheld GPS (Garmin: GPS 60), and positional accuracy of ±5 m were typically achieved. I used Google Maps (maps.google.com) and Google Earth to georeference GBIF records with missing or clearly imprecise occurrence points (for example, in a marine environment). Duplicate records were next removed with the duplicates function in the R package DISMO (Hijmans et al., 2017), and remaining records then thinned to reduce spatial autocorrelation using SPTHIN (Aiello-Lammens et al., 2015), such that adjacent occurrence points within 10 km of each other were pruned. All data manipulation, modelling and statistical analyses were conducted in R version 3.5.0 (R Core Team, 2019) unless otherwise stated.

Habitat suitability and distribution modelling algorithms

Various classes of models have been used in SDM; however, there is no agreement on which model yields the best predictive power (Qiao et al., 2015). I used three algorithms commonly employed in invasive SDM: a generalised linear model (GLM; linear regression), a random forest model (RF, bagging tree: Breiman, 2001), and Maxent (maximum entropy: Phillips et al., 2006). All three models are supervised ones, that is, they require a response variable, such as presence–absence data for training, although random forest is capable of unsupervised learning (Shi and Horvath, 2006), and Maxent has often been touted as a presence–only algorithm. In reality, Maxent generates background points to discriminate suitable habitat from possibly unsuitable ones (Elith et al., 2011). The distinction between presence–absence and presence–only is important, as absence records are difficult to obtain (Mackenzie and Royle, 2005).

Environmental predictors — data sources

The class of predictors, and the selection of a final set of predictors used in modelling (feature subsetting) determines the predictive prowess of a model (Petitpierre et al., 2017). Climate is traditionally thought to delimit niche space, and

thus govern species distribution; WorldClim (Hijmans et al., 2005), comprising nineteen bioclimatic predictors, has largely been the default predictor set over the last decade for species and niche modelling. However, advances in climatology has produced better bioclimatic datasets (Peterson and Nakazawa, 2008). In place of WorldClim, two recent environmental predictor datasets were used: ENVIREM (Title and Bemmels, 2018) and CHELSA (Karger et al., 2017). In addition, a soil predictor dataset (SoilGrids250m: Hengl et al., 2017) was included, as edaphic factors may delineate plant species distribution (Beauregard and de Blois, 2014, but see Ehrenfeld et al., 1997).

The ENVIREM (Title and Bemmels, 2018) dataset comprises sixteen climatic and two topographic predictors, which relate to a species' physiology, and therefore, niche space. For example, evapotranspiration has been physiologically linked to plant growth, and in particular I anticipated that topographic wetness index and terrain roughness index may be relevant to my study species, as they are found in mesic habitats. Although categorical predictors can be approximated to continuous ones (for example, coded to a dummy variable), I excluded the single categorical predictor, *monthCountByTemp10* (denoting the number of months above 10°C) for GLM and RF modeling, following Title and Bemmels (2018). However, as Maxent accepts categorical predictors, *monthCountByTemp10* was included in Maxent modeling.

Although ENVIREM complements, rather than replaces the WorldClim bioclimatic variables, I selected the CHELSA dataset as a companion dataset to ENVIREM. CHELSA (Karger et al., 2017) was built on newer statistical downscaling algorithms, and showed an improvement over WorldClim in a head-to-head comparison by the authors (see also Maria and Udo, 2017). The CHELSA dataset contains nineteen predictors, which are similar to the WorldClim bioclimatic variables.

For edaphic predictors, I downloaded the SoilGrids250m (Hengl et al., 2017) data set at 5 cm depth, as Iridaceae corms were usually found between 5–10 cm below the soil surface. (*Gladiolus gueinzii* corms are usually buried much deeper, but in a fairly homogenious sandy substrate; pers. obs.) The SoilGrids predictor layers enumerated standard soil properties such as bulk density, cation exchange capacity, organic carbon, pH, soil texture fractions, and coarse fragments.

All environmental datasets were downloaded at the highest resolution: 0.5 arc seconds for CHELSA and ENVIREM, and 0.125 arc seconds for SoilGrids250m, and then up-scaled to 2.5 arc minutes (*c.* 5 km), as this approximates the average spatial uncertainty in the GBIF records (Connor et al., 2018). The southern African raster layers were then cropped to a latitude just north of Windhoek, Namibia, at the boundary of the warm desert climate (Köppen

climate classification; Schulze, 1947). The Australian raster layers were cropped, and masked by the Australian country border GIS shape file (downloaded from gadm.org).

In addition, a custom predictor, *DIST_COAST* was formulated with *G. gueinzii* in mind. Pilot models showed that pre-packaged bioclimatic and edaphic variables predicted the distribution of the species in the hinterland, regardless of threshold value. *DIST_COAST* is a raster layer, with cell values measuring the distance of a cell from the nearest coast. The set of predictors used for species distribution and ecological niche modelling is shown on Table 4.1.

Environmental predictors — subsetting

There is now a plethora of feature-subsetting techniques (Cobos et al., 2019); it would be enlightening, but senseless to explore them in their entirety. Popular variable selection methods in SDM are filter methods such as correlation testing and principal component analysis (PCA: Guichón and Cassini, 1999). These assess the predictors for relevance prior to modelling itself, and eliminate those that are redundant, or do not meet some preconceived criterion. It may be quite arbitrary, when presented with two highly collinear variables, to choose which one to exclude, and collinear variables may not be entirely a bad thing — for example, in random forest (Li et al., 2016). There are questions whether regression-based variable selection methods, such as PCA or variance inflation factor analysis, can readily be applied to other model classes (Kuhn and Johnson, 2013). Here, tailored variable selection methods were explored to select variables, including wrapper methods, which perform feature subsetting during model run.

Models may be optimised by parameterisation (that is, tuning). Although models may perform quite well at their default settings, tweaking is advised, particularly for machine learning algorithms, as they are not easily interpretable (Kuhn and Johnson, 2013, p. 64). Where available, built-in tools were used for model tuning. For random forest, `tuneRF` in the R package RANDOMFOREST (Liaw and Wiener, 2002) selected the best *mtry* value (the number of variables randomly sampled as candidates at each branch split), and a custom script was written to identify the optimal *ntree* value (the number of trees to grow). For Maxent, ENMEVAL (Muscarella et al., 2014) calculated the best feature classes (lineal, quadratic, product, threshold, and hinge) and regularisation (beta) multiplier, as well as the optimal predictor number. Both R packages were run with all predictors, although a category predictor, *monthCountByTemp10* (from ENVIREM) was excluded from RF tuning, as this predictor was never selected during feature subsetting. The default settings for GLM were maintained (quadratic interactions between predictors), as higher-level interactions may lead to overly-complex models, overfitting, and ultimately, poor predictive

Table 4.1: Predictor set used in species distribution and ecological niche modelling.

Predictor	Abbreviation	Description
CHELSA		
CHELSA1	CH_AnnMT	Annual mean temperature
CHELSA2	CH_MDiur	Mean diurnal range
CHELSA3	CH_Isoth	Isothermality
CHELSA4	CH_Tsea	Temperature seasonality
CHELSA5	CH_WMtMT	Max temperature of warmest month
CHELSA6	CH_MiTColdMt	Min temperature of coldest month
CHELSA7	CH_TAnnRg	Temperature annual range
CHELSA8	CH_WetQMT	Mean temperature of wettest quarter
CHELSA9	CH_DryQMT	Mean temperature of driest quarter
CHELSA10	CH_WarmQMT	Mean temperature of warmest quarter
CHELSA11	CH_ColdQMT	Mean temperature of coldest quarter
CHELSA12	CH_AnnP	Annual precipitation
CHELSA13	CH_WetMtP	Precipitation of wettest month
CHELSA14	CH_DryMtP	Precipitation of driest month
CHELSA15	CH_Psea	Precipitation seasonality
CHELSA16	CH_WetQP	Precipitation of wettest quarter
CHELSA17	CH_DryQP	Precipitation of driest quarter
CHELSA18	CH_WarmQP	Precipitation of warmest quarter
CHELSA19	CH_ColdQP	Precipitation of coldest quarter
ENVIREM		
annualPET	EN_AnnPotEva	Annual potential evapotranspiration
aridityIndexThornthwaite	EN_Thornthwaite	Thornthwaite aridity index
climaticMoistureIndex	EN_MoistIndex	Relative wetness and aridity
continentality	EN_Continental	Temp difference between warmest and coldest months
embergerQ	EN_EmbergerQ	Emberger's pluviothermic quotient
growingDegDays0	EN_GrowDay0	Months above 0^{o}C mean temp sum × number of days
growingDegDays5	EN_GrowDay5	Months above 5^{o}C mean temp sum × number of days
maxTempColdest	EN_MaxTColdMt	Max temp of the coldest month
minTempWarmest	EN_MinTWarmMt	Min temp of the coldest month
monthCountByTemp10	EN_CountT10	Months with mean temp greater than 10^{o}C
PETColdestQuarter	EN_PETColdQ	Mean monthly PET of coldest quarter
PETDriestQuarter	EN_PETDryQ	Mean monthly PET of driest quarter
PETseasonality	EN_PETMtVar	Monthly variability in potential evapotranspiration
PETWarmestQuarter	EN_PETWarmQ	Mean monthly PET of warmest quarter
PETWettestQuarter	EN_PETWetQ	Mean monthly PET of wettest quarter
thermicityIndex	EN_Themicity	Compensated thermicity index
topoWet	EN_TopoWet	Terrain roughness index
tri	EN_Terrain	SAGA-GIS topographic wetness index
SOILGRIDS		
BLDFIE	SG_BulkDens	Bulk density
CECSOL	SG_Cation	Cation exchange capacity
CLYPPT	SG_Clay	Percentage of clay
CRFVOL	SG_Coarse	Percentage of coarse fragments
ORCDRC	SG_OrgCar	Organic carbon content
PHIHOX	SG_pHWater	pH index in water
PHIKCL	SG_pHKCl	pH index in KCl
SLTPPT	SG_Silt	Percentage of silt
SNDPPT	SG_Sand	Percentage of sand
CUSTOM		
DIST_COAST	DIST_COAST	Distance from coast
DIST_CITIES	DIST_CITIES	Distance from nearest city/cities

power (García-Callejas and Araújo, 2016).

All GLMs and RF models were run on BIOMOD2 (Thuiller et al., 2016), and Maxent was run on DISMO (Hijmans et al., 2017). For all three algorithms, I ran models with the full suite of predictors (less categorical ones for GLM and RF), versus models with pared predictor sets. Three filter methods were used for GLM: variance inflation factor (VIF) analysis (Brauner and Shacham, 1998), implemented in VIFSTEP and VIFCOR from the USDM package (Naimi et al., 2014), and the stepAIC function in MASS (Ripley, 2018), which implements stepwise model selection by AIC. Although armed with a common goal of eliminating highly correlated variables, VIFSTEP and VIFCOR mostly produced dissonant results, and there is no clear instruction in the literature how to reconcile this outcome. Therefore, predictor subsets produced by all three methods were fed into GLMs, which were later validated independently. In addition, BIOMOD2 enumerates variable importance by AIC as part of its model output (Thuiller et al., 2016), although the exact mechanism was not specified in the package manual or help. Predictors deemed important in a full–suite model were selected, and re-run in the next model iteration.

In its native R formulation, RANDOMFOREST calculates variable importance by order of accuracy or Gini coefficient; there is usually some semblance of similarity, but seldom concordance, between both rankings. Unlike VIFSTEP and VIFCOR above, there are algorithms, such as BORUTA (Kursa and Rudnicki, 2010) that provide a unified ranking of variable importance. I also used a backward selection algorithm, recursive feature elimination (RFE) in CARET (Kuhn and Johnson, 2013). RFE starts with a full model and calculates variable importance; here, I used the native RANDOMFOREST importance criterion. RFE iteratively builds new models from a pruned predictor set, until there is no improvement to the model. RFE returns the optimal number of predictors, and the predictors themselves.

Maxent too provides measures of variable importance by jackknifing, which gives the percent contribution of each predictor to the model (calculated based on AUC scores for a model running with that one predictor), and the permutation importance (the drop in AUC when that predictor was left out). Again, there was frequently close agreement between both rankings, but rarely perfect concordance. Here, I developed a heuristic distance-based measure of variable importance for Maxent (HDMM).

I combined (i) percent contribution and (ii) permutation importance, with the following model performance indices from the Maxent output file: training gain (iii) with and (iv) without predictor, test gain (v) with and (vi) without predictor, and AUC (vii) with and (viii) without the predictor. The larger the index, the more important the predictor, for (i) (ii), (iv), (vi) and (viii).

After adding one to all values to avoid division by zero, the reciprocal of these indices was calculated (so that an index value was now inversely proportional to its importance). Then the inverse covariance matrix of all indices was derived using var in R, and the Mahalanobis distance calculated to the origin of the multidimensional space. In this way, the most important variable is the farthest from the origin, and the least important ones closest to it.

The Mahalanobis distances were plotted in decreasing order, and the number of predictors to retain was estimated from an "elbow plot", akin to the estimation of retained axes in PCA (James et al., 2013, p. 384). Elbow plots typically show L–shaped graphs, when less than three predictors dominate the rankings. More predictors can be identified by superimposing a smoothing loess graph on the elbow plot. In addition to this heuristic method, feature selection was also performed during model tuning in ENMeval, a package specially coded for Maxent optimisation. ENMeval reports the predictor count for the model with the best parameters.

Lastly, I used a model–independent feature selection tool, varrank (Kratzer and Furrer, 2018), which implements a minimum redundancy maximum relevance (mRMRe) algorithm. In short, varrank scores a predictor by calculating how its relevance, against a global predictor set, may be negated by its redundancy, multiplied by a normalising function. I chose the peng method for the normalising function, as the default esteves method is computationally demanding. For the discretisation method, I chose a forward–search algorithm, the sturges rule, and mid method for conflating relevance and redundancy, following the example in Kratzer and Furrer (2018), as there was no apparent advantage in selecting other options. varrank returns a positive score for a relevant predictor, and a negative score for a redundant one. As the package does not identify an optimal number of predictors, I again used an elbow plot to estimate this parameter. These plots showed that varrank tended to be more conservative compared to variable ranking methods employed for GLM and RF, resulting in fewer favourable predictors. Because Maxent is capable of performing well with few variables, I used the varrank predictor subset mainly for Maxent modelling. In all, a total of 59 models were run.

Generating pseudo-absences

In addition to predictor subsetting, model performance is also governed by several factors. Much has been written on data quality and quantity (for example, Guisan et al., 2007; Cayuela et al., 2009); intuitively, the more records, and the more accurate and precise these records are makes for better predictions. Presence records are rarely complemented by true absence records, so generated pseudo-absence data need to reflect where a species is reasonably absent, and

model performance is contingent on the astute selection of a geographical boundary for populating pseudo-absence points (Barve et al., 2011). An ambitiously large extent may suppress the contribution of climatic variables in the core area of distribution (Acevedo et al., 2012), or produce spurious model predictions (Thuiller et al., 2004). Conversely, a small extent may result in overly conservative environmental covariation, so that the model cannot distinguish habitable (or potentially habitable) areas from uninhabitable ones.

Absence locations were simulated by background, or pseudo-absence points, generated by a three-step selection in MOPA (Iturbide et al., 2015) for each study species and algorithm. In the first step, pseudo-absence points were sampled over the study area. Then, the geographical extent over which pseudo-absences were sampled was delimited by environmental profiling. Lastly, the optimal threshold distance was defined to be that point when the model area under the receiver operating characteristic curve (AUC) exceeded the asymptotic AUC. Background point data were then extracted from MOPA at that threshold distance for downstream model input and validation.

The background extent, or the area allocated to pseudo–absence generation affects model performance (VanDerWal et al., 2009). Recently, it was recognised that species occurrence points in environmental space can be perceived as random points located on a mathematical space, and can therefore be described by a Poisson point process (Renner et al., 2015). In place of pseudo-absences, Poisson point process modelling (PPM) uses quadrature points to estimate model likelihood, or presence intensity; the model is thus not constrained by background size. For a study area, the number of quadrature points is increased, until model accuracy does not improve with more quadrature points; this then represents the optimal quadrature cut-off for that model. As maximum entropy has mathematical resemblance to Poisson PPM, Maxent co-opts Poisson PPM readily, with a slight modification to its parameters (Renner and Warton, 2013). Here, I integrated Poisson PPM in my Maxent models, using the code published in Renner et al. (2015).

Model validation

Presence–only occurrence data limit model validation, as one can never be sure that a study organism does not occupy geographic space outside its recorded occurrence pattern, even when distribution is assumed to be at or near equilibrium (Varela et al., 2009; Hastie and Fithian, 2013). Therefore, the use of discriminatory indices for model performance, such as the AUC, has been criticised, as background points are not necessarily synonymous with absence points (Lobo et al., 2008). Furthermore, evaluation metrics for presence–only HSMDs may only provide a relative measure of model performance, as background or

pseudo-absence data can be generated in various ways (for example, see Phillips et al., 2009; Barbet-Massin et al., 2012; Bariotakis and Pirintsos, 2018); thus models should only be compared across species within the same study area, or across models with the same species (Guisan et al., 2017). Other measures, such as model sensitivity and omission rate can circumvent the lack of absence data; however these indices require the prior assignment of a threshold to calculate false positive and false negative rates.

It is therefore possible to impute model performance, by the judicious setting of a threshold value (Liu et al., 2005). Given a model–generated predictive map, a threshold value is a cut-off value, such that probabilities of occurrence below that threshold are assigned as absence points, and those above as presence points. Typically, a mid-point value of 0.5 is adopted as the threshold; however, this is arbitrary. Based on Pearson (2010), I wrote an R script to test the significance of a sensitivity score, using an exact one-tailed binomial test. I start with threshold $\tau = 0.5$, with lower bound = 0 and upper bound = 1; if the test p-value $<\alpha$ ($= 0.5$), then the lower bound was moved to 0.5 and τ increased by moving it to the new mid-point (0.75). New τ values were then recursively computed, until the absolute change in p-value was <0.01. The last threshold value, τ thus calculated was used to calculate a confusion matrix, and model sensitivity, specificity, Kappa and true skill statistic (TSS = sensitivity + specificity - 1) values, using the PRESENCEABSENCE package (Freeman and Moisen, 2008).

Three other indices were also calculated: the absolute validation index (AVI: Hirzel and Arlettaz, 2003), the contrast validation index (CVI: Hirzel et al., 2004), and the proportion of the predicted occupancy area. AVI computes the proportion of presence points above a pre-selected threshold value, and ranges from 0 to 1. CVI is a modified AVI that corrects for the theoretical case of presence point prediction with prevalence = 1; CVI takes values from 0 to 0.5. I also calculated Boyce's index (Boyce et al., 2002), a threshold–independent measure of presence–only model performance. The Boyce method splits the model prediction into b bins (or classes), and computes the proportion of spatial cells with presences in each bin, and is implemented in ECOSPAT (Di Cola et al., 2017). A well-discriminating model will predict more presences in bins containing more suitable habitat, compared to bins with less suitable habitats. The Boyce index ranges from -1 to +1, signifying counter predictions (i.e., predicting presences where an organism should not be found), to a perfect prediction of habitat suitability. There are two ways to derive Boyce's index: by feeding the presence points and environmental rasters to ECOSPAT, or by supplying the predicted probabilities of occurrence of a subset of locations to the package. Both methods yielded different index values. For consistency, I chose the first method, which gave more conservative measures.

Lastly, the Akaike Information Criterion (AIC), the corrected AIC (AIC_c), and Bayesian Information Criterion (BIC) for all models were calculated in the stand-alone software NICHEA (Qiao et al., 2016). Although promulgated by Burnham and Anderson (2002) as a measure of model precision, these information–theoretic indices are not free from criticism; for example, they provide useful information only if the same sampling regime is maintained across disparate data sets (Brewer et al., 2016), a condition that is unlikely to hold in herbarium specimens. As such, these were not used to select the optimum model. Instead, the scores for each model's sensitivity, TSS and Boyce's index were averaged, and the optimal model for each study taxon was chosen on maximum score.

To ascertain whether algorithm or feature subsetting had an effect on model performance, I applied energy statistical tests using the ENERGY package (Rizzo and Székely, 2018), with threshold value, proportion of occupancy, AVI, CVI, AUC, model sensitivity and specificity, and Boyce's index as independent variables, algorithm or subset as response variables. Here, subset meant whether the full suite or a subset of predictors were used for modelling. Energy statistics are inferences based on energy distance, which is a metric that estimates the distance between statistical observations in multivariate space (Székely and Rizzo, 2004; Rizzo and Székely, 2010).

Finally, a statistical test for variation among predictor importance during subsetting was performed. First, predictor importance values for the optimal models per species were tabulated, and each predictor was weighted by its position (that is, contribution), as determined by the model algorithm. Unselected predictors were assigned a zero value. The weighted contributions per model were normalised, and a mean value was then calculated for all predictors. This mean value is indicative of the its importance across all models. To test for variability among predictor sets, I used an aligned rank transformation ANOVA (Wobbrock et al., 2011) encoded in the R package ARTOOL (Kay and Wobbrock, 2019), with species and predictors as factors. A post-hoc analysis was performed by pairwise contrasts of least-square means derived from a linear model, as suggested by the package manual. The post-hoc test was run on the EMMEANS package (Lenth, 2020).

Estimation of geographical occupancy

For each species, two estimates of geographical occupancy were quantified from the probabilistic and binary (threshold) rasters for the best model for that species. For the first estimate, the number of habitable cells on the raster was determined by subtracting the number of cells with zero probability of occurrence from the total number of cells. The relative probabilistic occupancy was calculated as

the number of occupied cells / total number of habitable cells ×100%.
Similarly, the relative threshold occupancy was calculated as

the number of occupied cells / total number of cells with absolute probability ×100%.
Calculations were performed on rasters with 2.5-arc second resolution in RASTER.

4.2.2 Ecological niche modelling

To assess niche characteristics, an ordination technique (principal component analysis, PCA) was used to create a predictive model for species occurrence patterns against environmental variation. First, the PCA was calibrated on both ranges against the predictor set associated with the species' optimum model. The southern African range was delimited by the bioregions where the species was recorded, and the Australian range was delineated by the amalgamation of the potential distribution (as predicted by the optimum model) and a convex hull of the presence points of the species. Next, the two-dimensional PCA space was divided into a 100 ×100 grid, and transformed by a kernel density function to create a smoothed occurrence density grid (Broennimann et al., 2012).

Niche overlap between southern African and Australian populations was quantified with Schoener's D (Schoener, 1968; Warren et al., 2008) in the ECOSPAT R package (Di Cola et al., 2017). Niche shifting was interpreted as a shift in occurrence density centroids between native and introduced ranges. Niche conservation (or divergence) however could only be inferred from a combination of two tests. The first test for niche equivalency randomised occurrence points between both regions, while the second test for niche similarity randomised background cells in the environmental grid between both regions. For both tests, the overlap indices for simulated runs were compared to the observed indices, and evidence for niche conservatism (or divergence) inferred from the resulting distribution of indices.

Null hypotheses for permutation tests assume that there is no effect of treatment on compared populations (Manly, 2007). Here, both niche tests operate on different null hypotheses. The null hypothesis for the niche equivalency test assumes that there is no effect of region on species distribution patterns (Warren et al., 2008). In other words, niche overlap is not expected to change significantly if individuals (occurrence points) are randomly allocated between both populations, and overlap indices recalculated from the new species occurrence density grids. A small p-value here indicates that niches across both ranges are more similar (equivalent) than expected from chance alone. For this test, I selected the "greater" option for the alternative hypothesis in ECOSPAT to test for niche conservatism, and the "lower" option to test for niche divergence.

On the other hand, the null hypothesis for the niche similarity test assumes

Table 4.2: Interpretation of niche equivalency test and niche (background) similarity test results in ECOSPAT, based on the critical region $\alpha = 0.05$. Upper-tailed refers to the "greater" alternative hypothesis (testing for niche conservation), and lower-tailed refers to the "lower" alternative hypothesis (testing for niche divergence). Twelve other combinations are inconclusive and not tabled here.

Scenario	Niche equivalency test ($p \leq 0.05$)	Niche similarity test ($p \leq 0.05$)	Inference
1	Upper-tailed	Upper-tailed	Niche conservation
2	Upper-tailed	Lower-tailed	Niche conservation
3	Lower-tailed	Upper-tailed	Niche divergence
4	Lower-tailed	Lower-tailed	Non-analogous environment

that there is no effect of the environment on species distribution, that is, the null hypothesis of niche similarity is rejected if the environments in both ranges are more similar than expected by chance (Broennimann et al., 2012). By contrast to the niche equivalence test, the similarity test is implemented in a different manner in ECOSPAT. Instead of simply allocating grid cells randomly, a new density centroid is randomly selected among grid cells, and a new species occurrence density distribution is generated around this centroid. In effect, the density pattern is shifted, and overlap indices recalculated. A small p-value here indicates that the species occupies environments in both regions that are more similar than by chance. For this test, I selected the "greater" alternative hypothesis to test whether the background was more similar than expected, the "lower" option to test whether it was more different than expected, for a one-way randomisation of density grids (only the Australia grid is randomised).

Realised niche conservation occurs when niches and background are more similar than expected; that is, when both niche equivalency and similarity tests are significant for upper one-tailed tests of conservation. Conversely, a "true" niche evolution may be possible if the niche equivalency test is significant for a lower one-tailed test, meaning that the species has expanded its niche into non-analogue environments (that is, environments not present in the native range) (Williams and Jackson, 2007). However, inference of niche divergence in non-analogue space is not well-founded, as comparisons of biological phenomena break down if there are no common environments (Guisan et al., 2014). Therefore, niche divergence is only conclusive when the niche equivalency test is significant for divergence, and the niche similarity test is significant for environmental similarity. Interpretations are shown on Table 4.2. For both tests, I used 500 iterations, as pilot simulations showed that the mean values of simulated D converged at that point.

ECOSPAT also calculates niche stability, expansion, and unfilling. Niche stability and expansion represent the proportion of the introduced-range niche overlapping, or non-overlapping, with the native-range niche, while niche unfilling concerns the amount of native-range niche not realised in the introduced range

(Guisan et al., 2014). Here, niche proportions were calculated (1) over the entire environment extent, (2) over analogous environment, and (3) while progressively excluding marginal environments (5, 10, 15, 20 and 25 percentiles of outlying environments).

Niche analyses were compared to multivariate environmental similarity surfaces (MESS: Elith et al., 2010). MESS were generated by calculating multidimensional distances between environmental values at a species' occurrence points in its native range and those in the entire introduced range. The MESS metric is a relative measure proportional to the environmental similarity between regions. A negative MESS value denotes dissimilarity. MESS was run on DISMO.

4.3 Results

4.3.1 Species distribution modelling

There was no consistent "best" algorithm for the five optimal models (Tables 4.3 and 4.4) or across all models (Fig. 4.1). For the optimal models, the highest mean score (92.8%) across Boyce's index, model sensitivity and true skill statistic (TSS) was achieved by RANDOM FOREST with BIOMOD-generated subsetting. Although three models scored an average of over 90% for Boyce's index, sensitivity and true skill statistic independently, no single model achieved a score of 90% over all three measures in tandem. Random forest was the most consistent performer, and placed first for three taxa, but with different predictor subsetting methods (boruta, varrank and BIOMOD), but only achieved top ranking for Boyce's index for *Watsonia meriana* var. *bulbillifera*. While GLM for *W. meriana* var. *meriana* modelled with the full set of predictors scored best for sensitivity and TSS, it had the lowest score for Boyce's index.

Energy tests showed that there was no significant difference among models for predictor subsetting method for the optimal models (Table 4.4). On the other hand, there were differences in relation to algorithm and overall model performance indices. Algorithms were inferred to be important for *Gladiolus gueinzii* (p-value: 0.0020) and *G. undulatus* (p-value: 0.0020). The variation among algorithms is likely to arise from the poorer performance from GLMs for Boyce's index, sensitivity and TSS for these two species, which occurred across all models (Fig. 4.2). Overall optimal model performance was significantly different only for *G. undulatus* across 13 model parameters, and was likely due to extremities in AUC, specificity, TSS and Boyce's indices. This was reflected in the poor scores for Boyce's index (GLM), model sensitivity (GLM and Maxent) and TSS (GLM) for all models for this species (Fig. 4.2). In particular, all GLMs for *G. undulatus* reported negative values for Boyce's index.

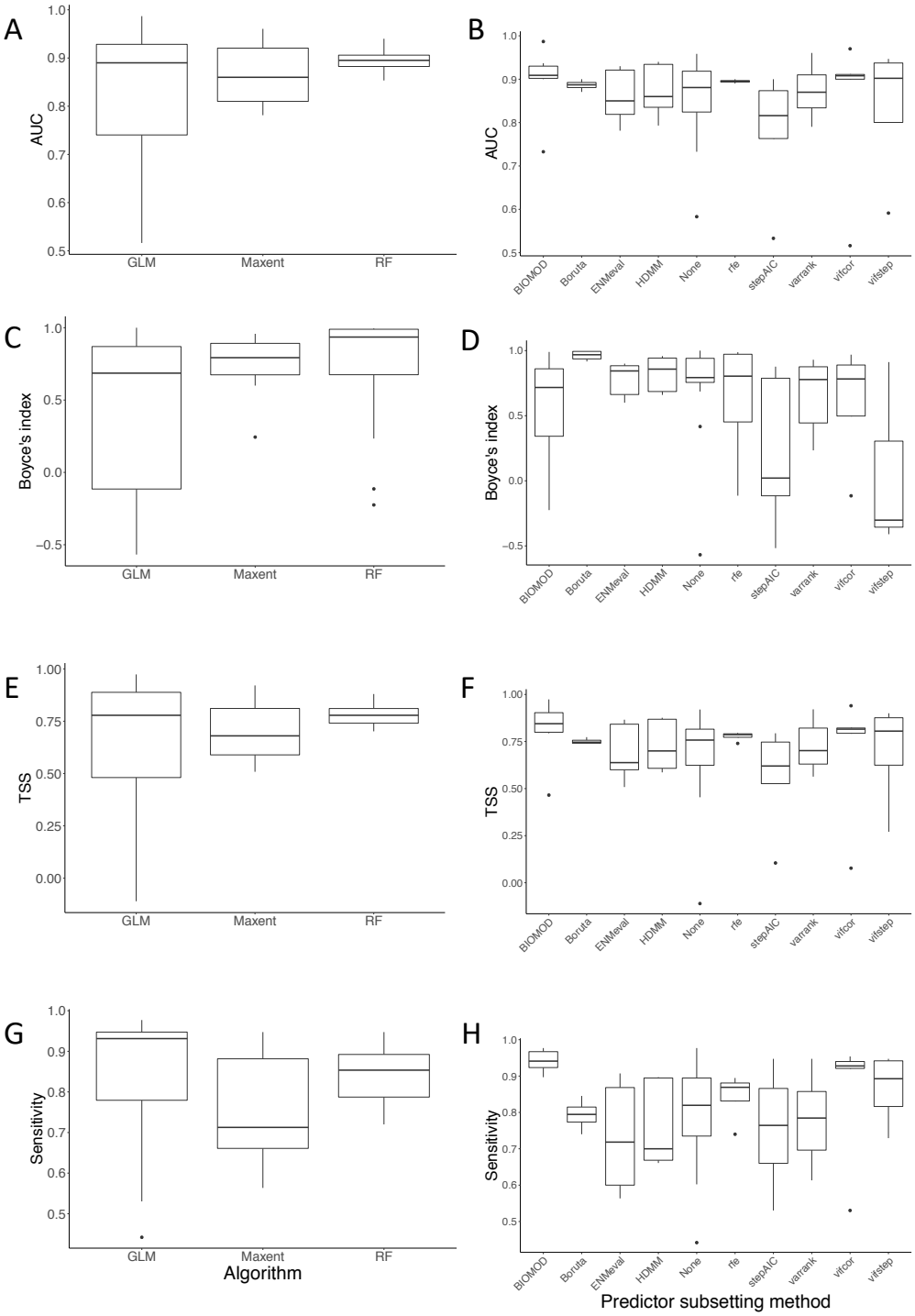

Figure 4.1: Model performance over all species distribution models (total 59) for *Gladiolus gueinzii*, *G. tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*, as measured by area under curve (AUC), Boyce's index, true skill statistic (TSS) and model sensitivity, with respect to algorithm (Panels **A**, **C**, **E**, **G**) and predictor subsetting method (Panels **B**, **D**, **F**, **H**). For algorithms, GLM = generalised linear model, and RF = random forest.

Table 4.3: The best performing species distribution model for predicting the distribution of introduced Iridaceae in Australia, by the main model algorithm and the predictor selection algorithm. The number of predictors used in modelling stemmed from the predictor selection process. Models were assessed based on the average of Boyce's index, model sensitivity, true skill statistic (TSS), with a higher average value signifying better predictive performance. The species modelled are textitGladiolus gueinzii, *G. tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*.

Species	Model algorithm	Subsetting algorithm	Predictors	Boyce's index	Sensitivity	TSS	Mean
G. gueinzii	Random forest	varrank	11	0.930	0.880	0.880	0.897
G. tristis	Maxent	HDMM	15	0.958	0.897	0.876	0.910
G. undulatus	Random forest	boruta	43	0.943	0.785	0.751	0.826
W. meriana var. *meriana*	Random forest	BIOMOD	21	0.990	0.920	0.874	0.928
W. meriana var. bulbillifera	GLM	None	45	0.885	0.974	0.895	0.918
			Mean	0.941	0.891	0.855	

Table 4.4: Energy tests for species distribution model performance variability on five introduced Iridaceae taxa: textitGladiolus gueinzii, *G. tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*. Three tests were run for each species: by algorithm (GLM, Maxent or random forest), predictor set (all, or a subset of predictors used in modelling), and by model results (threshold, occupancy level, AVI, CVI, AUC, sensitivity, specificity, TSS and Boyce's index values). All tests were run using the ENERGY package in R.

	Algorithm		Predictor		Overall model		
Species	E-statistic	p-value	E-statistic	p-value	N	E-statistic	p-value
G. gueinzii	6.515	0.002	0.318	0.683	12	23.306	0.172
G. tristis	767.260	0.176	57.833	0.435	12	10711.000	0.453
G. undulatus	303.240	0.002	7.373	0.567	12	1405.200	0.004
W. meriana var. *bulbillifera*	90.151	0.100	7.210	1.000	11	883.000	0.530
W. meriana var. *meriana*	26.690	0.321	5.351	0.485	12	529.840	0.279

In addition, there was no support for an optimal predictor selection method (Tables 4.3 and 4.3). Indeed, there was no significant difference among methods giving rise to model optimality. There was also no indication of consistency among predictor subsetting methods (Fig. 4.1). In other words, there was no evidence that the heuristic distance-based measure (HDMM) of variable importance for Maxent performed any better than other feature subsetting methods operating under Maxent or other algorithms. However, HDMM was used for variable selection for the sole case where Maxent outperformed other algorithms for *G. tristis*, with model sensitivity and Boyce's index above 0.9. HDMM also scored the highest Boyce's index (0.943) for *W. meriana* var. *meriana*, but was outperformed here by GLM (BIOMOD2/STEPAIC) and Maxent with VARRANK. Overall, HDMM performance was middling (Fig. 4.1).

The three most important predictors for each species were (Fig. 4.3): dis-

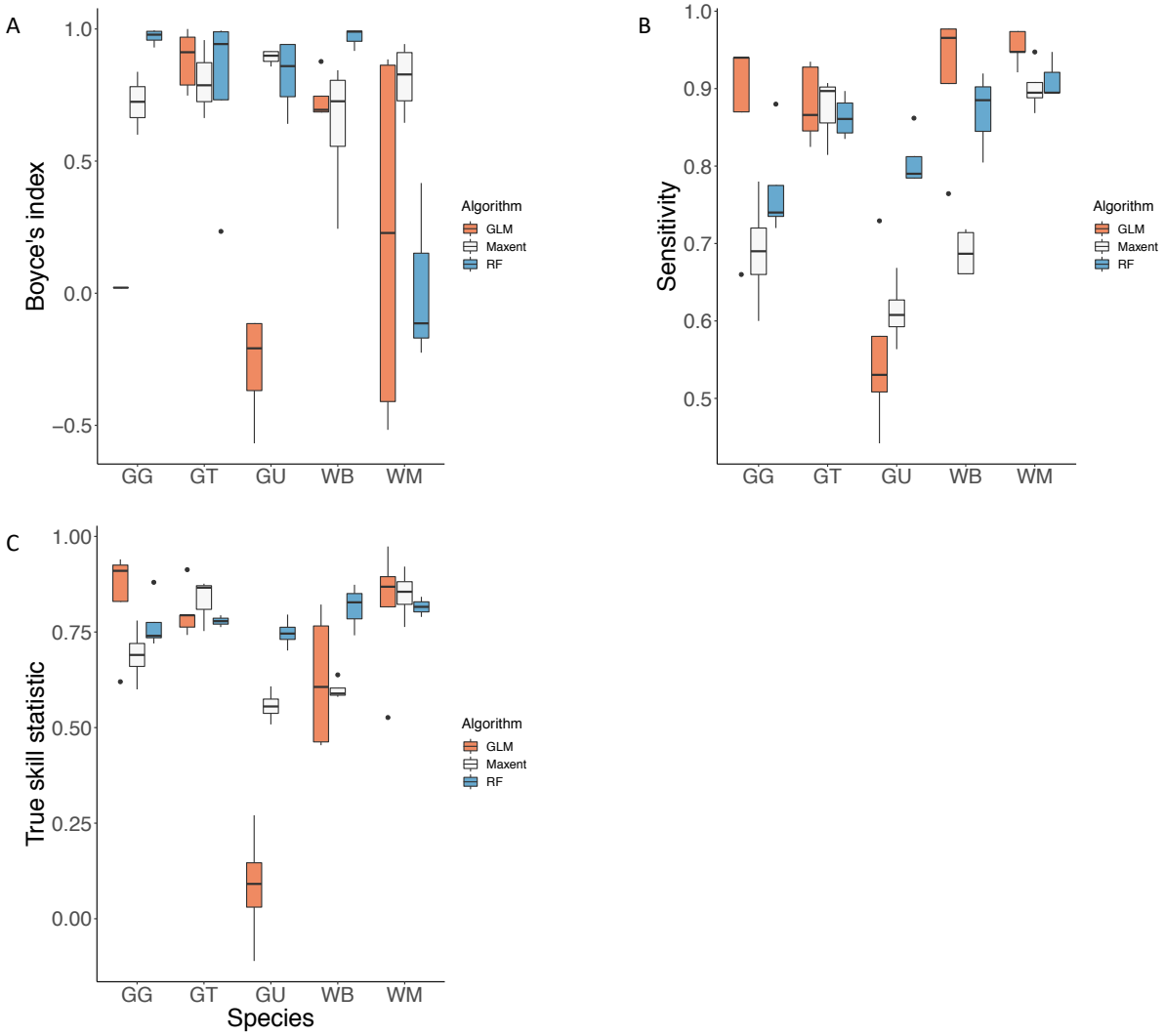

Figure 4.2: Model performance over all species distribution models (total 59) for *Gladiolus gueinzii*, *G. tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*, as measured by **A**: Boyce's index, **B**: model sensitivity and **C**: true skill statistic (TSS), with respect to algorithm and focal species. For algorithm, GLM = generalised linear model, RF = random forest. For species, GG = *G. gueinzii*, GT = *G. tristis*, GU = *G. undulatus*, WB = *W. meriana* var. *bulbillifera* and WM = *W. meriana* var. *meriana*.

tance from coast, the PET of the warmest quarter and continentality (*G. gueinii*); precipitation of the coldest quarter (CHELSA19), the number of months with a mean temperature above 10 °C, and PET for the wettest quarter (*G. tristis*); the mean temperature of the wettest quarter (CHELSA8), PET for the warmest quarter and the mean temperature of the driest quarter (CHELSA9) (*G. undulatus*); the mean temperature of the driest quarter (CHELSA9), precipitation in the wettest quarter (CHELSA16) and precipitation of the wettest month (CHELSA13) (*W. meriana* var. *bulbillifera*); and precipitation seasonality (CHELSA15), pH index in water and PET for the warmest quarter (*W. meriana* var. *meriana*).

Overall, the twelve variables comprising the first quartile by rank across all species were: warmest quarter PET, pH index in water, seasonal precipitation,

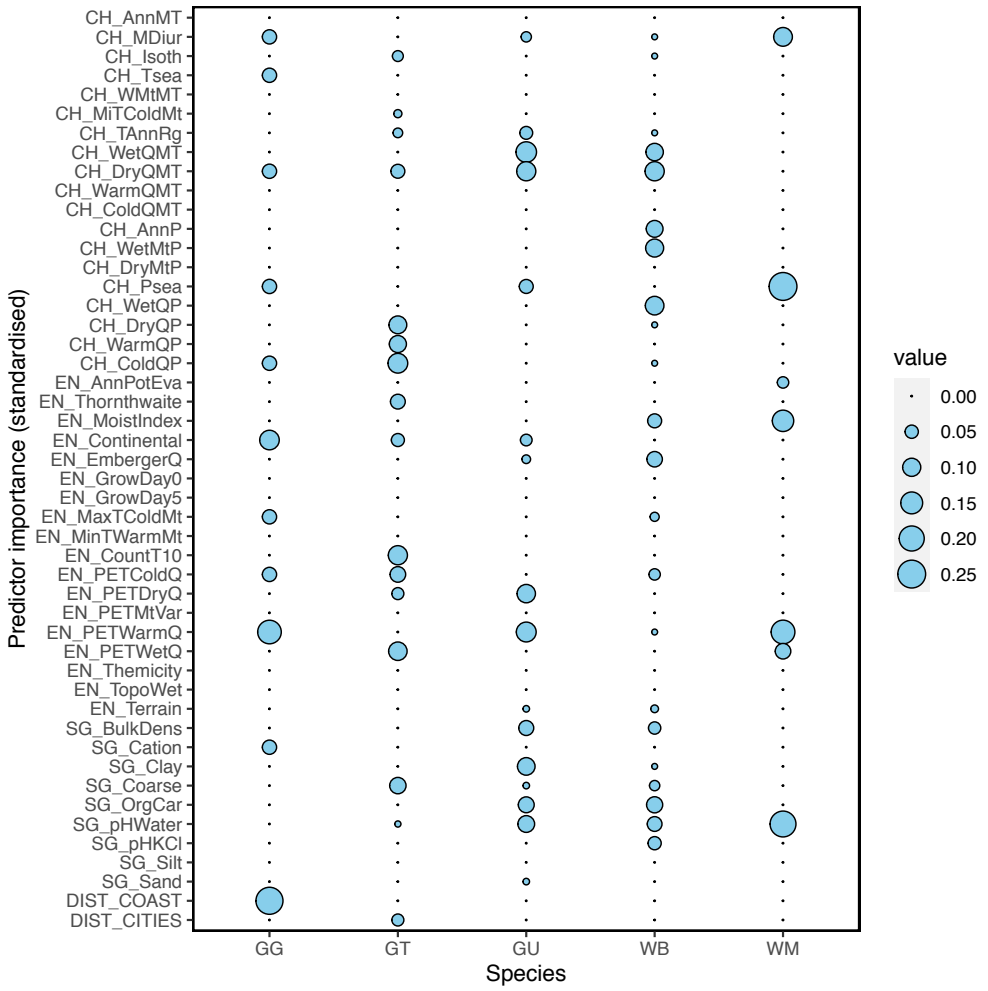

Figure 4.3: Predictor importance calculated based on the best model for *Gladiolus gueinzii* (GG, 11 predictors used), *G. tristis* (GT, 15 predictors used), *G. undulatus* (GU, 43 predictors used), *Watsonia meriana* var. *bulbillifera* (WB, 21 predictors used) and *W. meriana* var. *meriana* (WM, 45 predictors used). The more important a predictor, the higher its score for a species. Scores were standardised during ranking; see the text for details. Also see Table 4.1 for the full name of predictors and their source data set.

the mean temperature of driest quarter, distance from coast, the mean temperature of wettest quarter, continentality (difference between coldest and warmest months), the mean diurnal range, relative wetness and aridity, precipitation of the coldest quarter, PET of the wettest quarter, and PET of the wettest quarter. Five variables originated from the ENVIREM and CHELSA datasets each, and one from SoilGrids.

In general, the optimal models were in better agreement with present distribution records when thresholding was applied (Figs. 4.4 and 4.5). Without thresholding, it was predicted that *G. gueinzii* would colonise all coastlines in Australia (Fig. 4.4A). However, with thresholding applied (Fig. 4.4B), its projected occupation was constrained to Fraser Island off Queensland in the north, and sporadically westwards past the tip of southwestern mainland Australia,

near Bunbury. By contrast, the current distribution of *G. tristis* (Fig. 4.4D), *G. undulatus* (Fig. 4.4F) and *W. meriana* var. *bulbillifera* (Fig. 4.5B) were not predicted by thresholding. In particular, the abundant populations of *G. undulatus* along coastal New South Wales were not predicted by the model, as were three populations of *W. meriana* var. *bulbillifera* in New South Wales and Queensland.

By contrast, all optimal models suggested a high potential for range infilling along contiguous corridors. Eastern populations were projected to expand westwards into South Australia, and western populations (except *G. gueinzii*) eastwards towards Esperance. However, the portion of the Nullarbor Plain straddling the Nullabor National Park was expected to present a barrier to Eastern and Western population confluence.

Not surprisingly, *distance from coast* was the highest-ranked predictor for the coastal dune species *G. gueinzii*, followed by PET warmest quarter and continentality. Generally, most models concurred on the top variable but with dissimilar emphasis on its importance. Where GLMs predicted that the species could be found along the coastlines of mainland and insular Australia, Maxent models were more conservative, predicting the absence of the species west of the Lower Eyre Peninsula. The projection by the optimal algorithm, RANDOM FOREST/VARRANK agreed with MESS analysis (Fig. 4.6A), which showed greater envionmental distance (and therefore resistance) towards north-western Australia.

For *G. tristis*, the top three predictors were coldest quarter precipitation, number of months above 10 °C and wettest quarter PET. Species distribution models generally agreed with MESS (Fig. 4.6B), where environmental distance increased northwards. MESS however did not identify the Nullarbor Plain as dissimilar to native range environmental conditions. On the other hand, with thresholding applied, Maxent with HDMM failed to account for populations north of the Dividing Range in south-eastern Australia. However, species distribution pattern in Tasmania matched model predictions well.

For *G. undulatus*, wettest quarter mean temperature, warmest quarter PET and driest quarter mean temperature were the top-ranked predictors, indicating an intimate relationship with temperature and water availability. MESS analysis showed a clear longitudinal boundary across Australia that coincided roughly with the transition to an arid interior (Fig. 4.6C). In agreement with the optimal RANDOM FOREST model and Maxent models, MESS indicated higher environmental dissimilarity for the New South Wales populations not predicted by thresholding. By contrast, some GLMs correctly predicted their presence, but greatly over-projected species distribution in tropical Australia.

Despite their phylogenetic relatedness, predictor ranking for *W. meriana*

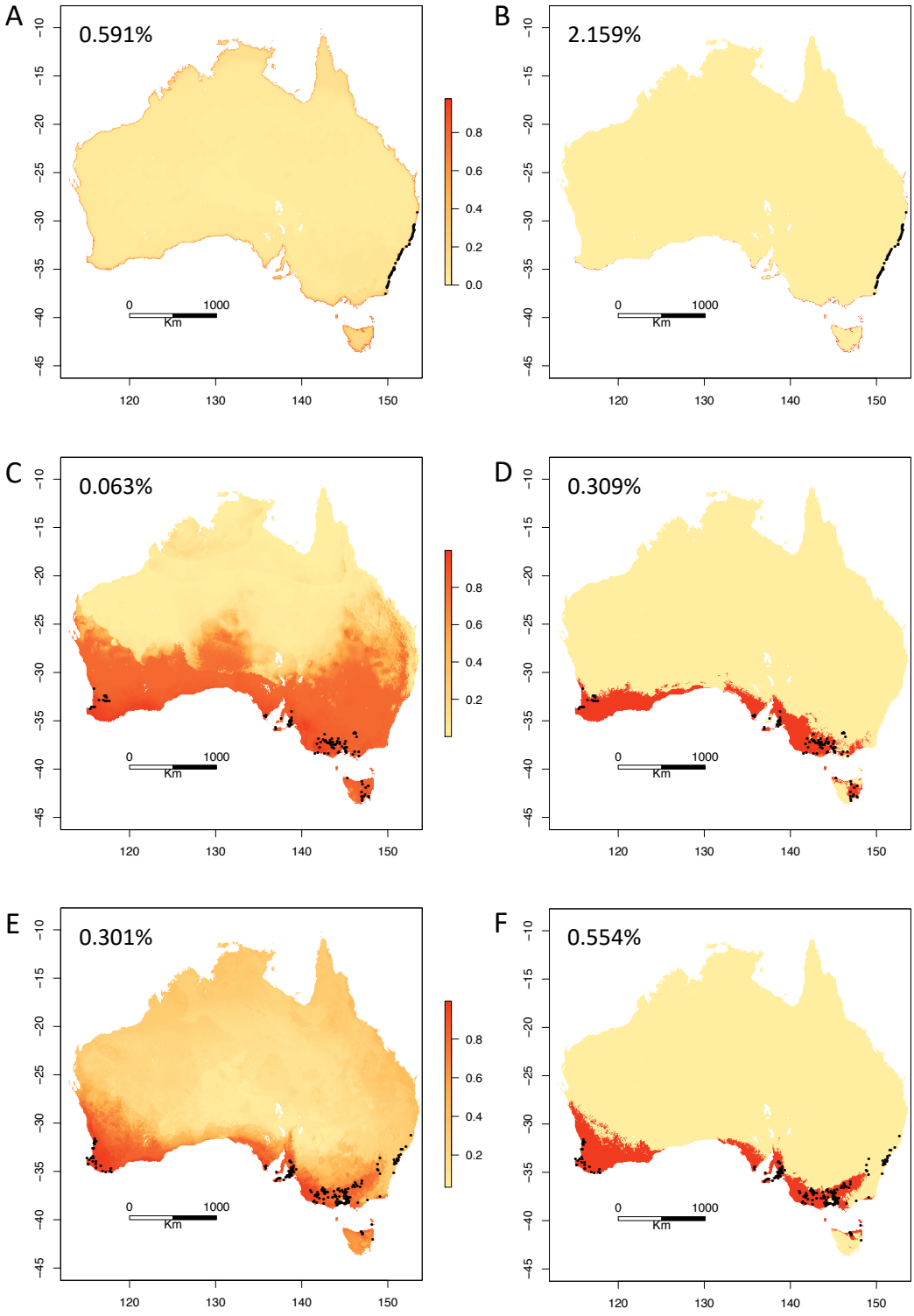

Figure 4.4: Potential distribution as computed by the optimum model for each taxon. The left panels show the probability distribution, and the right panels show the binary distribution, after a threshold is applied to the probability model. **A** and **B**): *Gladiolus gueinzii*, **C** and **D**: *G. tristis* and **E** and **F**: *G. undulatus*. Points in black represent presence records. The value at the top-left corner of each panel is the relative occupancy (probabilistic for the left panels and threshold for the right panels). Cell size of rasters are 2.5 × 2.5 arc seconds. Figures are generated by the RASTER package.

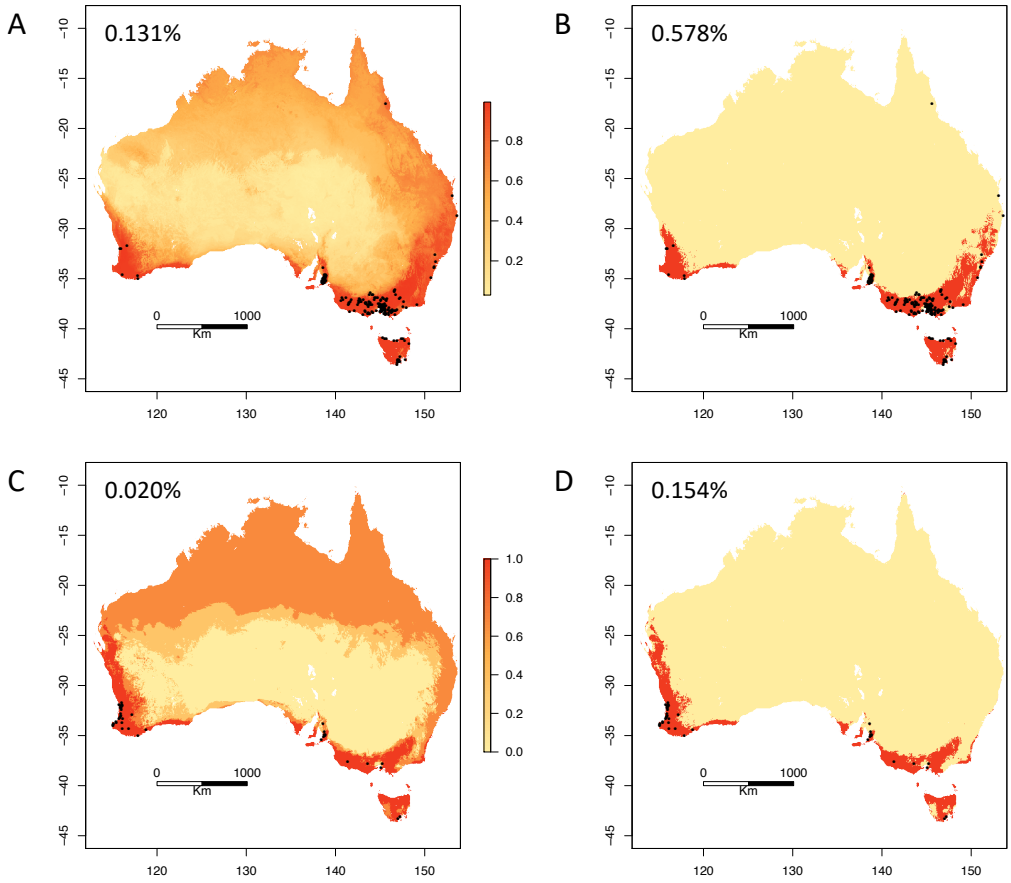

Figure 4.5: Potential distribution as computed by the optimum model for each taxon. The left panels show the probability distribution, and the right panels show the binary distribution, after a threshold is applied to the probability model. **A** and **B**): *Watsonia meriana* var. *bulbillifera*, and **C** and **D**: *W. meriana* var. *meriana*. Points in black represent presence records. The value at the top-left corner of each panel is the relative occupancy (probabilistic for the left panels and threshold for the right panels). Cell size of rasters are 2.5 × 2.5 arc seconds. Figures are generated by the RASTER package.

var. *bulbillifera* and *W. meriana* var. *meriana* were different. While driest quarter mean temperature, wettest quarter precipitation and wettest month precipitation were the three most important predictors for *W. meriana* var. *bulbillifera*, precipitation seasonality, pH index in water and warmest quarter PET determined *W. meriana* var. *meriana* distribution pattern. In support of their relationship, MESS maps were highly similar in pattern, but different in severity of environmental dissimilarity: environmental distances were about 1.6 times greater for *W. meriana* var. *bulbillifera* at their extremities (Figs. 4.7A and 4.7B). However, despite the elevated dissimilarity, RANDOM FOREST correctly predicted *W. meriana* var. *bulbillifera* populations in central New South Wales, but not the Queensland populations. Although Maxent models accurately captured western Australian populations, they failed to predict north-eastern seaboard populations. In comparison, RANDOM FOREST/BIOMOD

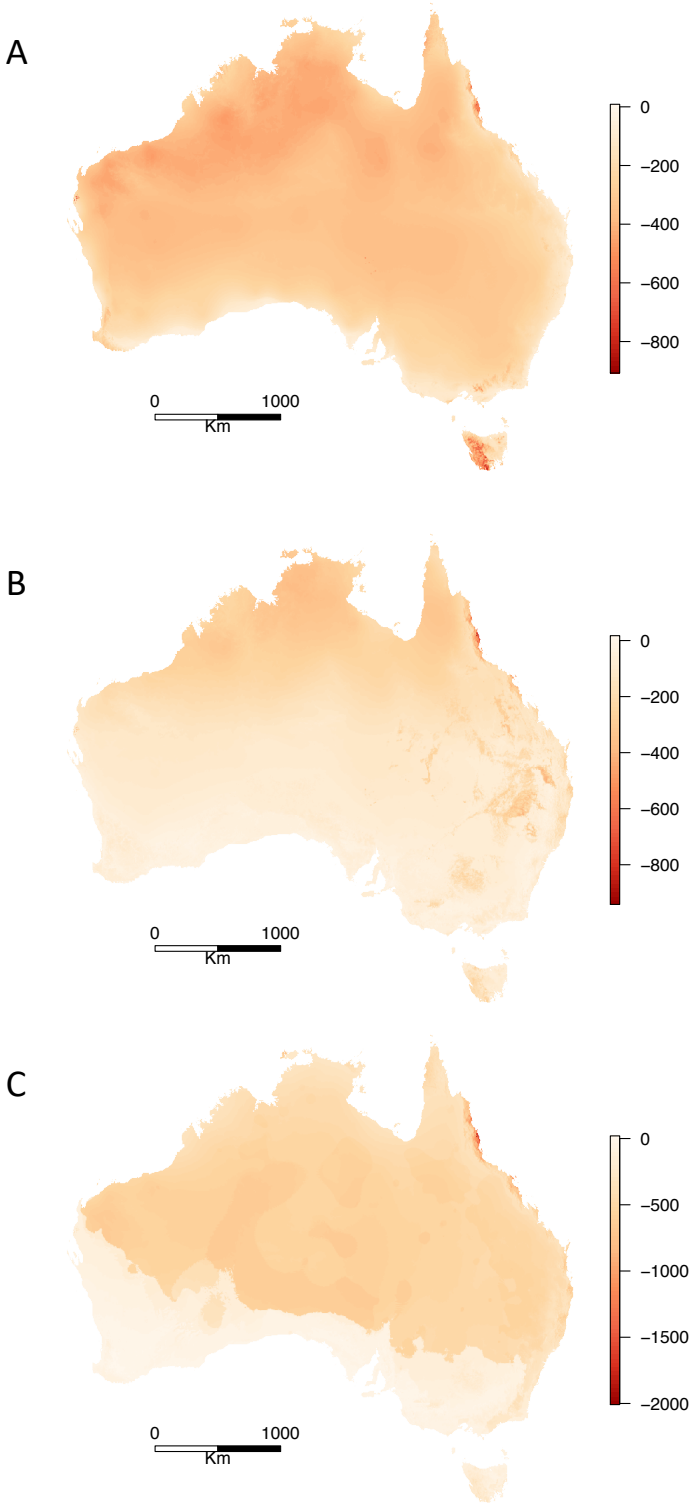

Figure 4.6: MESS analysis of environmental similarity for **A**: *Gladiolus gueinzii*, **B**: *G. tristis* and **C**: *G. undulatus*, based on bioclimatic and soil predictors in Table 4.1. MESS and map rendering were executed with the DISMO package.

prediction aligned with GLM (all predictors) for *W. meriana* var. *meriana*, but with a greater potential distribution in the south-western corner of the Nullarbor Plain. Conversely, GLM (all predictors) over-projected potential *W. meriana* var. *meriana* distribution, such that the variety was expected in northern tropical Australia.

Both the probabilistic and threshold estimations for the degree of geographical occupancy showed that all five species have low levels of occupancy (Figs. 4.4 and 4.5). Over the probabilistic surfaces, *W. meriana* var. *meriana* has only occupied 0.020% of its potential distribution, while *G. gueinzii* has colonised 0.591% of its potential distribution. When a threshold has been applied, *W. meriana* var. *meriana* consistently has the lowest occupancy level (0.154%), and *G. gueinzii* has the highest (2.159%). All models and their test statistics are listed in Appendix D.

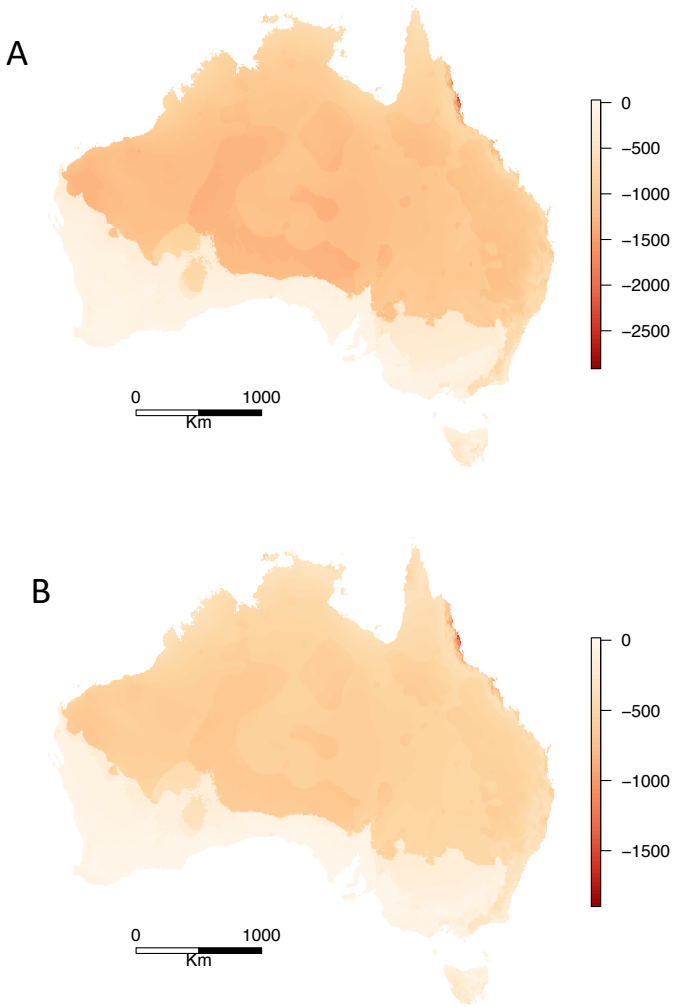

Figure 4.7: MESS analysis of environmental similarity for **A**: *Watsonia meriana* var. *bulbillifera* and **B**: *W. meriana* var. *meriana*, based on bioclimatic and soil predictors in Table 4.1. MESS and map rendering were executed with the DISMO package.

Table 4.5: Niche dynamics for study Iridaceae taxa. D = Schoener's D overlap index. Equiv. = niche equivalency, and Simil. = niche similarity. Niche expansion, stability and filling were calculated at the intersection between native and introduced space. All computations were performed in ECOSPAT.

		Conservation test p-value		Divergence test p-value				
Taxon	D	Equiv.	Simil.	Equiv.	Simil.	Expansion	Stability	Unfilling
G. gueinzii	0.0000	1.0000	1.0000	0.0020	0.6747	1.0000	0.0000	1.0000
G. tristis	0.4704	0.0002	0.0060	1.0000	0.9980	0.1042	0.8958	0.1166
G. undulatus	0.3244	0.9900	0.0040	0.0060	0.9980	0.4695	0.5305	0.1654
W. meriana var. *bulbillifera*	0.1635	0.8343	0.1198	0.1637	0.8822	0.2353	0.7647	0.1882
W. meriana var. *meriana*	0.2695	0.8882	0.0590	0.0878	0.9621	0.3102	0.6898	0.2844

4.3.2 Ecological niche modelling

For *Gladiolus gueinzii*, there was no niche overlap between ranges (Schoener's D = 0; Table 4.5). All randomisation trials for the upper-tailed niche equivalence test resulted in D values greater than the observed D (p_D = 1.000, Fig. 4.8A), indicating that niches were not equivalent between its native range in southern Africa and introduced range in Australia. By contrast, the lower-tailed test for equivalency was significant, indicating niche divergence (p_D = 0.002, Fig. 4.8B). However, the upper-tailed niche similarity test showed that both environments were more different than expected by chance (p_D = 1.000; Fig. 4.8C). Therefore, there was no conclusive evidence that its niche was conserved or divergent across regions. Between regions, there was a total shift in niche space (solid mauve, Fig. 4.8E). When marginal environment densities were progressively removed, the level of niche expansion and unfilling remained steady (juxtaposed emerald and bronze line, Fig. 4.8H). There was a niche shift in the species distribution centroid towards an environment with greater continentality (difference between the warmest and coldest months) and coldest quarter precipitation, and towards a lower maximum temperature of the coldest month (red arrow, Fig. 4.8E). By contrast, the Australian study area environmental centroid has shifted towards higher precipitation in the coldest quarter and soil cation exchange capacity (black arrow, Fig. 4.8E).

Around half of native and introduced range niches overlapped for *G. tristis* (D = 0.470; Table 4.5). Both upper-tailed niche equivalency test (p_D = 0; Fig. 4.9A) and niche similarity test (p_D = 0.006; Fig. 4.9C) were significant at α = 0.05, indicating that niches and backgrounds were more similar than expected by chance. Therefore, niche conservation was operating between native and introduced ranges in *G. tristis* (Scenario 1, Table 4.2). Indeed, comparison of occurrence density grids showed a high degree of niche overlap (solid mauve, Fig. 4.9E), and relatively lesser expansion (solid bronze) and unfilling (solid

emerald). There was more unfilling than expansion as common environmental space increased (Fig. 4.9H). Although niche was conserved, there was evidence of population and environmental shifts towards higher precipitation levels in the coldest quarter, and isothermality (red and black arrows respectively; Fig. 4.9E).

Niche overlap was low between ranges for *G. undulatus* ($D = 0.324$; Table 4.5). The upper-tailed niche equivalency test was not significant at $\alpha = 0.05$ ($p_D = 0.990$; Fig 4.10A), but the niche similarity test found the opposite ($p_D = 0.004$; Fig. 4.10C). By contrast, there was strong evidence for niche divergence (Lower-tailed equivalency test: $p_D = 0.006$, Fig. 4.10B; Scenario 3, Table 4.2). Although not evident from the plot of niche dynamics (Fig. 4.10E), niche overlap (solid mauve) was greater than expansion (solid bronze) or unfilling (solid emerald). This trend prevailed as more marginal environments were excluded (Fig. 4.10H). The *G. undulatus* population in Australia has shifted towards an environment with greater soil silt content and topographic wetness indices, and lower soil water pH (red arrow, Fig. 4.10E). The background environment on the other hand has tended towards a higher mean temperature of the wettest quarter (black arrow, Fig. 4.10E).

For *W. meriana* var. *bulbillifera*, niche overlap between regions was low ($D = 0.164$; Table 4.5). Both upper-tailed and lower-tailed niche equivalency tests were insignificant at $\alpha = 0.05$ (Upper: $p_D = 0.834$, Fig. 4.11A; lower: $p_D = 0.164$, Fig. 4.11B), and the niche similarity tests showed similar trends (Upper: $p_D = 0.120$, Fig. 4.11C; lower: $p_D = 0.882$, Fig. 4.11D). Thus, neither niche conservation nor divergence was inferred. From Figure 4.11E, there was greater niche overlap (solid mauve) than expansion (solid bronze) or infilling (solid emerald). When marginal environments were progressively excluded, niche stability (overlap) prevailed, while infilling remained fairly constant (Fig. 4.11H). Compared to its native range, populations in Australia inhabit environments with greater soil organic carbon content, and topographic wetness indices (red arrow, Fig. 4.11E). The background environment showed an opposite trend towards higher soil bulk density (black arrow, Fig. 4.11E).

The trends were largely repeated in *W. meriana* var. *meriana*, compared to *W. meriana* var. *bulbillifera*. Niche overlap between regions was low ($D = 0.270$; Table 4.5). While the niche equivalency tests were insignificant at $\alpha = 0.05$ (Upper: $p_D = 0.888$, Fig. 4.12A; lower: $p_D = 0.088$, Fig. 4.12B), the upper-tailed niche similarity test was almost significant ($p_D = 0.058$; Fig 4.12C), signalling no niche conservation or divergence despite close environmental similarity between regions. Although niche expansion (solid bronze) was apparent from Fig. 4.12E, it was lower compared to niche stasis (solid mauve). By contrast to *W. meriana* var. *bulbillifera*, range dynamics changed drastically with environmen-

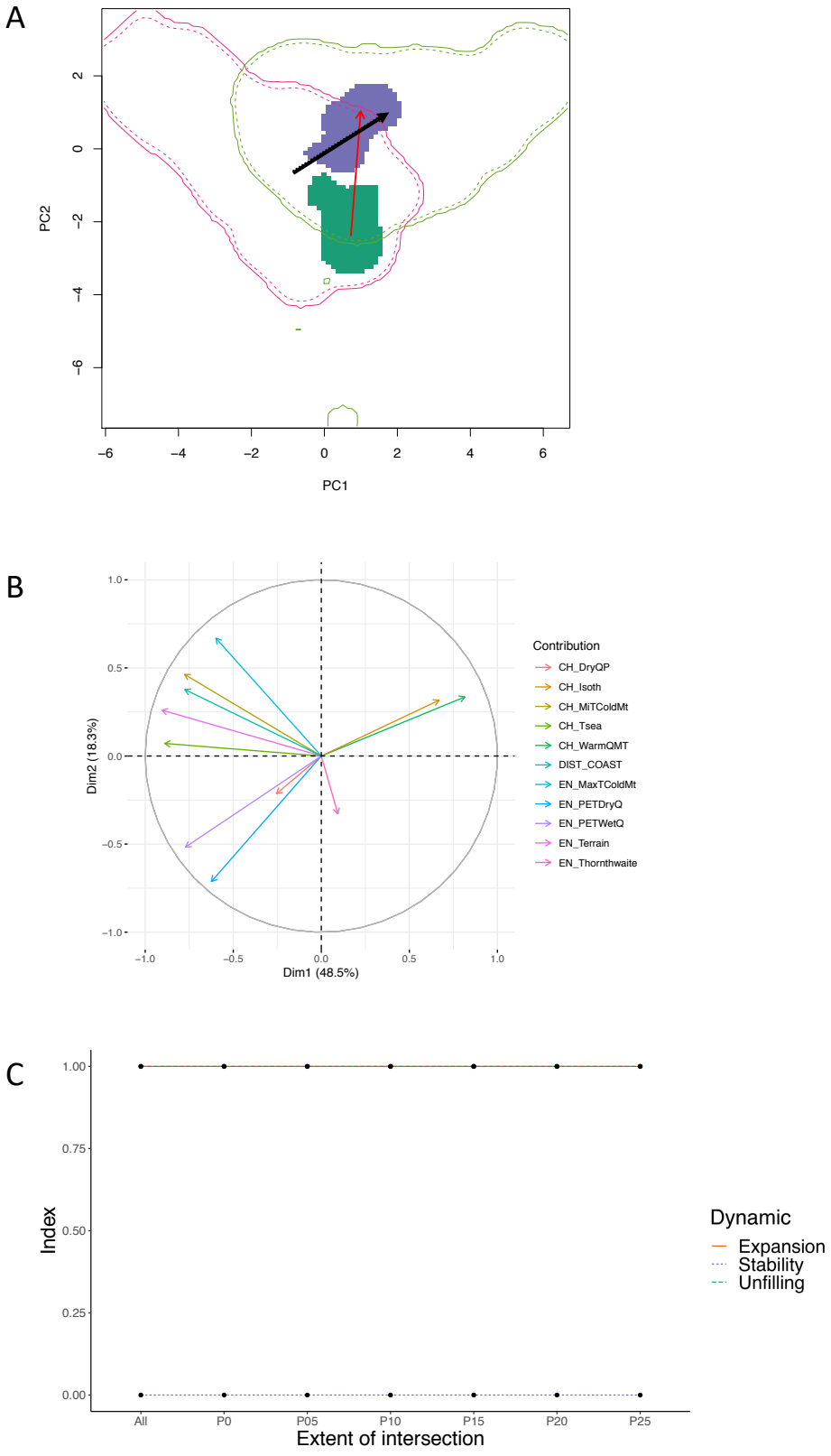

Figure 4.8: Niche dynamics for *Gladiolus gueinzii*. **A**: Population kernel density on two-dimensional environmental space. Solid mauve = niche stasis (overlap), and solid emerald = niche unfilling. Rose-coloured lines delimit the native-range environmental space: Solid = entire space, dotted = exclusion of marginal (first quantile) environmental space. Green-coloured lines delimit introduced-range environmental space: Solid = entire space, dotted = exclusion of marginal (first quantile) space. The red arrow links the centroid from the native- (southern African) to the introduced-range (Australia) distributions; the black arrow links the centroid from the native- (southern Africa) to the introduced-range extent. **B**: Contribution by predictors to PCA variation. **C**: Niche dynamics with progressive elimination of marginal environments. All = entire space, P0 = intersection of native and introduced-range space, P05 = 5% of marginal space eliminated, P10 = 10% of marginal space eliminated and so on, until the first quartile.

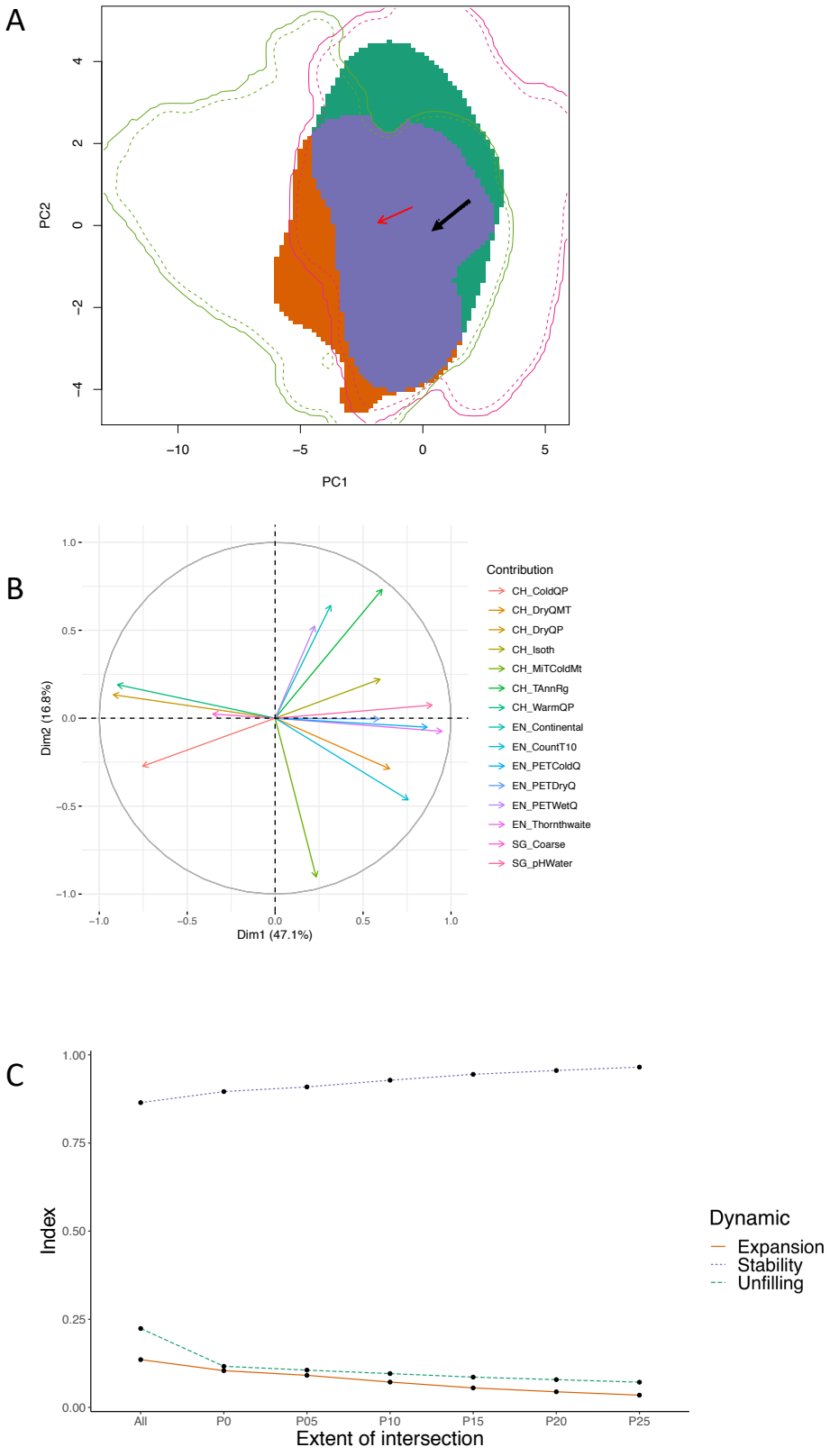

Figure 4.9: Niche dynamics for *Gladiolus tristis*. **A**: Population kernel density on two-dimensional environmental space. Solid mauve = niche stasis (overlap), solid bronze = niche expansion, and solid emerald = niche unfilling. Rose-coloured lines delimit the native-range environmental space: Solid = entire space, dotted = exclusion of marginal (first quantile) environmental space. Green-coloured lines delimit introduced-range environmental space: Solid = entire space, dotted = exclusion of marginal (first quantile) space. The red arrow links the centroid from the native- (southern African) to the introduced-range (Australia) distributions; the black arrow links the centroid from the native- (southern Africa) to the introduced-range extent. **B**: Contribution by predictors to PCA variation. **C**: Niche dynamics with progressive elimination of marginal environments. All = entire space, P0 = intersection of native and introduced-range space, P05 = 5% of marginal space eliminated, P10 = 10% of marginal space eliminated and so on, until the first quartile.

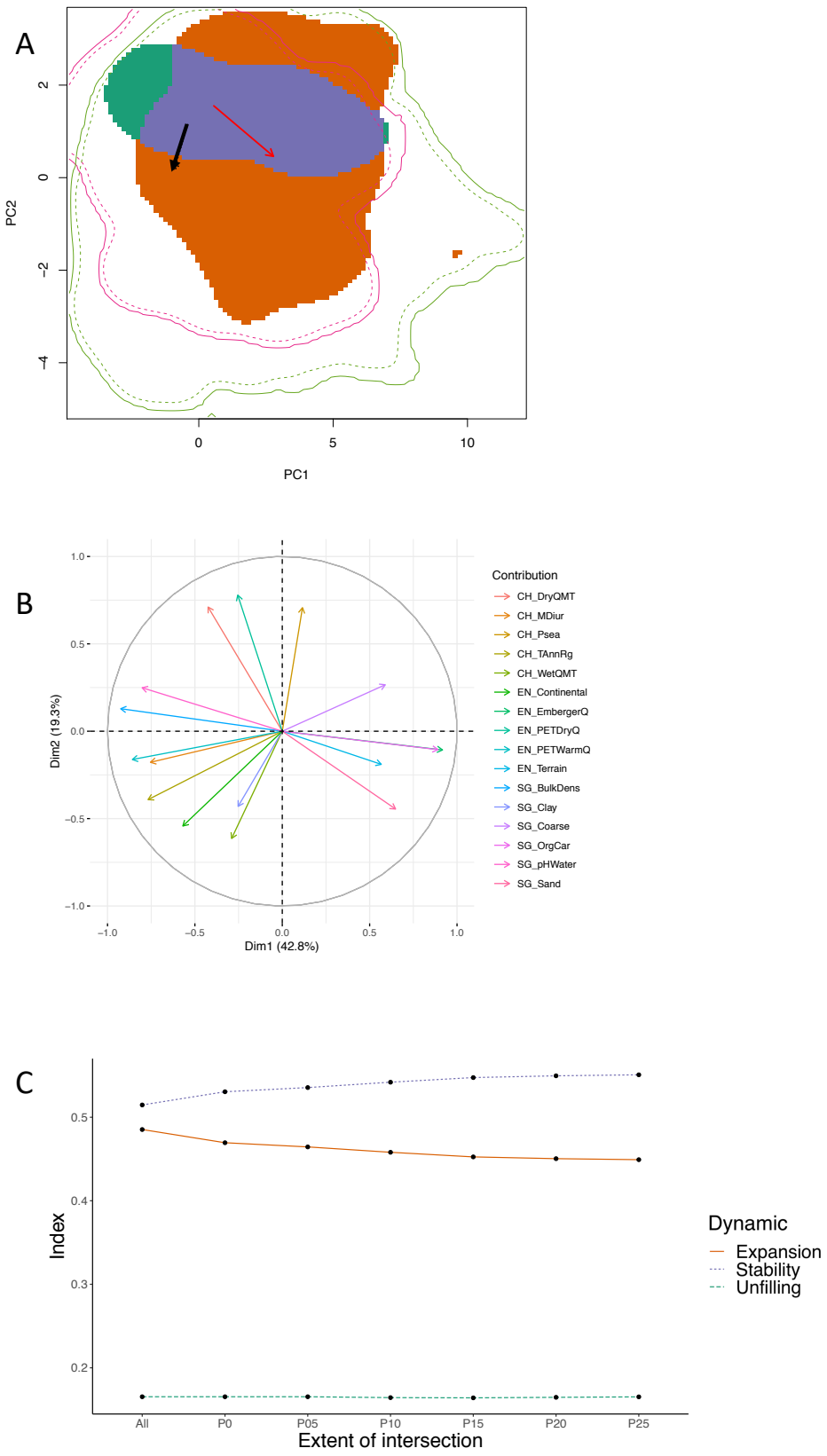

Figure 4.10: Niche dynamics for *Gladiolus undulatus*. **A**: Population kernel density on two-dimensional environmental space. Solid mauve = niche stasis (overlap), solid bronze = niche expansion, and solid emerald = niche unfilling. Rose-coloured lines delimit the native-range environmental space: Solid = entire space, dotted = exclusion of marginal (first quantile) environmental space. Green-coloured lines delimit introduced-range environmental space: Solid = entire space, dotted = exclusion of marginal (first quantile) space. The red arrow links the centroid from the native- (southern African) to the introduced-range (Australia) distributions; the black arrow links the centroid from the native- (southern Africa) to the introduced-range extent. **B**: Contribution by predictors to PCA variation. **C**: Niche dynamics with progressive elimination of marginal environments. All = entire space, P0 = intersection of native and introduced-range space, P05 = 5% of marginal space eliminated, P10 = 10% of marginal space eliminated and so on, until the first quartile.

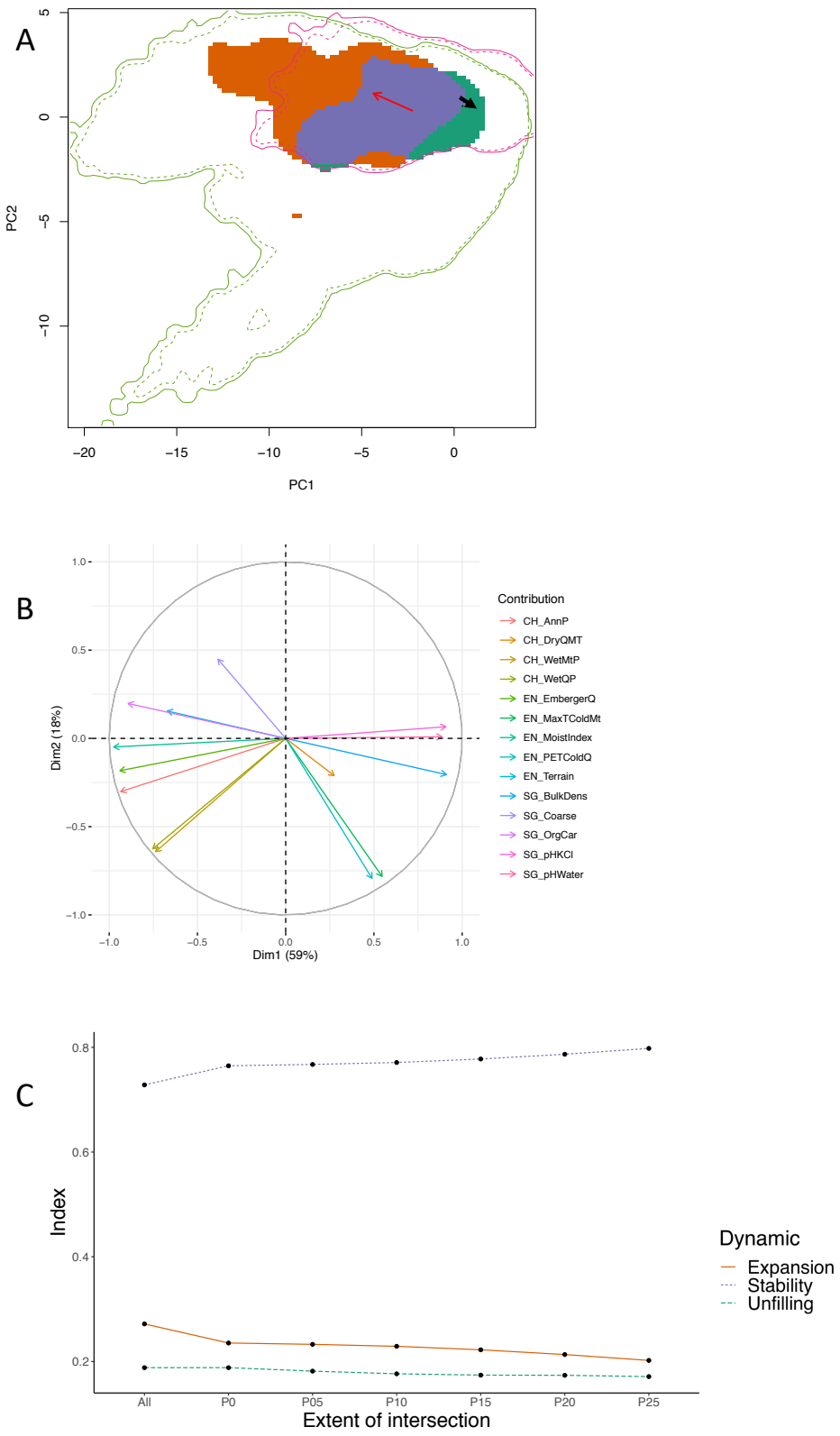

Figure 4.11: Niche dynamics for *Watsonia meriana* var. *bulbillifera*. **A**: Population kernel density on two-dimensional environmental space. Solid mauve = niche stasis (overlap), solid bronze = niche expansion, and solid emerald = niche unfilling. Rose-coloured lines delimit the native-range environmental space: Solid = entire space, dotted = exclusion of marginal (first quantile) environmental space. Green-coloured lines delimit introduced-range environmental space: Solid = entire space, dotted = exclusion of marginal (first quantile) space. The red arrow links the centroid from the native- (southern African) to the introduced-range (Australia) distributions; the black arrow links the centroid from the native- (southern Africa) to the introduced-range extent. **B**: Contribution by predictors to PCA variation. **C**: Niche dynamics with progressive elimination of marginal environments. All = entire space, P0 = intersection of native and introduced-range space, P05 = 5% of marginal space eliminated, P10 = 10% of marginal space eliminated and so on, until the first quartile.

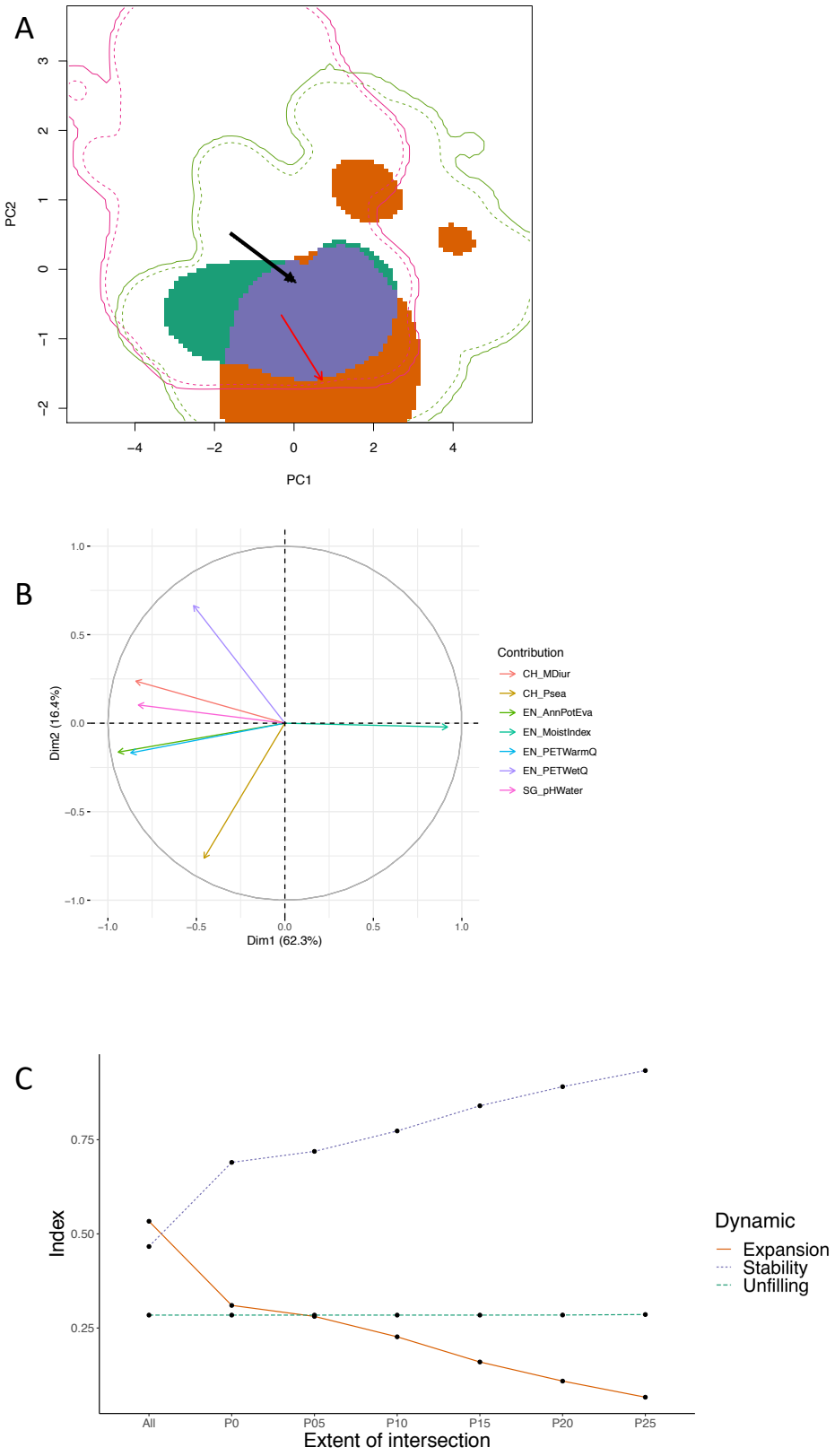

Figure 4.12: Niche dynamics for *Watsonia meriana* var. *meriana*. **A**: Population kernel density on two-dimensional environmental space. Solid mauve = niche stasis (overlap), solid bronze = niche expansion, and solid emerald = niche unfilling. Rose-coloured lines delimit the native-range environmental space: Solid = entire space, dotted = exclusion of marginal (first quantile) environmental space. Green-coloured lines delimit introduced-range environmental space: Solid = entire space, dotted = exclusion of marginal (first quantile) space. The red arrow links the centroid from the native- (southern African) to the introduced-range (Australia) distributions; the black arrow links the centroid from the native- (southern Africa) to the introduced-range extent. **B**: Contribution by predictors to PCA variation. **C**: Niche dynamics with progressive elimination of marginal environments. All = entire space, P0 = intersection of native and introduced-range space, P05 = 5% of marginal space eliminated, P10 = 10% of marginal space eliminated and so on, until the first quartile.

tal marginality (Fig. 4.12H), with expansion decreasing and overlap increasing rapidly with progressively more common environment. The occurrence density centroid and background environment of *W. meriana* var. *meriana* in Australia shifted to one with lower wettest quarter PET (red and black arrows, Fig. 4.12E).

4.4 Discussion

For species distribution modelling, there was no consensus among algorithm or predictor set associated with the best model for each species. Current occupancy levels were found to be low; for probability-based potential distribution surfaces, occupancy ranged from 0.020% (*W. meriana* var. *meriana*) to 0.591% (*G. gueinzii*). For threshold-based potential distribution surfaces, occupancy ranged from 0.154% (*W. meriana* var. *meriana*) to 2.159% (*G. gueinzii*).

The key hypothesis for this study set the expectation of niche evolution in outcrossing species, and niche conservation or reduction in selfing and asexual species. In general, there was no support for this hypothesis. In fact, niche divergence in Australia for the asexual *G. undulatus* and facultative selfing *G. gueinzii*—although inconclusive in the latter—undermined the premise of the hypothesis. There was no clear signal of niche evolution in either the predominantly asexual *W. meriana* var. *bulbillifera* or the outcrossing *W. meriana* var. *meriana*, although niche expansion was greater in the sexual variety. Only *G. tristis* met the expectation of niche conservation in an asexual species. Indeed, *G. tristis* demonstrated the highest niche overlap between native and introduced ranges, and the lowest niche expansion among the focal species.

4.4.1 Species distribution modelling and occupancy degree

The lack of consensus in algorithm and predictor selection in driving the best model for each species was in agreement with Qiao et al. (2015), who found that there was no optimal algorithm when tested against virtual species distributions. Working with "real-world" community-level data sets, Norberg et al. (2019) similarly inferred a large variation in the predictive performance of 33 presence–absence models; however, they could not explain the causes for the differential performance. In addition to uncertainty in algorithm choice, predictor (variable) selection is also a point for contention, with proponents for climatic variables (Bucklin et al., 2015) and for predictors that are biologically more meaningful (Fourcade et al., 2018; Gardner et al., 2019). Besides biologically relevant predictors, Petitpierre et al. (2017) suggested using either eight

"state-of-the-art" variables (commonly used in SDMs for plants relating to temperature, precipitation and moisture index) or an orthogonalised subset (for example, from a PCA). While the "state-of-the-art" variables were not directly used in my SDMs, and therefore could not be compared against other predictor subsetting methods here, I found that such variables were not consistently inferred as important in my SDMs (for example, annual mean temperature). It must be borne in mind that the study species were unlikely to be in equilibrium with their environment, and that a better understanding of their biology and distribution would lead to better models (Rinnhofer et al., 2012).

Even with a sizeable lag time of over 100 years for four of the focal species (*G. tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*), species distribution modelling with thresholding revealed that they have so far managed to occupy less than 1% of their potential geographical extent. On the other hand, *G. gueinzii*, first recorded in 1950, has the highest relative occupancy level among the study species. While some studies have found that the spatial extent of introduced plant species was positively correlated with their minimum residence time (Castro et al., 2005; Pyšek and Jarošík, 2005), others have not found such an association (Thuiller et al., 2006; Gassó et al., 2012; Terzano et al., 2018).

It was also unexpected that the seed-producing *W. meriana* var. *meriana* registered the lowest relative probabilistic and threshold occupancies, as seeds are considered natural dispersive structures, in contrast to cormels. As *W. meriana* var. *meriana* seeds demonstrated high germinability (Chapter 2), it is not known what factors constrain its distribution; it is possible that plantlets from cormels and vegetative fragments are more viable than from seeds (Wan et al., 2019). In contrast to *W. meriana* var. *meriana*, the only other predominantly seed-producing species, *G. gueinzii* has the greatest relative occupancies. However, the disparity could be explained by its propagule output, as its cormels could also be dispersed by an assured dispersal vector (ocean currents: Heyligers, 1999), and its potential distribution extent was smaller in magnitude, so that the comparative increase in relative occupancy was greater than for *W. meriana* var. *meriana*. While my study has suggested that spatial occupancy was not associated with propagule type, it could not be ruled out that human dispersal has promoted the spread of the clonal species more than the sexual species (see for example Barbosa et al., 2019).

Many hypotheses have been proposed to explain species invasiveness and habitat invasibility (Catford et al., 2009; Enders et al., 2018, 2020), and relative occupancy can be obscured by factors beyond propagule type or residence time. While Gassó et al. (2009) found a positive relationship between distribution range and residence time, the association only applied to alien flora that were

introduced more than 100 years ago in Spain, as later introductions were possibly not in equilibrium with their environment, thus affecting species modelling performance. In South Africa, Terzano et al. (2018) inferred relative occupancies of 18.8–83.5% for introduced flora with residence time between 79–337 years. The greater magnitude in their results could be explained by the derivation of relative occupancy by biome, rather than by spatial extent. Similarly, they failed to find an association between residence time and relative occupancy, which they attributed to a higher invasibility of certain biomes, uncertainty in old first records, and disequilibrium of more recent introductions with their environment.

There is presently little research on the mechanistic distribution patterns of vegetatively-reproducing terrestrial and non-riparian plants, which can inform future species distribution models for such taxa. By cultivating the geophyte *Oxalis pes-caprae* along an elevational gradient, Ross et al. (2008) found that bulbil biomass production fell below a sustainable level above 750 m and bulbil production was sensitive to the vagaries in climate above 600 m, while soil had no significant effect on plant performance. In general, their results were in agreement with the predictors used for Iridaceae distribution modelling, where climatic variables were generally more important than edaphic ones.

4.4.2 Ecological niche modelling

All five taxa consistently showed niche shifting in Australia. Niche shifting appears to be common in introduced and invasive species. Although the invasive spotted knapweed (*Centaurea stoebe*) in eastern North America inhabited conditions similar to its native range (Europe), the western population spread to warmer climates with higher aridity/humidity in the east (Broennimann et al., 2014). Niche shifting was also substantial in a study of 51 naturalised species, where niche expansion in analogous climate exceeded 50% for 19 species (22 species for non-analogous climate) (Early and Sax, 2014). In another study of 815 species globally, Atwater et al. (2018) demonstrated that niche shifts were ubiquitous: over 90% of 2364 comparisons between native and invasive ranges were significant for overlap and stability were significant, showing that niche models were highly dissimilar between ranges. Niche shifting is therefore neither a sufficient nor necessary condition for niche evolution (Guisan et al., 2014).

Niche divergence (albeit not conclusive) was observed in the mixed-mating *G. gueinzii*. Rather than it being the signature of rapid evolution, such a divergence might be a "non-evolutionary niche shift" (Herrando-Moraira et al., 2019), which is a niche change arising from environmental distinction between native and introduced ranges. The high degree of unfilling and expansion against stability within the short time since introduction (*c.* 70 years) pointed to the species occupying a different environment in Australia, although not significantly dis-

similar or similar to its native environment. In another study, the autogamous orchid *Disa bracteata* experienced a niche shift from southern Africa to Australia arising from differential distribution of climates, even though both regions share climatic zones (Konowalik and Kolanowska, 2018). The long-term costs of self-fertilisation can only be surmised (Igic and Busch, 2013; Park et al., 2018; Grant and Kalisz, 2019). However, an expedient benefit may be the capacity for niche shifting at range fronts, as the genetic or plastic switch to selfing erects a pre-zygotic barrier against maladaptive gene flow from core populations (Levin, 2010; Peterson and Kay, 2015). Unlike *D. bracteata*, *G. gueinzii* boasts a mixed mating system, which can ameliorate inbreeding depression from self-fertilisation.

Rapid increases in self-fertilisation can boost additive genetic variation, which can alleviate the effects of a genetic bottleneck (as during long-distance colonisation; Lande, 1977). In a glasshouse experiment, Tabassum and Leishman (2019) inferred increased selfing and seed dispersal potential, but no difference in seed size or production in range-edge populations in *G. gueinzii*. Likewise, Darling et al. (2008) deduced higher selfing levels in peripheral populations along a latitudinal gradient in the coastal dune plant *Abronia umbellata*, but were unable to find a robust link between seed and mating traits. Reproductive traits can also vary with latitude (Hockett and Ahokas, 1979). The present distribution of *G. gueinzii* in Australia extended its South African by about 2° latitude south (Africa: 29.7–34.7°S, Australia: 29.1–37.6°S). It is not known how climatic differences along this latitudinal gradient might affect reproduction in *G. gueinzii*, in addition to life history trait (Barrett et al., 1996) and demographic (Cadet et al., 2003) variation, which can trigger evolutionary changes in colonising populations and thus drive niche dynamics in the species (Grossenbacher et al., 2015).

Also unexpected was the variability among asexual lineages, which precluded a strong signal between mating system and niche dynamics in introduced populations. While niche conservation in *G. tristis* was anticipated, divergence in *G. undulatus* was surprising. Niche divergence might have arisen from the numerous populations in New South Wales that were not predicted by species distribution modelling, an area that MESS analysis showed to be environmentally different from predicted areas. By contrast, consistent niche differentiation was found in a comparative study of invasive apomictic and sexual congeneric or confamilial species (Dellinger et al., 2016). While their study investigated the association between niche dynamics and reproductive mode, all apomictic species reported significant niche equivalency test scores. In single-species studies, Datta et al. (2019) found that the invasive apomictic plant *Ageratina adenophora* had undergone niche expansion in its introduced range, while Escobar et al. (2016) inferred

a shift into novel environments in the introduced asexual alga *Nitellopsis obtusa*. Therefore, niche shifting is no stranger to asexual flora.

Precisely how niche shifting may occur in asexual invasive species is at present not clear (Yu et al., 2016). Plants may be more labile in their physiology than assumed, with tolerance for environments found beyond their present geographical distribution (Bush et al., 2018; Coiner et al., 2018). Evolutionary potential may be common in asexual plant species. By quantifying morphological changes in herbarium specimens for introduced species over time, Dalrymple et al. (2015) found that half ($N = 8$) of their focal species demonstrated significant trait changes, some of which were known to be under genetic control (for example, leaf size and shape: Wu, 2000). While invasiveness has been attributed to phenotypic plasticity in clonal plants (Geng et al., 2007), epigenetics has been found to modulate cold tolerance and consequently invasiveness in apomictic *Ageratina adenophora* (Xie et al., 2015).

Another mechanism for niche evolution in asexual plants is polyploidisation. Asexuality—especially apomixis—is strongly associated with polyploidy (Asker and Jerling, 1992; Kearney, 2005). Polyploids have been observed to have wider ecological amplitude (Karunarathne et al., 2018), greater range size (Lowry and Lester, 2006) and different niche patterns (Glennon et al., 2014), compared to their diploid progenitors. It is not surprising that polyploidisation has been linked to invasive potential (Treier et al., 2009; Pandit et al., 2011; te Beest et al., 2012). Among the focal taxa, there was evidence that *G. tristis* might be triploid, and *G. undulatus* pentaploid. Studies of invasive polyploids usually involve even-level polyploids (e.g. Treier et al., 2009; Hahn et al., 2012), as odd-level polyploids are rarer compared to even ploidy levels (Karunarathne et al., 2018). Indeed, triploid plants are cultivated for their robustness and sterility, which suppresses invasiveness (Kurokochi et al., 2014; Wang et al., 2016), and may therefore undermine niche evolution. A study of the invasive triploid tiger lily (*Lilium lancifolium*) uncovered a two-step niche shifting during globally colonisation (Herrando-Moraira et al., 2019). Only the triploid form of *L. lancifolium* was transported from its native range (Korea) to East Asia, when it intriguingly experienced a 62% niche expansion (the authors attributed this to climatic mismatch). However, niche stasis in *L. lancifolium* was maintained during subsequent global invasion, in agreement with *G. tristis* but not *G. undulatus*. In other species, genetic variability and invasiveness can be elevated in odd-level polyploids via intermittent sex (Chapman et al., 2004) or ingression of sexual forms (Castro et al., 2016).

The focal comparison between sexual and asexual Iridaceae threw up some surprises. Rather than a clear-cut expansion, niche dynamics for predominantly-outcrossing *W. meriana* var. *meriana* was inconclusive. Its degree of niche over-

lap between native and introduced regions was actually higher than conspecific asexual *W. meriana* var. *bulbillifera* and self-fertile *G. gueinzii*. However, *W. meriana* var. *meriana* demonstrated greater niche expansion compared to *W. meriana* var. *bulbillifera*, although it was lower than *G. undulatus*. The latter was unexpected, as its profuse production of winged seeds should reduce barriers to dispersal (Eriksson and Jakobsson, 1998; Lavergne et al., 2004), although range expansion may be contingent on population size and seed longevity at the invasion front (Dostál, 2005). Dispersal limitation despite seed production has been widely documented (Primack and Miao, 1992; Yakimowski et al., 2005; Kirchner et al., 2006; Butterfield et al., 2019). In addition, *W. meriana* var. *meriana* populations were mainly recorded adjacent to roads in Victoria and in the Adelaide Hills in South Australia. While roads may serve as a conduit to invasive spread (Lavoie et al., 2007; Rauschert et al., 2017), they can also disrupt dispersal vectors or mechanisms (von der Lippe and Kowarik, 2012; Chen et al., 2019). Moreover, road verges can undergo intensive weed management (Cooke et al., 2014).

The higher degree of unfilling in *W. meriana* var. *meriana* in contrast to *W. meriana* var. *bulbillifera* suggested that the sexual conspecific was occupying suitable habitats at a slower rate than its asexual relative. Indeed, the number of spatial grids occupied by *W. meriana* var. *meriana* was 0.12% of probable grids, based on the optimum model from species distribution modelling, compared to 0.55% for *W. meriana* var. *bulbillifera*. The level of unfilling also indicated that invasive spread was still very much a work in progress; Broennimann et al. (2014) suggested a period of 120 years was required to attain environmental equilibrium for *Centaurea stoebe*. The idea of a lag time before proper spread was compelling in the case of *G. gueinzii*, which was introduced prior to 1950 (Heyligers, 1999), and was found to have the highest unfilling (0.815) among the study taxa. Thus, the difference in niche dynamics between *W. meriana* var. *meriana* and other asexual relatives might boil down to a more recent introduction period for the former.

However, there is evidence that their introductions were contemporary: a review of GBIF records showed that the earliest herbarium specimen for *W. meriana* var. *meriana* was collected in 1899 (Keysbrook, South Australia), 1900 for *W. meriana* var. *bulbillifera* (Port Arthur, Tasmania), 1841 for *G. tristis* (near Hobart, Tasmania) and 1879 for *G. undulatus* (Tamar River, Tasmania), thus mitigating time lag as a factor. However, there is a caveat: with the exception of *G. gueinzii*, the focal taxa were most likely introduced as ornamentals, and it was unknown when they "jumped the garden fence" (*sensu* Groves et al., 2005). The selection for climatic suitability and robust growth in horticulture may in reality select for a predilection to invade (van Kleunen et al.,

2007), and widely-planted ornamentals can form invasion foci over a broad area and augment natural spread (Kowarik, 2003). Therefore, a species may attain a relatively greater invasiveness through more popular human use. Human use might apply to outlying *G. undulatus* populations in New South Wales and to *W. meriana* var. *bulbillifera* populations in Queensland. While the proximity of *G. undulatus* populations in New South Wales suggested autonomous local dispersal, the distances between northern range-edge populations of *W. meriana* var. *bulbillifera* indicated human transport might be implicated.

Another factor for the absence of niche conservation in *W. meriana* var. *bulbillifera* was the paucity of records (seven) in its native range. This might result in biased data, when collection/recording was carried out over a fraction of its true occupancy and environmental amplitude, which would degrade inferences from species distribution and ecological niche modelling (Stolar and Nielsen, 2015). Poor sampling may unintentionally simulate environmental non-equilibrium (when suitable environments are not occupied by a species due to dispersal barriers or biotic exclusion; Qiao et al., 2017), which leads to erroneous estimation of fundamental niches (de Andrade et al., 2019). Rapid temperature rise affects many aspects of plant reproduction, including phenology (Lustenhouwer et al., 2018), sexual reproduction in plants (Hedhly et al., 2009; Zinn et al., 2010), propagule germination (Walck et al., 2010) and pollinator service (Stuble et al., 2017), but response may be idiosyncratic (Robinson and Henry, 2018).

Work on introduced *Senecio inaequidens* showed that different ecological and selection pressures acted over the course of invasion (Lachmuth et al., 2011). This may explain the relatively low level of niche expansion observed in *W. meriana* var. *meriana*, particularly in Victoria, where population spread was negligible. Although self-incompatibility was suspected to be compromised, it remained strongly self-incompatible; thus genetic diversity was not expect to decrease remarkably by way of selfing (Zhu et al., 2017). However, this diversity and geographical isolation among populations might be insufficient to propel secondary spread, despite at least *c.* 120 years since introduction to Australia. While genetic supplementation via multiple introductions and interpopulation gene flow was implicated in niche expansion into formerly unsuitable climates in self-incompatible *Ambrosia artemisiifolia* (Gallien et al., 2016), little is known about the introduction history for *W. meriana* var. *meriana*. GBIF data revealed long intervals between records: *c.* 40 years between the first collection from Keysbrook and the third (1938, Gawler, also South Australia), while the first interstate record was made in 1948 (Margaret River, Western Australia). It is not known whether this was a new introduction, or a long-distance dispersal from an eastern population.

Moreover, environmental non-equilibrium cannot be discounted in the study species. In the Cape region, herbaceous (including Iridaceae) underground storage organs are consumed by mole rats (Lovegrove and Jarvis, 1986). As mole rats and similar subterranean herbivores are absent in Australia (Begall et al., 2007), introduced Iridaceae are liberated from herbivory (enemy release: Keane and Crawley, 2002). This can increase their realised niche, which may be interpreted as niche shifting (Guisan et al., 2014). However, mole rats also cache corms and other geophytic storage organs (Bennett and Jarvis, 1995), and may unintentionally facilitate plant spread when caches are neglected. How release from enemy may affect Iridaceae niche dynamics requires the inclusion of biotic predictors for distribution and niche modelling (Dormann et al., 2018).

The present work suggests that niche shifting and expansion can occur in clonal and selfing species, at similar levels to (or even supersede) outcrossing species, in agreement with Dellinger et al. (2016) and Grant and Kalisz (2019). Additionally, it also found that the focal species have yet to attain their fundamental niche in their native range. Within the asexual species (*G. tristis, G. undulatus* and *W. meriana* var. *bulbillifera*), conflicting results implicate processes that are driving differential niche dynamics. While the elucidation of these mechanisms await future work, some lessons may be drawn from the present study. First, asexuality does not pose any constraint to invasive spread. At present, *W. meriana* var. *bulbillifera* can be considered to be more successful in range expansion compared to *W. meriana* var. *meriana*. Second, asexual and selfing lineages may be far from being evolutionary dead-ends. *G. gueinzii*, *G.undulatus* and *W. meriana* var. *bulbillifera* were found to inhabit environments not predicted by species distribution modelling, thereby countering argument that asexual taxa are adapted only to homogeneous environments. Third, a multifaceted approach across spatial (Kirchheimer et al., 2016) and temporal (Herrando-Moraira et al., 2019) scales is required to elucidate complex phenomena such as invasion niche dynamics. While this study is one of few to incorporate mating system, species distribution modelling and ecological niche modelling, it would benefit from a comprehensive range-wide analysis of population genetic structure and diversity across continents.

Chapter 5

Dispersal characteristics of introduced Iridaceae in Victoria, Australia

5.1 Introduction

How terrestrial plants move in space has attracted long-standing interest (Good, 1931; Harper, 1977; Cousens et al., 2008), and the derivation of dispersal kernels (the probability distributions of dispersal distances) of invasive species can explain distribution patterns and inform weed risk assessment and management (Coutts et al., 2011; Crossman et al., 2011). One of the defining characteristics of a biological invasion event is the human-mediated movement of organisms. Even as natural long-distance dispersals continue to operate, they are now beginning to pale in comparison to biotic homogenisation wrought by intentional and unintentional human transport (Ricciardi, 2007; Seebens et al., 2017). The association of such organisms with humans may linger after the introduction to their adventive range, particularly for sessile biota such as plants. The mechanisms giving rise to invasive spread are thus of interest, as greater management effort will be required to subdue autonomous range expansion, compared to species that face dispersal limitations (Auffret et al., 2014).

Humans may also facilitate invasive spread by modifying the backdrop against which natural dispersals take place. Anthropogenic intervention can take many forms. Beyond transport to an adventive region, humans can continue to act as a dispersal agent. In addition to enhancing pre-adaptation by artificial selection (Guo et al., 2019), garden cultivation increases the spatial distribution and the number of focal points for range expansion (van Kleunen et al., 2018). In general, human use of non-indigenous flora in settings such as agriculture (Cook and Dias, 2006), forestry (Richardson, 1998), medicinal use (Groom et al., 2019) and biofuel production (Raghu et al., 2006) can enlarge the spatial distribution

of introduced species.

The influence of humans on terrestrial biomes is now pervasive (Sanderson et al., 2002), and anthropogenic transformation of landscapes threatens biodiversity and promotes invasibility (With, 2002). In particular, human activity can modulate natural processes, and create new disturbances or disturbance regimes, which in turn may increase the number of microsites for non-indigenous species (Hobbs and Huenneke, 1992), suppress biotic resistance by releasing unused resources (Davis et al., 2000, but see Liu et al., 2018) or alter pollination patterns (Aizen and Vázquez, 2006), paving the way for encroachment by non-indigenous plants capable of uniparental reproduction (Issaly et al., 2019, also see Chapter 2). The trend towards urbanisation is also thought to promote biological invasion, as urban areas contain heterogeneous and highly disturbed habitats, and typically act as gateways for introduced biota (Wang et al., 2011).

Anthropogenic structures such as roads can serve as habitats or conduits for indigenous species movement (Gustafsson and Hansson, 1997). At the same time, roads can also act as corridors for invasive spread of motile species, such as cane toads (*Rhinella marina*) (Brown et al., 2006). For plants, in addition to providing germination sites, roads may enhance the long-distance movement of propagules by vehicles (von der Lippe and Kowarik, 2007), road grading (Rauschert et al., 2017) or secondary wind dispersal (Kowarik and von der Lippe, 2011). For example, the range expansion of the introduced common ragweed (*Ambrosia artemisiifolia*) in Quebéc, Canada was concomitant with the development of the road network in the region, suggesting the role of transport corridors for invasive spread (Lavoie et al., 2007). However, the efficacy of roads as dispersal corridors is likely to be idiosyncratic. In a study of three common non-indigenous species along forest roads in Ohio, Christen and Matlack (2009) found that the extent and spatial configuration of invasive spread were driven by both intrinsic factors (specific reproductive strategy, habitat requirements and seed traits) and extrinsic ones (dispersal vectors and habitat quality).

Current research on terrestrial plant movement has very much focused on seeds as dispersal propagules (e.g. Ridley, 1905; Howe and Smallwood, 1982; Vittoz and Engler, 2007; Bullock et al., 2017), even though plants can disperse as fragments, bulbils or clonal growth, with many species spreading by more than one type of propagule (Bullock et al., 2006). Plant fragments and seeds of riparian plant species are well suited to long-distance dispersal by hydrochory (Thomas et al., 2005; Nilsson et al., 2010; Aronson et al., 2017). On the other hand, the derivation of dispersal kernels for non-riparian vegetative spread has focused on short-distance dispersal events (less than 20 m) (Mizuki and Takahashi, 2009; Matlaga et al., 2017), which explained local population expansion, but not how introduced plants achieved long-distance spread, which can control

the rate of population expansion (Kot et al., 1996).

An alternative way for inferring dispersal kernels is the inverse modelling of such kernels based on observed patterns of individuals, coupled with genetic maternity analysis (Robledo-Arnuncio and García, 2007). The accuracy of such models were found to be highly sensitive to seed sampling strategy, and to the proportion of long-distance dispersal events to shorter ones when assessed with the animal-dispersed tree species *Prunus mahaleb* (Robledo-Arnuncio and García, 2007). Even when genetic analyses could identify invasion pathways, they were generally unable to uncover the causes of long-distance dispersal events, as in the invasion of the clonal vine *Pueraria montana* var. *lobata* in North America (Bentley and Mauricio, 2016), or the invasion of the heterostylous polyploid geophyte *Oxalis pes-caprae* in the western Mediterranean Basin (Papini et al., 2017). While human-mediated long-distance dispersals are distinctive to natural dispersal (arising from landscape permeability, animal vectors, extreme meteorological events or ocean currents) with notably higher vector displacement velocity (movement characteristics of the vector) and longer seed passage time (interactions between seed and vector traits), it remains difficult to identify all important vectors and their contribution to long-distance dispersal (Nathan et al., 2008; Niggemann et al., 2009).

In Australia, introduced species from the geophytic family Iridaceae from mediterranean-climate southern Africa have colonised both the south-eastern and south-western regions of the continent (see Chapter 1). The reproductive ecology, population genetic structure and niche dynamics of five taxa (*Gladiolus gueinzii*, *G. tritis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*) have been studied in south-eastern Australia (Victoria and New South Wales; see Chapters 2, 3 and 4 respectively). The lack of seed set in *G. tristis* and *G. undulatus* indicated that spatial spread was vegetative (in the form of cormels), which was supported by the high degree of genetic uniformity in these species. Similarly, *W. meriana* var. *bulbillifera* was found to regenerate primarily by aerial cormels, with rare seed set. By contrast, *W. meriana* var. *meriana* reproduced only via seed.

Ecological niche modelling showed that the clonal and predominantly clonal taxa (*G. tritis*, *G. undulatus* and *W. meriana* var. *bulbillifera*) have already occupied a substantial portion of their native-range niche space (Table 4.5), with *G. tritis* and *G. undulatus* demonstrating higher niche stability (0.8958 and 0.7647 respectively) compared to the sexual *G. gueinzii* and *W. meriana* var. *meriana* (0.0000 and 0.6898 respectively). Surveys conducted by the author between 2011 to 2015 in Victoria, Australia, suggested that the study taxa (with the exception of the coastal *G. gueinzii*) were more likely to be found along roads than in natural areas. To answer the question of how clonal reproduction could

promote the dispersal of introduced Iridaceae, I investigated: (1) the tempo of colonisation of Australia by *G. gueinzii*, *G. tritis*, *G. undulatus*, *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana* with reference to curated records; (2) the dispersal kernel, and the degree of anthropogenic vs. non-anthropogenic dispersal modes for *G. tritis*, *G. undulatus*, *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana* in Australia; and (3) whether the distribution of *G. tritis*, *G. undulatus* and *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana* populations are associated with road corridors in Victoria.

5.2 Methods

5.2.1 Tempo of colonisation

Species occurrence records with year of collection were downloaded from the Global Biodiversity Information (see Figs 1.3 and 1.4). For each taxon, the data sets were filtered to retain only Australian records, partitioned into 20-year bins and mapped.

5.2.2 Dispersal kernels, and natural vs. anthropogenic dispersal

To infer natural versus anthropogenic dispersal components, I used a recent method established by Butikofer et al. (2018). This framework comprised two main steps. The first step inferred the degree of anthropogenic contribution to extant spatial distribution by an expectation-maximisation (EM) algorithm. The second step estimated the dispersal kernel for naturally-dispersed populations. The framework was incorporated as the R package BIOLINV (Butikofer and Jones, 2018).

Briefly, dispersal arising from anthropogenic origins were assumed to have a uniform distribution $g_y(d)$ described by a Poisson point process, where d is the distance between such populations. The probability of establishment of a new population is thus independent of other extant populations. By contrast, dispersal arising from natural origins was modelled by a single-tail Gaussian distribution $f(d, \sigma)$, where σ is the standard deviation for the distribution.

The nearest-neighbour distances for anthropogenic and natural components can be described by a mixture distribution

$$L_y(d) = (1 - \pi)g_y d + \pi f(d, \sigma)$$

with π as the proportion due to natural origins.

Based on the relative likelihood of natural and anthropogenic distributions

for its nearest-neighbour distances, the probability that a point was of natural origin was estimated by

$$W_i = \frac{\pi f(d_i, \sigma)}{(1-\pi)g(d_i) + \pi f(d_i, \sigma)}$$

where π and σ were initial guesses.

The parameters π and σ were updated by computing

$$\pi = \frac{\Sigma W_i}{n}$$

and

$$\sigma = \sqrt{\frac{\Sigma(W_i d_i^2)}{\Sigma W_i}}.$$

The computations of W_i, π and σ were iterated, until estimates of π and σ did not change by more than 0.00001 during successive iterations. Butikofer et al. (2018) found that these estimations were robust to a range of initial estimates of π and σ.

While the EM algorithm had the capacity to discriminate anthropogenic and natural dispersals, the nearest neighbour to a point could not be interpreted as the source population for that point. Therefore, the dispersal kernel could not be estimated from nearest-neighbour configurations. Instead, points were classified as anthropogenic if W_i is less than 0.5, and natural otherwise.

The dispersal distance probability distribution was described by the function

$$f(x) = \frac{C}{2\alpha\Gamma(\frac{1}{C})} e^{-|\frac{x}{\alpha}|^C}$$

where Γ is the gamma function, C the slope parameter and α the distance parameter.

Following the authors' recommendation, I set $C = 2$ (which represented a Gaussian distribution to account for kurtosis), leaving a single variable α to be estimated. To compute the dispersal kernel, simulations across a range of α values were run, and the similarity between actual and simulated distribution was measured by Ripley's K-function. The α value returning the greatest similarity was taken as the dispersal kernel. Graphically, this was the minimum inflexion point on a plot of dissimilarity against α values.

To reduce computation cost, the initial α values were set from one km to 80 km in intervals of 10 km. The global minimum inflexion point was estimated from the dissimilarity graph, and additional simulations were re-run, with α values centred around the global minimum. The search radius for Ripley's K-function was set to a lower bound of one km and an upper bound equal to that for α. For global searches, search intervals were set to one km, and reduced as

appropriate for local searches (for example, 0.1 km intervals for $\alpha < 5$).

The BIOLINV package allowed for the generation of random points based on habitat suitability values from zero (not suitable) to one (suitable). Excess points were generated per model run, and deleted with a probability corresponding to the reciprocal of the habitat suitability value of the cell they fell into. Remaining points were then filtered by random sampling to the required number.

As the study taxa were introduced to Australia as ornamentals (with the likely exception of *G. gueinzii*; see Chapter 1), anthropogenic influence on their spatial distribution were incorporated by proximity to roads (see above) and to cities. City data for Australia were filtered from the data set *world.cities* from the R package MAPS (Brownrigg, 2018), and geographic coordinates were obtained from a Google API via the `geocode` function from the GGMAP package (Kahle and Wickham, 2013). A city distance raster was generated in the manner described in Section 5.2.1.

The habitat suitability map (HSM) was created by the element-wise multiplication of the species' ecological probability raster (see Chapter 4), the proximity to roads raster and the proximity to cities raster:

$$r_{i,j} = \Pi(r_{\text{ecology}_{i,j}})(r_{\text{roads}_{i,j}})(r_{\text{cities}_{i,j}})$$

where $r_{i,j}$ represents the value of a cell at position (i, j); rasters were scaled to [0, 1] prior to multiplication. Finally, the HSM was re-projected to a projected coordinate system (Albers: EPSG 3577) for distance measurement by the BIOLINV package.

Species occurrence data were downloaded from the Global Biodiversity Information Facility (GBIF; www.gbif.org) for *G. tristis* in November 2017 and May 2018 for the other study taxa (see Figs 1.3 and 1.4). Duplicate records were removed, and remaining records were checked for plausible georeferencing (see Chapter 4). While Butikofer et al. (2018) recommended that only records with uncertainty of less than the model cell size be tested, I found that this procedure resulted in the removal of many early records, particularly before the 1950s. As these early records might capture long-distance anthropogenic dispersal, records with uncertainty below 10 km were retained for analyses. A total of 101 records for *G. gueinzii*, 138 for *G. tristis*, 472 for *G. undulatus*, 987 for *W. meriana* var. *bulbillifera* and 81 for *W. meriana* var. *meriana* were retained for testing (Figs. 5.1 and 5.2).

For each species, three jackknifed data sets were run, and the mean and standard error for α, and the number of anthropogenic and natural origins were calculated.

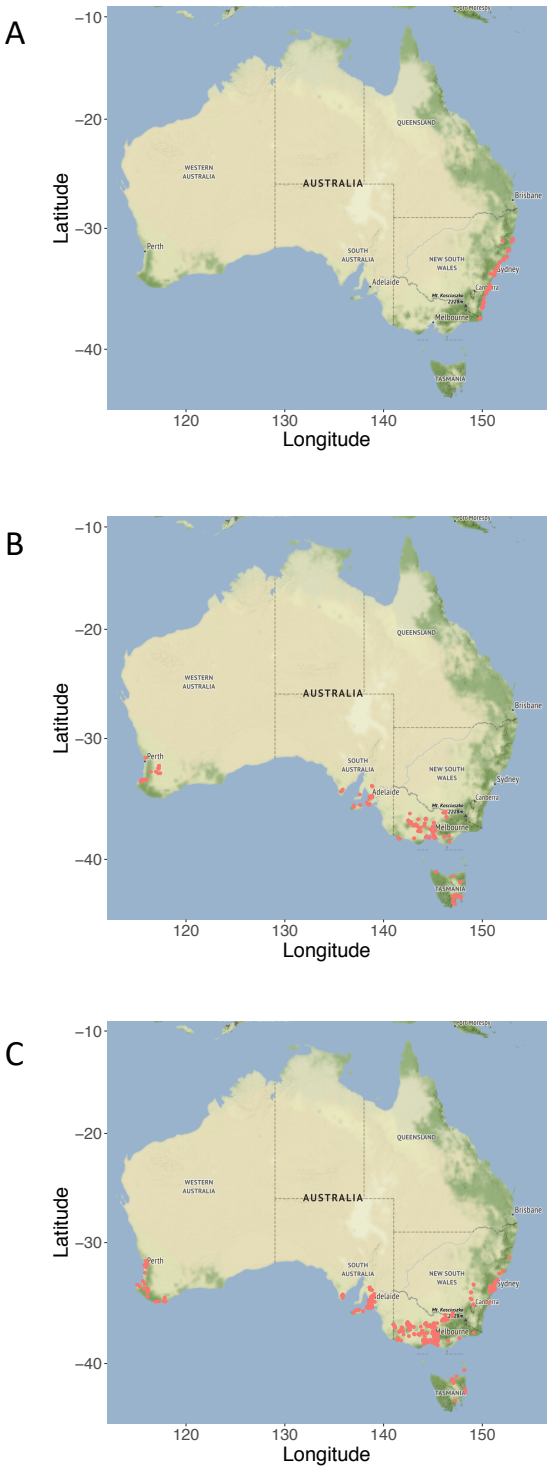

Figure 5.1: Sample points for the estimation of dispersal kernel and the proportion of anthropogenic vs. natural dispersal events for **A**: *Gladiolus gueinzii*, **B**: *G. tristis* and **C**: *G. undulatus*. Note that *G. gueinzii* was excluded from this analysis due to a unresolvable runtime error; see the text for details.

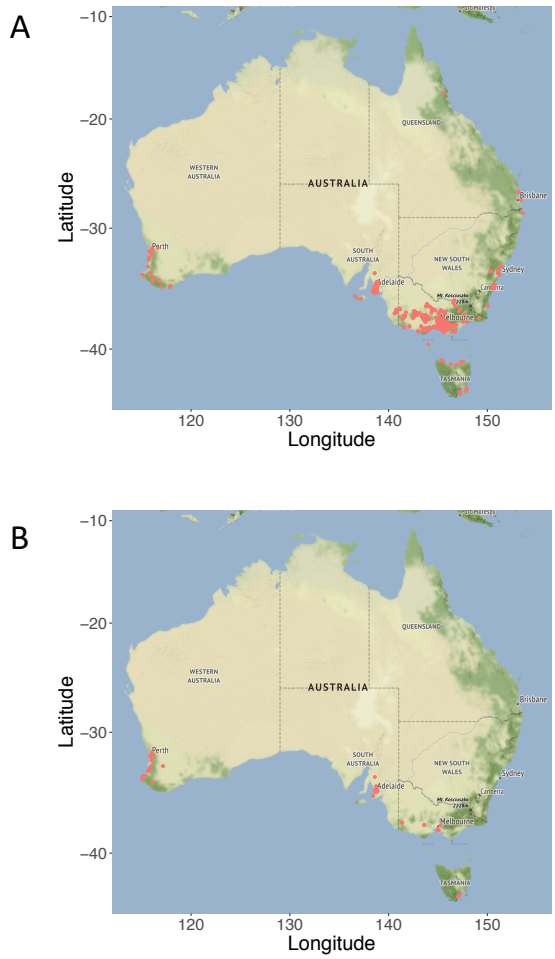

Figure 5.2: Sample points for the estimation of dispersal kernel and the proportion of anthropogenic vs. natural dispersal events for **A**: *Watsonia meriana* var. *bulbillifera* and **B**: *W. meriana* var. *meriana*.

5.2.3 Proximity to roads

Occurrence records for my study taxa were downloaded from the Victorian Biodiversity Atlas (VBA) in September 2018. These records were collected by Department of Environment, Land, Water and Planning (DELWP) staff, government agencies and partner organisations, non-government organisations, ecological consultancies, university students and community wildlife survey groups. As such, the VBA presented a comprehensive database of biodiversity across Victoria.

As *G. gueinzii* was only recorded in one location in Victoria (Mallacoota), this species was excluded from further analyses. Scrutiny of the VBA data set revealed that some samples were classified only as "*Watsonia meriana*". As the infraspecific identification for these records was indeterminate, they were removed from further analyses. In addition to the VBA records, I added my records from field surveys during 2011–15 (see Appendix A). Records with a geographical precision of $\leq$ 100 m were retained, except for *W. meriana* var.

meriana, where all VBA records were ≥ 500 m in precision. Duplicates were then removed using the duplicated function in R (R Core Team, 2019). Final population sizes were 52 for *G. tristis*, 149 for *G. undulatus*, 580 for *W. meriana* var. *bulbillifera* and 7 for *W. meriana* var. *meriana* (Fig. 5.3).

Road data for Australia were downloaded from OpenStreetMap (www.openstreetmap) under an Open Database License in September 2019, and delimited to Victoria by computing their intersection with a spatial shape file for the state (obtained from www.gadm.org), using the st_intersection function from the R package SF (Pebesma, 2018). The truncated spatial polygon was then converted to a raster object with rasterize from the RASTER package (Hijmans, 2020). A distance raster of 30" resolution was created by computing the distance for each empty cell to the nearest non-empty cell (that is, containing road datum), using the distance function in the same package. Finally, the mean distance for the occurrence points of each taxon was calculated by averaging the cell values on which the points fell.

I used a randomisation procedure to test the association between road proximity and the spatial distribution for each study species. To generate plausible random points, the road distance raster was masked by the species' distribution raster (see Chapter 4), using the mask function in the RASTER package. A total of 9999 randomisation trials were run per species. For each trial, an equal number of random points to occurrence points were generated with the randomPoints function in the DISMO package (Hijmans et al., 2017), and the mean distance of random points to nearest road was calculated as above. A p value was computed, based on a one-tailed test.

5.3 Results

5.3.1 Tempo of colonisation

Sampling records for the study species showed that both short- and long-distance dispersals occurred in tandem.

While invasive spread was limited in *G. gueinzii* during the decade after introduction around 1950 (Fig. 5.4A), it began to accelerate from the 1960s, with a long-dispersal event to South West Rocks (Fig. 5.4C). In the 1980s, there was infilling of the intervening region and southwards expansion (Fig. 5.4D–E), followed by consolidation from the 1990s, but with no evident range expansion at the margins (Fig. 5.4F–G).

For *G. tristis*, a long-distance dispersal event signalled the colonisation of the mainland more than 20 years after introduction to insular Tasmania (Fig. 5.5B). Over the next two decades, the species consolidated its presence in Victoria and South Australia (Fig. 5.5C), but it was only after the 1940s that it was

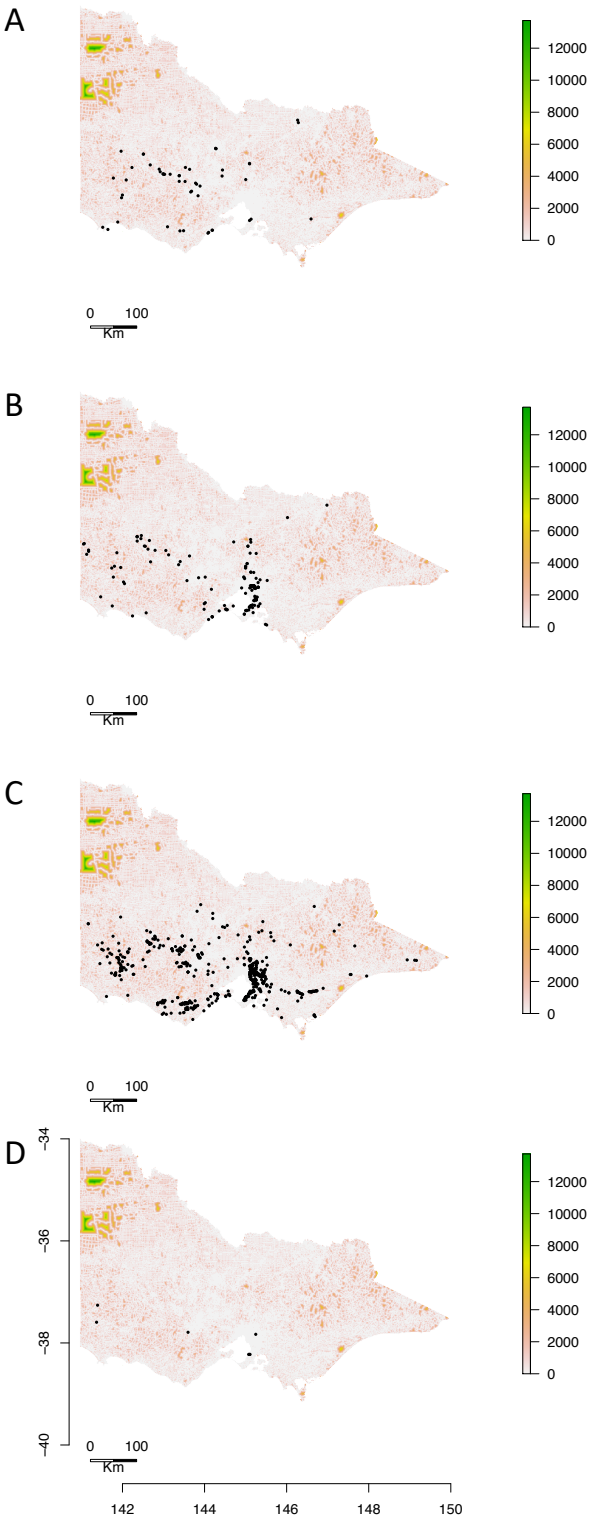

Figure 5.3: Sample points for quantifying proximity of populations to roads. The points were downloaded from the Victorian Biodiversity Atlas and supplemented with survey data conducted between 2011 and 2015. Duplicate records and records with precision of > 100 m were removed prior to analysis; see the text for details. **A**: *Gladiolus tristis*, **B**: *G. undulatus*, **C**: *Watsonia meriana* var. *bulbillifera* and **D**: *W. meriana* var. *meriana*. The legends show the distance of a cell from the nearest road in meters. Note that *G. gueinzii* was excluded from this analysis.

introduced to Western Australia (Fig. 5.5D). Regional encroachment took place from the 1960s to the present (Fig. 5.5E–G).

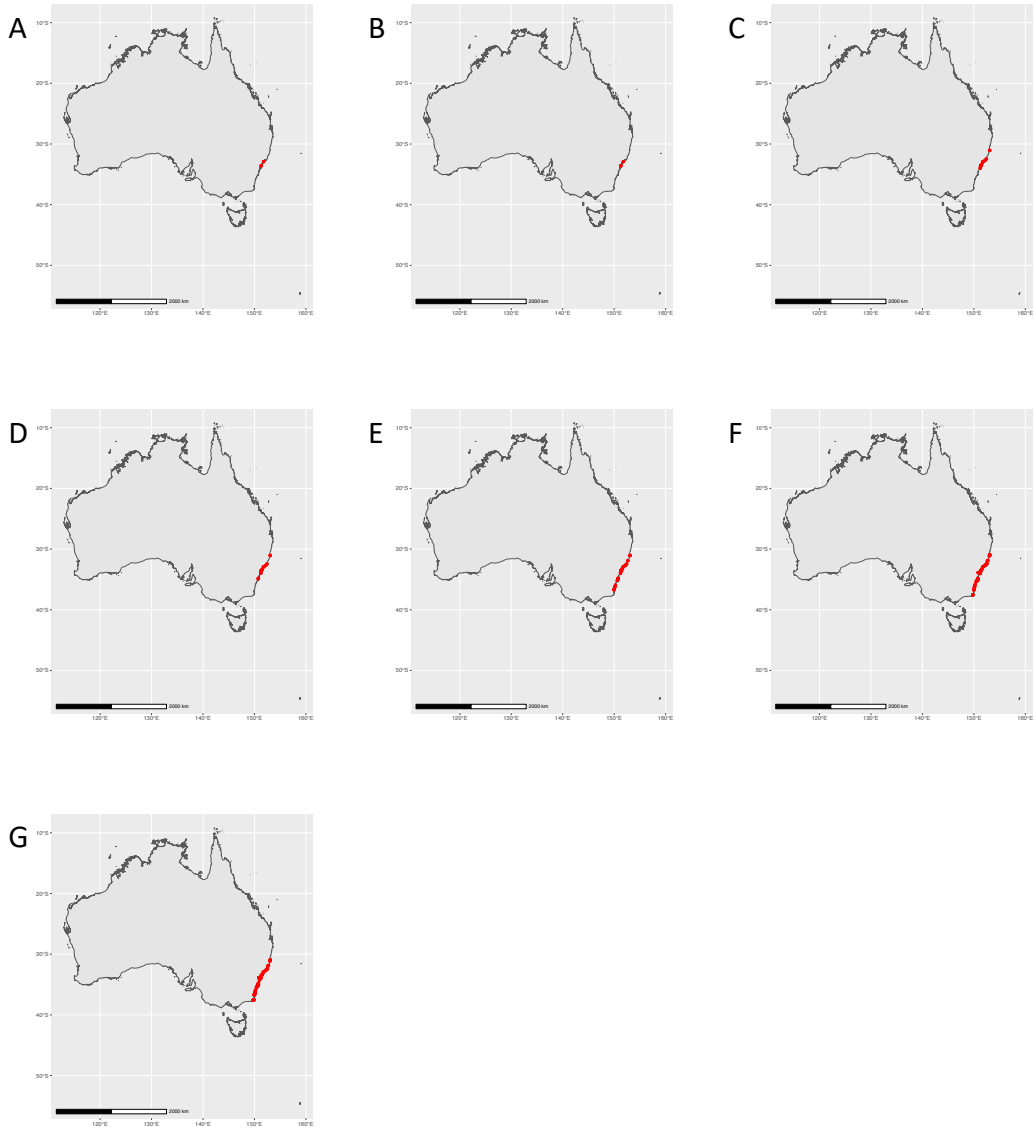

Figure 5.4: Records of *Gladiolus gueinzii* collection over time. **A**: 1950–1955, **B**: 1955–1960, **C**: 1960–1972, **D**: 1972–1984, **E**: 1984–1996, **F**: 1996–2008 and **G**: 2008–2017.

By contrast, *G. undulatus* experienced long-distance dispersal soon after introduction (Fig. 5.6A–C), with a period of consolidation from the 1920s to the 1940s (Fig. 5.6D). Similar to *G. tristis*, the species was introduced to Western Australia after 1940 (Fig. 5.6E), followed by regional spread from then on (Fig. 5.6F–H).

W. meriana var. *bulbillifera* also achieved a high degree of geographical spread after its introduction to Tasmania (Fig. 5.7A–B). The first record in Western Australia appeared after 1940 (Fig. 5.7C). While regional spread occurred from the 1960s, a long-distance dispersal event placed the variety above -30° latitude (Fig. 5.7E). Following a period of consolidation (Fig. 5.7F), *W.*

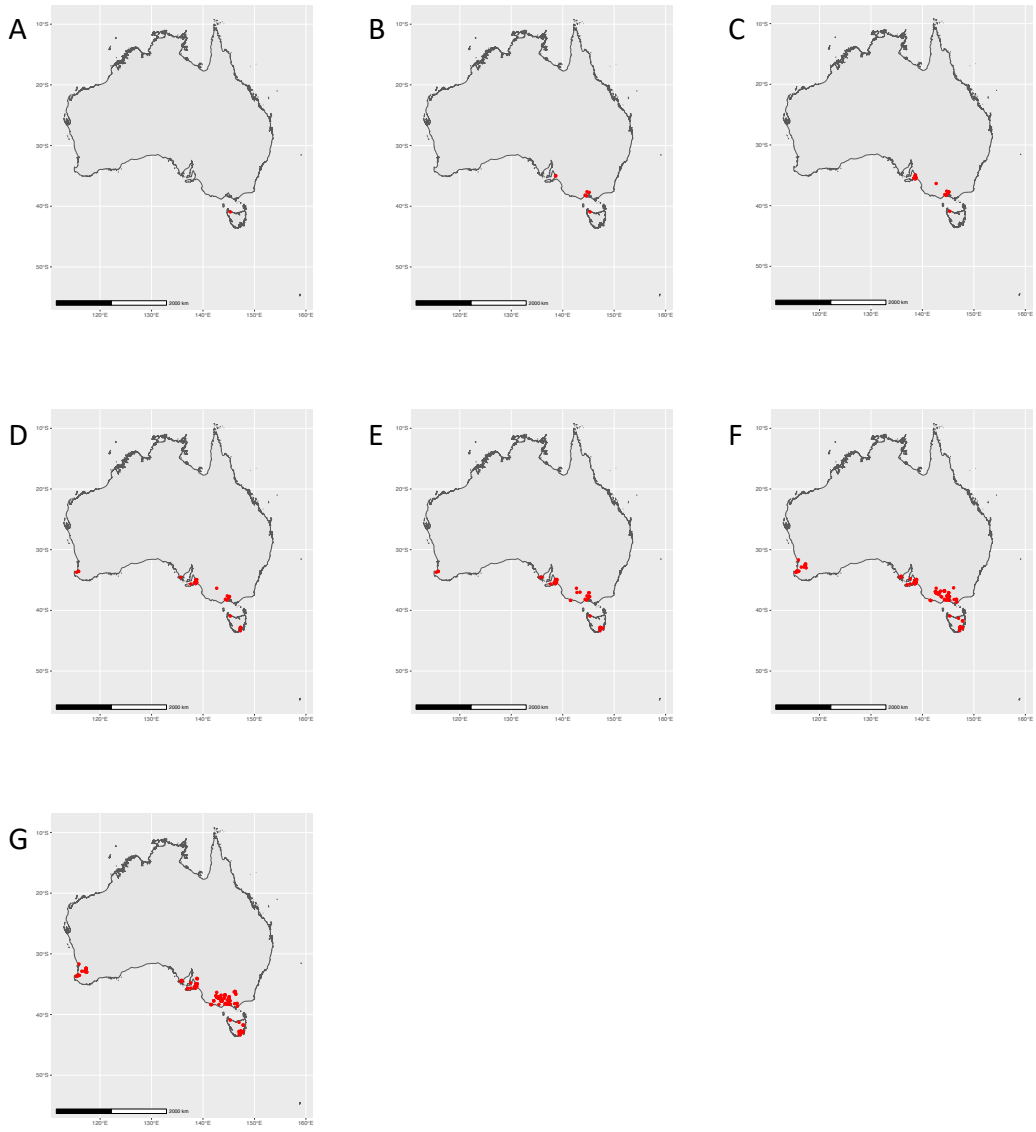

Figure 5.5: Records of *Gladiolus tristis* collection over time. **A**: 1880–1900, **B**: 1900–1920, **C**: 1920–1940, **D**: 1940–1960, **E**: 1960–1980, **F**: 1980–2000 and **G**: 2000–2013.

meriana var. *bulbillifera* was recorded above the -20° latitude from the turn of the century (Fig. 5.7G).

By contrast to other taxa, *W. meriana* var. *meriana* was first recorded in Western Australia (Fig. 5.8A), and eastwards in South Australia after 1920 (Fig. 5.8B). Regional spread took place until 1980, with the variety recorded in Tasmania and Victoria during this period (Fig. 5.8C–E). Since then, regional spread and movement of < 500 km have characterised the spatial distribution of *W. meriana* var. *meriana* (Fig. 5.8F–G).

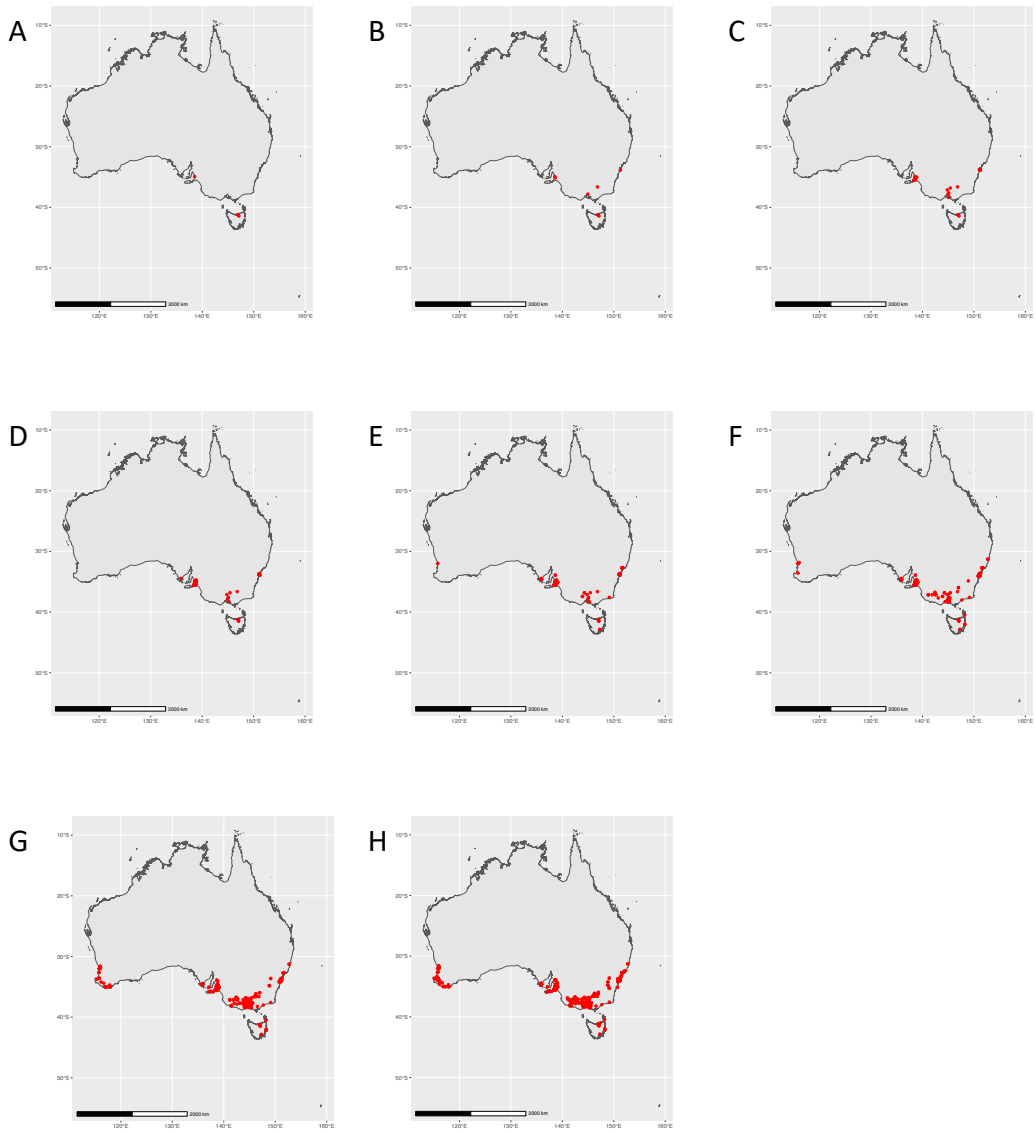

Figure 5.6: Records of *Gladiolus undulatus* collection over time. **A**: 1860–1880, **B**: 1880–1900, **C**: 1900–1920, **D**: 1920–1940, **E**: 1940–1960, **F**: 1960–1980, **G**: 1980–2000 and **H**: 2000–2015.

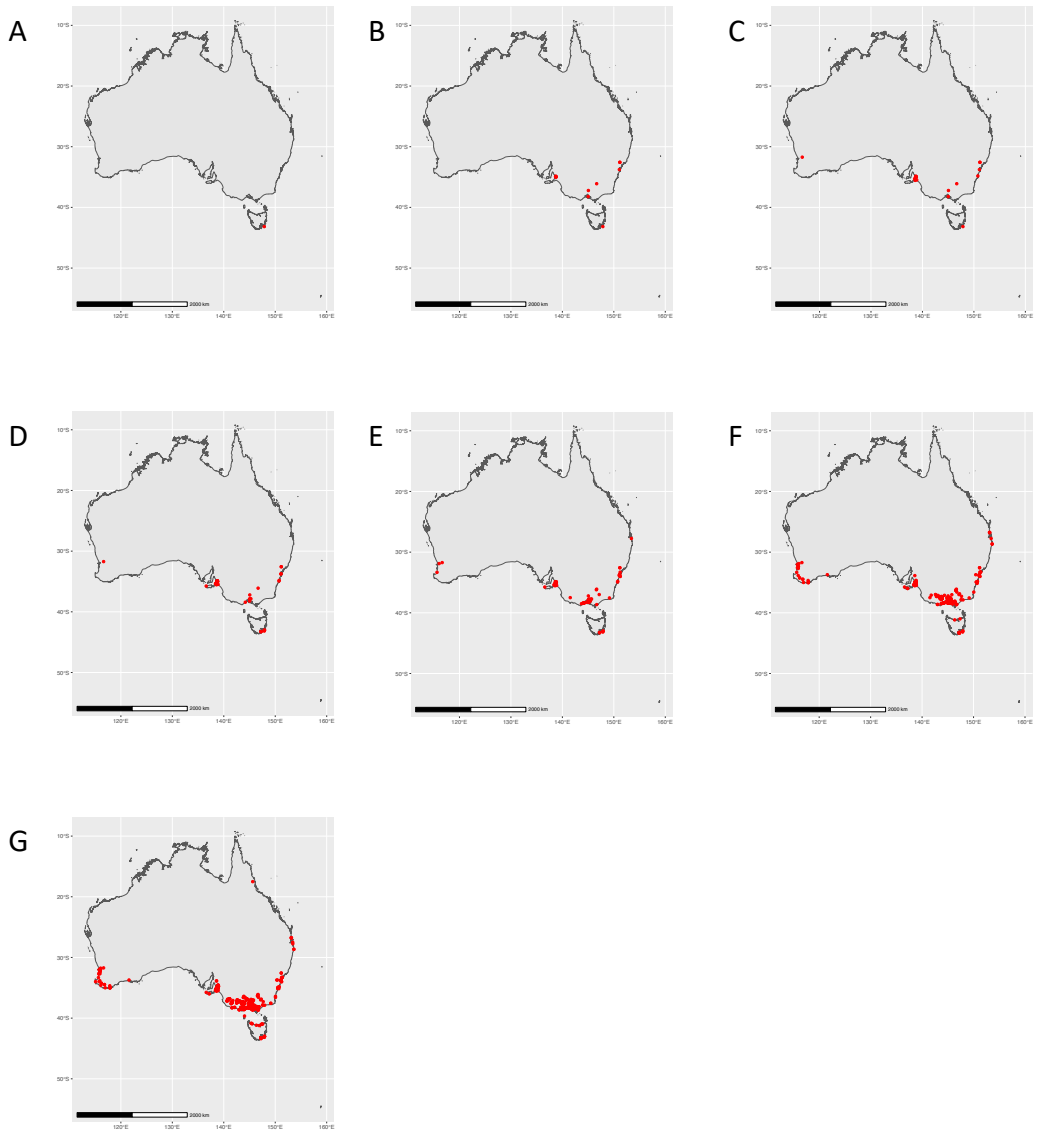

Figure 5.7: Records of *Watsonia meriana* var. *bulbillifera* collection over time. **A**: 1880–1900, **B**: 1900–1920, **C**: 1920–1940, **D**: 1940–1960, **E**: 1960–1980, **F**: 1980–2000 and **G**: 2000–2017.

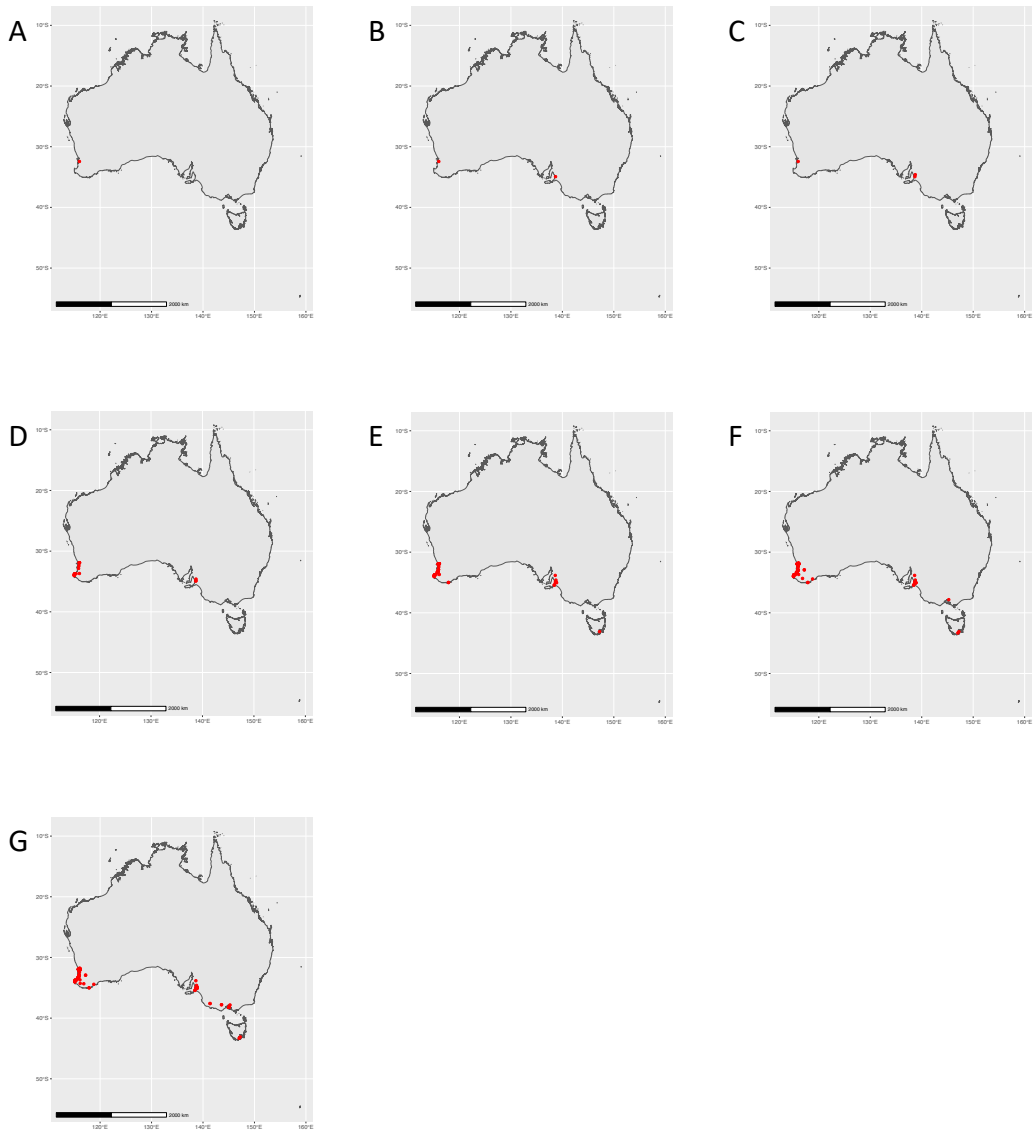

Figure 5.8: Records of *Watsonia meriana* var. *meriana* collection over time. **A**: 1880–1900, **B**: 1900–1920, **C**: 1920–1940, **D**: 1940–1960, **E**: 1960–1980, **F**: 1980–2000 and **G**: 2000–2015.

5.3.2 Dispersal kernels, and natural vs. anthropogenic dispersal

Due to a run-time error thrown by the EM algorithm, the proportion of anthropogenic versus natural origins, as well as the dispersal kernel for *G. gueinzii* could not be computed.

For *G. tristis*, anthropogenic origins made up 8.33% of recorded spatial occurrence points, with the highest dispersal kernel (> 70 km) recorded among all taxa (Table 5.1).

A similar level for anthropogenic origin was inferred for *G. undulatus* (9.03%) (Table 5.1). However, the dispersal kernel (22 km) was substantially lower than that found for *G. tristis*.

Anthropogenic origins constituted 8.04% of recorded populations for *W. meriana* var. *bulbillifera*, with a dispersal kernel of over 2 km (Table 5.1).

While *W. meriana* var. *meriana* experienced the greatest proportion of points of anthropogenic origin (13.64%) among the study taxa, it was found to have the shortest dispersal kernel (1 km) (Table 5.1).

Table 5.1: Classification of origins of occurrence points and kernel estimation for *Gladiolus tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*. Based on three jackknifed populations per species, the first two columns show the number of points from anthropogenic and natural dispersal with standard error. For natural dispersal, α is an estimation of the dispersal kernel.

	Anthropogenic origin	Natural origin	α (km)
G. tristis	10.333 ± 0.192	113.667 ± 4.141	71.667 ± 2.546
G. undulatus	38.333 ± 0.770	386.333 ± 12.894	21.667 ± 0.962
W. meriana var. *bulbillifera*	75.333 ± 0.192	862.000 ± 28.868	2.167 ± 0.096
W. meriana var. *meriana*	9.000 ± 0.456	57.000 ± 1.399	1.050 ± 0.171

5.3.3 Proximity to roads

For *G. tristis*, *G. undulatus*, *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana*, randomisation tests were significant at $\alpha = 0.05$ (Table 5.2 and Fig. 5.9), supporting the hypothesis that their spatial distributions were not random, but were clustered around road structures. In general, the distances from roads were less than 100 m, while simulated populations were 400 m or more from roads.

5.4 Discussion

With the exception of the coastal geophyte *G. gueinzii*, I found that four other introduced Iridaceae (*G. tristis*, *G. undulatus*, *W. meriana* var. *bulbillifera* and

Table 5.2: Proximity to roads for *Gladiolus tristis*, *G. undulatus*, *Watsonia meriana* var. *bulbillifera* and *W. meriana* var. *meriana*. Results from randomisation tests, for the null hypothesis that the spatial distribution of recorded occurrence points were independent of proximity to road structures.

		Distance from road (m)		
	N	Actual	Randomised	p
G. tristis	52	23.33	588.84	< 0.001
G. undulatus	149	80.67	432.48	< 0.001
W. meriana var. *bulbillifera*	580	22.56	398.58	< 0.001
W. meriana var. *meriana*	7	0.00	400.78	< 0.001

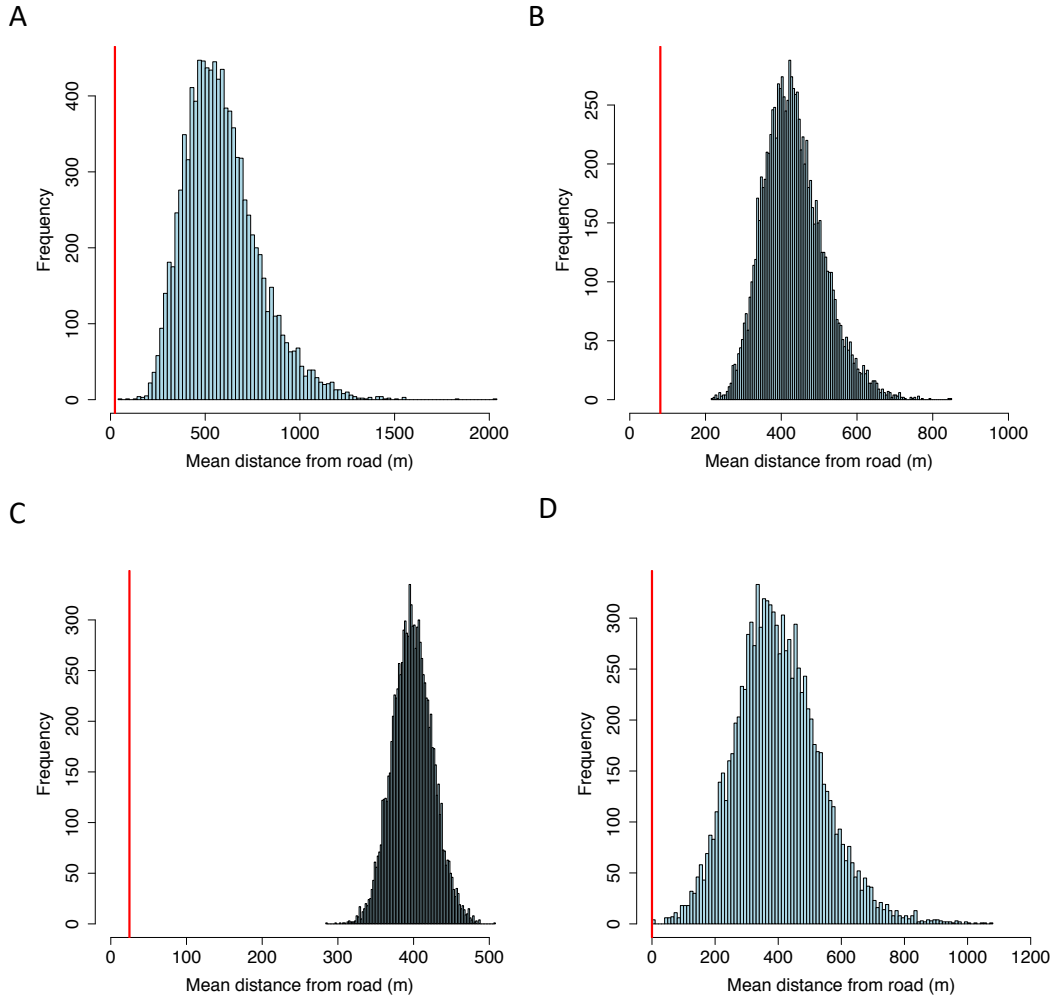

Figure 5.9: Frequency distribution of permutation tests for distance from population to nearest road. **A**: *Gladiolus tristis*, **B**: *G. undulatus* and **C**: *Watsonia meriana* var. *bulbillifera* and **D**: *W. meriana* var. *meriana*. The vertical red lines represent the mean distance of Australian populations from the nearest road for each species. For each species, 9999 trials were run.

W. meriana var. *meriana*) demonstrated short- and long-distance dispersal patterns in their adventive range in Australia. Both dispersal modes appeared to proceed simultaneously, giving rise to regional and local colonisation. Using an EM algorithm, it was also found that their spatial patterns bore anthropogenic and natural origins, although the contribution from natural dispersal

was substantially greater compared to human-mediated movement by an order of magnitude. Dispersal kernels of the study species were highly variable, and their magnitudes were not consistent across their reproductive modes. While *G. tristis* and *G. undulatus* reproduced vegetatively by cormels, the dispersal kernel of *G. tristis* was inferred to be three times larger than that for the *G. undulatus*. When compared to *W. meriana* var. *meriana* (which reproduced via seeds), the dispersal kernel of *G. tristis* was found to be sevenfold higher. By contrast, the dispersal kernels for *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana* were more similar, despite different reproductive strategies (aerial cormels versus seeds respectively). Finally, permutation tests demonstrated that the study taxa (excluding *G. gueinzii*) were significantly arrayed along roads than expected by chance alone.

It is not implausible for congeners (or confamilials) to demonstrate high variance in dispersal kernels. When they applied their framework to introduced frogs in New Zealand, Butikofer et al. (2018) found that *Litoria ewingii* had twice the kernel size compared to *L. aurea*. By contrast, dispersal characteristics were similar in the invasive thistles *Carduus nutans* and *C. acanthoides* (Skarpaas and Shea, 2007). Generally for terrestrial plants, seed dispersal distance is governed by plant height (Thomson et al., 2011), dispersal agent (García et al., 2007), dispersal syndrome and seed traits (Tamme et al., 2014), which may not be homologous across closely-related taxa.

As no result has been published for dispersal characteristics or population genetic structure of the Iridaceae in its native range, the high variance in kernel size inferred from Australian records could not be corroborated here. However, I argue that the estimated kernel of 70 km for *G. tristis* is likely to be an overestimation. Both *G. tristis* and *G. undulatus* occupy overlapping ecoregions, and it is not unreasonable to assume that their cormels are transported by functionally similar vectors, and should therefore result in comparable kernel magnitudes. The overestimation of *G. tristis* kernel may stem from the implementation of the assumption in the EM algorithm that new populations of natural origins are spatially closer than those of anthropogenic origins. A consequence of this assumption is that the algorithm may infer anthropogenic points as natural ones, when the former are highly aggregated (Butikofer et al., 2018). It is not difficult to imagine the scenario where local spread (that is, points of natural origin) is poorly (or not) captured by the recording or collection of a single sample from a population (Nic Lughadha et al., 2019), leading to the erroneous inference of natural origins for proximal but anthropogenically-dispersed points. I suggest that the user be permitted to define a prior probability distribution in a future implementation of the EM algorithm, based on biological knowledge of propagule dispersal patterns (for example, Vittoz and Engler, 2007).

The finding that the dispersal kernel of *W. meriana* var. *meriana* was the smallest among the focal taxa was also unexpected, given that seeds are considered to be specialised dispersal structures. However, the magnitudes of the dispersal kernel for *W. meriana* var. *bulbillifera* and *W. meriana* var. *meriana* suggest that they too were overestimated. Seed trapping showed a dispersal limit of 80 m for the shrubs *Calluna vulgaris* and *Erica cinerea* (Bullock and Clarke, 2000), and mean dispersal distance was around 2 m for *Carduus nutans* and *C. acanthoides* (Skarpaas and Shea, 2007). By contrast, Ronsheim (1994) found that there was no significant difference between mean dispersal distance for seeds (37.5 cm) and bulbils (35.1 cm) in the geophyte *Allium vineale*, with the maximum dispersal distance greater for seeds (130 cm) compared to bulbils (95 cm). In another study, even when *Dioscorea japonica* bulbils were secondarily dispersed by rodents, Mizuki and Takahashi (2009) found that they were moved at most 7.4 m, with a mean distance of 1.1 m (S.E. = 1.3). Another study on the neotropical understorey herb *Goeppertia marantifolia* found that the maximum mean dispersal distance of bulbils was 124 cm (Matlaga et al., 2017). Given that the dispersal kernels of my study species were up to three orders greater in magnitude relative to other findings, it is suggested that these results be treated with caution.

While the permutation tests were in agreement with field observations, the proximity of my study taxa to transport routes was only tested at a regional scale (the State of Victoria). By contrast, incursion of geophytes into bushland has been recorded in south-west Australia (Brown and Brooks, 2002), and in particular the encroachment of *W. meriana* var. *meriana* into *Banksia* woodland (Brown, 2006) and of *W. meriana* var. *bulbillifera* into clay-based wetlands (Brown et al., 2008) on the Swan Coastal Plain 200 km south of Perth. It was not known whether these incursions occurred naturally, but it was thought that regenerative plant materials were transported to these sites as garden rubbish or soil, with secondary spread by water, soil movement, birds and human activity (Brown and Brooks, 2002). In California, *W. meriana* var. *bulbillifera* has been recorded along an arterial highway (Madison, 1996), but the confamilial *Romulea rosea* was found in an otherwise undisturbed forest plot in Texas (Singhurst et al., 2009).

While the proximity of invasive Iridaceae to roads suggests an anthropogenic element in range expansion, such influence may be indirect rather than direct (where humans are the dispersal vectors). Road-based anthropochory can be measured by quantifying propagule transport by machinery (Zwaenepoel et al., 2006), human movement (Pickering et al., 2011) or soil movement (Rauschert et al., 2017). While such a study was beyond the capacity of this work, it was likely that direct and indirect components contributed to long-distance disper-

sal in the focal taxa. In Australia, rural roads are frequently delineated by drainage lines (for example in montane areas: Johnston and Johnston, 2004), which can form naturally from changes to hydrology following disturbance, such as road building (Burch et al., 1987). These drainage lines can facilitate propagule movement by hydrochory at local and regional scales, akin to the dispersal of vegetative fragments along riparian habitats (Pyšek and Prach, 1996; Howell and Benson, 2000) or by ephemeral non-riparian water dispersal (Tekiela and Barney, 2013). Conversely, spatial patterns at landscape scales can be reasonably attributed to humans, such as the colonisation of Western Australia by *G. tristis*, approximately 2000 km from the nearest population in South Australia in the 1940s with no intervening colonies being recorded. However, these patterns could have arisen from stochastic events such as multiple introductions from their native range, or from horticultural trade (Okada et al., 2007). Future work with molecular data will be required to decipher the invasion history and corroborate the natural and anthropogenic contributions to the spatial patterning of introduced Iridaceae to the continent (Estoup and Guillemaud, 2010; Bras et al., 2019).

5.4.1 Implications

In agreement with my observations during field population surveys, the focal taxa (with the exception of *G. gueinzii*) were shown to occupy habitats with greater proximity to roads than expected. In its native range, *G. tristis* occupies a wide range of altitudes and soils (Goldblatt and Manning, 1998), while *G. undulatus* is primarily restricted to mesic substrates of coarse or sandstone soils (Goldblatt and Manning, 1998), and *W. meriana* s.l. has affinity to seasonally-moist sand or thin rocky soils (Goldblatt, 1989). The proximity to roads might have arisen as an artefact of anthropogenic introduction and dispersal; however, it also suggests that these taxa are more labile in their habitat requirements than expected.

Roads have different ecologies compared to natural areas (Forman and Alexander, 1998). Roads are relatively impervious, and channel rain drainage to their shoulders. These drainage lines are expected to provide suitable germination sites for the study taxa, as *G. tristis*, *G. undulatus* and *W. meriana* s.l.[1] are associated with mesic habitats in their native range (Goldblatt, 1989; Goldblatt and Manning, 1998). In addition, the absence of dense canopies above roads increases solar penetration, which simulates the open to mid-dense cover in fynbos and renosterveld environments in southern Africa (Moll et al., 1984). On the other hand, how altered disturbance regimes impinge the persistence of introduced Iridaceae is less clear. While changes in disturbance regimes is positively

[1]Goldblatt does not recognise any infraspecific delineation of *Watsonia meriana*.

associated with site invasibility (Moles et al., 2012), the direction and magnitude of change in disturbance regimes along road habitats in Australia relative to natural communities in southern Africa is not presently known.

Alterations in disturbance regime along Australian roads include changes to fire regime, herbivory and soil properties. Rural roads in Victoria, Australia are used as fire breaks, and are subjected to prescribed burning (Country Fire Authority, 2011), which has been found to enhance biological invasion of roadside vegetation by introduced grasses (Milberg and Lamont, 1995). While mole rats and similar subterranean herbivores are absent in Australia (Begall et al., 2007), the mowing of roadside forbs simulate herbivory by the removal of foliar material, although underground storage organs are unlikely to be affected. However, specific response to herbivory is likely to be governed by adaptation to such interactions. Grazing in eastern Mediterranean grasslands maintain geophyte diversity (Noy-Meir and Oron, 2001), but the converse may be true for South Africa, where elephants were observed to avoid feeding on geophytes (Landman et al., 2008).

Invasion between southern Africa and Australia is enhanced by similar climatic and infertile landscapes (Thuiller et al., 2005; Hopper, 2009). However, roadside disturbance can alter the physical, chemical and biological properties of soil. A study in the Australian Alps showed that soil particles became coarser nearer to roads; in addition, relative to natural vegetation, road verges and drainage lines had higher pH, lower levels of nutrients (nitrogen and phosphorus) and organic matter, and significantly different levels of exchangeable cations (Johnston and Johnston, 2004). Another study in southern Brazil found that elevated levels of calcium was linked to lower aluminium toxicity and greater non-indigenous plant richness (Barbosa et al., 2010). Roadside flora may also experience heavy metal pollution, although toxicity can be ameliorated by physiological tolerance (Yang et al., 2007) and by the low mobility of heavy metals (Pagotto et al., 2001).

A switch in habitat type is by no means rare in invasive species. In a literature-based study of 286 invasive species, Hejda et al. (2015) found that many species invaded habitats different from their native-range habitats; for example, there was an increase in the species number in adventive-range scrublands (120) compared to that in the native ranges (60). As their study was deliberately performed at a coarse (bioregional) scale, it is also possible that invasive populations remain affiliated to pockets of similar native-range habitats, such as microrefugia nested among more dissimilar environments. A ramification of such affiliation to rare or special habitats can create an incongruence between occupancy patterns and species distribution or ecological niche model predictions (Worth et al., 2014, but see Soley-Guardia et al., 2016). A second

implication pertains to the management of invasive Iridaceae in Australia, as guidelines exist for controlling road invasions (Panetta and Hopkins, 1991), although the linear and often extensive nature of transport corridors will require substantial resources and concerted efforts (Joly et al., 2011).

In conclusion, this chapter has presented evidence that the focal species (with the exception of *G. gueinzii*) have attained invasive spread via transport corridors, and that there was an anthropogenic component in their dispersal (which was likely to be underestimated by the EM algorithm). Unfortunately, it is presently beyond the capacity of this study to compare the response curves of the focal species between native and adventive ranges. Thus, future studies are needed to elucidate their ecological amplitude in their native range (Hejda et al., 2009), as a basis for a biogeographical understanding of invasion success (Hierro et al., 2005).

Chapter 6

Synthesis

To paraphrase Sexton et al. (2009), biological invasions, while regrettable, provide useful lessons in evolutionary ecology and biogeography. Invasion biology itself is a fairly new discipline, and its inauguration was usually credited to Charles Elton's seminal work, *The Ecology of Invasions by Animals and Plants* (Elton, 1958). However, the transport of biota by humans had begun long before Elton's work, and records of non-native species started appearing in the 1700s (Davis, 2009). Due to the voluminous number of naturalised floras globally (over 13,000 species, van Kleunen et al., 2015, 2018), natural long-range colonisation can be confounded with human-mediated transport (for example, Wasowicz et al., 2018) and range expansion by native species (Nackley et al., 2017; Tong et al., 2018). This obfuscation has engendered debate about the nature and definition of invasive species (Colautti and MacIsaac, 2004; Hoffmann and Courchamp, 2016a; Essl et al., 2018).

Wilfully disregarding differences in semantics, it is clear that many non-native species are able to thrive, despite the high failure rate in colonisation success (Zenni and Nuñez, 2013). In this work, I define a species as invasive if it is able to reproduce and spread to new locations (*sensu* Richardson et al., 2000b). In terrestrial plants, invasive spread is predicated on the progressive dispersal of propagules, usually over generations, which in turn is underpinned by the reproductive mode (or modes) available to a species (Barrett, 2011). Traditionally, the role of mating system in non-native taxa has focused on the incipient stages of invasion (introduction and naturalisation) (Baker, 1955; Razanajatovo et al., 2016). The goal of my thesis was to explore the relationship between invasiveness and reproductive and pollination systems. Invasive South African Iridaceae in Australia provide a compelling basis for such studies, as they boast diverse pollination syndromes, mating systems and ecological amplitudes. Additionally, South African Iridaceae were introduced during colonial settlement (around 150 years ago), thereby circumventing time lag issues, but also at once identifiable as neophytes. My thesis addresses the questions: (1) does pollinator decoupling

promote a transition to self-fertilisation in self-incompatible species? (2) is there an association between invasiveness and mating system? and (3) how do asexual plant species generate invasive spread?

By pollinator exclusion and manual pollen supplementation (Chapter 2; Questions 1 and 2, Section 1.6), I ascertained that self-incompatible Iridaceae (*Gladiolus tristis* and *G. undulatus*) did not evolve self-fertility in their introduced range; therefore, invasive spread relied on the dispersal of cormels. Through pollinator observation, I also found no evidence that *Gladiolus* with specialised pollination syndromes were visited by pollen vectors, whereas *Watsonia meriana* var. *meriana* and *W. meriana* var. *bulbillifera* received attention from honey bees (*Apis mellifera*) and the New Holland honeyeater (*Phylidonyris novaehollandiae*). It is doubtful if *G. tristis* and *G. undulatus* were able to set seed even with pollinator visitation, as their pollen viabilities were found to be very low. By contrast, the presence of pollen vectors and high pollen viability in *W. meriana* var. *meriana* ensured profuse seed set, in agreement with Harmon-Threatt et al. (2009), who found that invasive flowering plants were more likely to be capable of uniparental reproduction, or were successful in attracting resident generalist pollinators.

In addition, the absence of seed set in *G. tristis* in south-eastern Australia countered the assertion by van Kleunen and Johnson (2007b) that naturalisation success in this species was due to rapid and profuse seedling emergence. While the comparison of non-native species with differential invasiveness is the most direct test for factors driving invasions (van Kleunen et al., 2010a), comparisons between native and introduced ranges remain important (Hierro et al., 2005). While two moth species from the genus Noctuidae were observed pollinating *G. tristis* in its native range, Goldblatt and Manning (2002) admitted that "details of moth visits are limited because of the difficulty of observing their activity in the dark or under low intensity red light" (p. 118). They did not observe any diurnal visitors to *G. tristis*. On the other hand, Coombs and Peter (2010) found in South Africa that the introduced tropical vine *Araujia sericifera* (the 'mothcatcher') was able to attract and exploit native honeybees as a diurnal pollinators, whereas its large nectar volume, white flowers and nocturnal scent suggested that it was moth-pollinated in its native range, on which little is known. Although cursory observations were made, I did not ascertain in any detail whether *G. tristis* was visited by diurnal pollinators. In addition, the reason why *G. tristis* was not observed to be visited by the cosmopolitan hawk moth *Agrius convolvuli* remains a conjecture, although the early flowering period of the species may present a phenological mismatch with potential pollinators (Kudo and Ida, 2013), or that *G. tristis* is not pollinated by *A. convolvuli* in South Africa. Supplementary observations across more populations in their

native and introduced ranges may help to resolve the issue of pollinator absence for *G. gueinzii*, *G. tristis* and *G. undulatus* in Australia.

Robust inferences from molecular work (Chapter 3; Question 3, Section 1.6) was not possible, as it could not be ascertained if the microsatellite loci used were indeed homologous to *G. longicollis*. However, the agreement of expected and observed amplicon sizes, coupled with amplification in most loci gave some assurance that the molecular data could be interpreted, albeit not in a profound manner. The data showed a high degree of molecular uniformity in *G. gueinzii* and *G. undulatus* populations in New South Wales and Victoria, respectively. On the other hand, there were two distinct clusters in *G. tristis*. The presence of fixed heterozygous molecular phenotypes in *G. tristis* and *G. undulatus*, and general lack of variation among populations indicated obligate clonal reproduction, while molecular homogeneity suggested that invasive spread in *G. gueinzii* was via seeds locally, and cormels distally. Coupled with the isolated nature of *G. tristis* and *G. undulatus* populations, the spatial molecular structure also suggested that these species achieved spread by jump dispersal events; given the more immobile nature of cormels as compared to seeds, such spread was likely to be mediated by humans, such as vehicular carriage (von der Lippe and Kowarik, 2007).

Moreover, more than two amplicons were detected in Australian accessions of *G. tristis* and *G. undulatus*, suggesting polyploidy. In addition, a three-amplicon electropherogram pattern was also detected in a South African accession of *G. tristis*, leading to the suspicion of polyploidy in its native range. If polyploidy can be confirmed, it will be the first time that polyploidy has been recorded in these species. Polyploidy in their native range is particularly intriguing, as it was expected that polyploidisation would have arisen from horticultural practices, although polyploidy was detected in natural populations of *Gladiolus* outside of Africa, for example triploid and tetraploid *G. tenuis* in the Caucasus (Kutlunina et al., 2017) and tetraploid *G. palustris* in Europe (Malkócs et al., 2019). If polyploidy is indeed extant in Australian *Gladiolus*, it raises the question of the association between polyploidy and mating system for the focal species. In particular, it is not clear if polyploidisation triggers clonal reproduction, or if clonal reproduction promotes polyploidisation (Herben et al., 2017; van Drunen, 2018; van Drunen and Husband, 2018). However, the observation of low pollen viability and absence of seed set in *G. tristis* and *G. undulatus* would be supported by the inference of triploidy.

As a measure of invasiveness, I used niche occupancy (Chapter 4; Question 4, Section 1.6), rather than spatial occupancy (for example, Pyšek et al., 2011a), although they are likely to be correlated (Slatyer et al., 2013). In contrast to niche breadth, geographic expanse does not entail evolutionary adaptation; for

example, apomictic plants usually have larger range sizes compared to their sexual relatives (geographical parthenogenesis: Hörandl et al., 2008), despite the low potential for adaptive evolution. Additionally, I also used presence-only correlative species distribution modelling to predict the potential distribution of the focal species, optimising for accessible area (background region size), environmental predictors and model algorithm. Four innovations were made here: a heuristic predictor ranking algorithm based on Mahalanobis distance, a convergent algorithm to locate the optimum threshold probability value for generating binary presence/absence maps on the R platform (based on Pearson, 2010), the use of energy statistics (Rizzo and Székely, 2018) to evaluate model performance, and the use of two-tailed tests of niche equivalency and similarity, which was inspired by Herrando-Moraira et al. (2019). The use of the ECOSPAT package (Di Cola et al., 2017) to conduct niche equivalency and similarity tests has gained popularity, as it provides programmed functions for these tests. However, these tests could be performed in four ways (equivalency test: upper-tailed or lower-tailed; similarity test: upper-tailed or lower-tailed), and authors have occasionally neglected reporting their operating null hypothesis (which determined the directions of the tests), leaving doubt as to the veracity of their findings. Similar to the call to define or circumscribe "invasive" in the invasion biology literature (van Kleunen et al., 2018), there is a need to define and report the null hypotheses for randomisation tests (Manly, 2007).

I found that there was neither an optimum prediction selection nor model algorithm, in agreement with Qiao et al. (2015); thus, the extemporaneous use of the "algorithm of the day" may result in sub-optimal results. The species distribution models indicated that none of the focal species had achieved equilibrium, that is, their observed distribution fell short of their potential distribution, in agreement with Bradley et al. (2015). Several distal populations for *G. undulatus* and *W. meriana* var. *bulbillifera* were not predicted by the models. Niche results were mixed, and did not show a clear association between niche dynamics and mating system in the study species. While *G. tristis* demonstrated niche conservation, both *G. gueinzii* and *G. undulatus* experienced niche divergence in Australia. For *G. undulatus*, niche divergence likely stemmed from northern populations (in New South Wales) that were unpredicted by the species distribution models. By contrast, environmental difference between native and introduced range probably led to niche divergence for *G. gueinzii*. Despite the presence of a few populations in Queensland, niche qualification was inconclusive for *W. meriana* var. *bulbillifera*, and for *W. meriana* var. *meriana*. Niche characterisation and distribution modelling in *W. meriana* var. *bulbillifera* may in particular be hampered by the paucity of records (Hernandez et al., 2006) or sampling bias (Beck et al., 2014). However, the isolation of these northern

populations again suggested long-distance dispersal by human agency.

In general, there was no concordance between molecular data and niche dynamics. Adaptation to new environments depends on the level of genetic variation in a population, and molecular data suggested that *G. gueinzii* and *G. undulatus* were not genetically diverse in Victoria. However, the molecular assessment was performed on a limited number of individuals and populations in south-eastern Australia (although *G. gueinzii* was sampled over its adventive range). This would unlikely capture the genetic diversity of the species in Australia. Moreover, it is difficult to relate niche dynamics of invasive to native populations without the knowledge of genetic diversity extant in both ranges. The presence of unique amplicons in South African accessions and relatively low genomic diversity in south-eastern Australia indicated that a sampling of the genetic pool during introduction to Australia, that is, a genetic bottleneck was enforced. Furthermore, the contribution of adaptive and non-adaptive plasticity (Ghalambor et al., 2007) was unknown in this study. The proper elucidation of the role of mating system in driving invasiveness will require the elucidation of population genetic structure, genetic diversity, phenotypic plasticity and potentially epigenetic variability across native and adventive ranges (for example, Amsellem et al., 2000; Addison et al., 2008).

With the exception of *W. meriana* var. *meriana*, other members of the focal species belong to the "dead-end" class of organisms. In evolutionary terms, asexuality (*G. tristis* and *G. undulatus*), self-fertility (*G. gueinzii*, mixed mating), pollination specialisation (all study taxa) and polyploidy (putatively *G. tristis* and *G. undulatus*) are cul-de-sacs (Stebbins, 1957; Wagner, 1970; Futuyma and Moreno, 1988; Maynard-Smith, 1978). Yet, there are instances where species have persisted in spite of drawbacks associated with dead-ends, or when the premise of the hypothesis has been questioned (Takebayashi and Morrell, 2001; Zietara et al., 2006; Tripp and Manos, 2008; Soltis et al., 2014).

Although by no means conclusive, my study suggested that rather than a clear-cut association between invasiveness and mating system, asexual taxa are more labile than expected, and asexuality should not be interpreted as an evolutionary impasse. Moreover, invasive spread was in all probability aided by stochastic factors, such as anthropogenic long-distance dispersals, which made prediction problematic. By using the expectation-maximisation (EM) algorithm (Chapter 5), I found that the spatial distributions of the focal taxa (excluding *G. gueinzii*, which was not evaluated) bore signatures of human-mediated dispersal, although dispersal events were predominantly natural. (However, the levels of anthropochory were in all likelihoood underestimations.) And although the robustness of the EM algorithm may be questioned, the dispersal kernel for *W. meriana* var. *meriana* (which disperses by seed) was similar to *W. meriana* var.

bulbillifera (which disperses mainly by aerial cormels), thus providing support that vegetative fragmentation can play a major role in invasive spread (Question 5, Section 1.6).

The potential for evolution, if not outrightly assessed, is often intimated in weed assessment protocols. For example, the Australian Weed Risk Assessment (AWRA) system (Pheloung et al., 1999) asks if the assessed species is known to have "broad climate suitability (environment versatility)" (Question 2.03), in the affirmative if it is "found to grow naturally in a broad range of climate types" (Gordon et al., 2010). It is also encouraging that the AWRA recognises the use of modelling techniques for risk assessment ("Output from a climate matching program may be used for this question." (Question 2.03), Gordon et al., 2010), and the role of reproductive assurance in invasive spread (Question 6.04: Self-fertilisation, Pheloung et al., 1999) and reproductive limitation ("6.05 Requires specialist pollinators", He et al., 2018). While there is an obvious need to balance accessibility and knowledge, future weed risk assessment protocols should in addition query whether a taxon has been recorded outside of its modelling distribution, or if has demonstrated niche divergence, as a proxy for potential to evolve—at least until genomic tools become routine.

Appendix A

Introduction

Figure A.1: Map of mainland Australia. Reproduced from Google Maps. Map was downloaded on 12 Oct. 2020.

Table A.1: Locations of study sites for *Gladiolus gueinzii*. All sites are in New South Wales, Australia, except Mallacoota (GGAV01) in Victoria, Australia, and Jeffreys Bay (GGZJ01) and Plettenberg Bay (GGZP01) in South Africa.

Population ID	**Population name**	**Latitude**	**Longitude**
GGAN01	Shoalhaven Heads	-34.86083	150.74689
GGAN02	Pretty Beach	-35.57033	150.36426
GGAN03	Gap Beach	-30.90619	153.08129
GGAN04	Lighthouse Beach	-31.47783	152.93159
GGAN05	Booti Booti	-32.29435	152.52257
GGAN06	Birdie Beach	-33.21086	151.59906
GGAN08	Pambula Beach	-36.93705	149.90720
GGAN09	Beares Beach	-36.43531	150.07628
GGAN10	Sandon Point	-34.33194	150.92725
GGAN11	Bennetts Beach	-32.67850	152.17686
GGAN12	Mystery Bay	-36.30093	150.13390
GGAN13	Palm Beach	-33.59225	151.32501
GGAN14	Currarong Beach	-35.00794	150.80273
GGAN15	Lake Cathie	-31.54352	152.86396
GGAN16	Narooma Beach	-36.22048	150.13978
GGAN17	Stockton Beach	-32.91365	151.78983
GGAN18	Middle Beach	-36.64578	150.01165
GGAN19	Werri Beach	-34.72780	150.83844
GGAN20	Durras Beach	-35.64312	150.30183
GGAV01	Bastion Point	-37.56363	149.76483
GGZJ01	Jeffreys Bay	-34.05507	24.92440
GGZP01	Plettenberg Bay	-34.08657	23.37124

Table A.2: Locations of study sites for *Gladiolus tristis*. All sites are in Victoria, Australia, except Swellendam (GTZS01) and Plettenberg Bay (GGZ01) and Plettenberg Bay (GGZP01) in South Africa.

Population ID	**Population name**	**Latitude**	**Longitude**
GTAV01	Pomonal	-37.12641	142.67401
GTAV02	Ararat	-37.25996	142.91671
GTAV03	Glenlisle	-37.17714	142.22881
GTAV04	Gringegalgona	-37.38431	141.77554
GTAV05	Noradjuha	-36.85773	141.96363
GTAV06	Hamilton	-37.76986	141.97431
GTAV07	Portland	-38.34877	141.51628
GTAV08	Portland	-38.24592	141.88210
GTAV09	Cobden	-38.33871	143.09506
GTAV11	Beaufort	-37.44857	143.38024
GTAV12	Tarrawa East	-37.45078	143.52217
GTAV13	Ballarat	-37.50408	143.80299
GTAV14	Nerrina	-37.54104	143.88761
GTAV15	Chute	37.32733	143.38659
GTAV16	Wallan	-37.41336	145.00090
GTAV17	Miners Rest	-37.48277	143.77876
GTAV18	Lamplough	-37.17597	143.53136
GTAV19	Kyneton	-37.22631	144.43463
GTAV20	Caralulup	-37.21338	143.63354
GTAV21	Bungaree	-37.42103	142.08826
GTAV22	Bayindeen	-37.30902	143.15941
GTAV23	Armstrong	-37.20975	142.89149
GTAV24	Tylden	-37.34424	144.42481
GTAV25	Hamilton	-37.71645	141.99558
GTAV26	Tallarook	-37.10542	145.09520
GTAV27	Dobie	-37.31137	143.02303
GTAV28	Tallarook West	-37.09699	145.09241
GTZS01	Swellendam, Western Cape	34.01000	20.43000
GTZP01	Plettenberg Bay	-34.07000	23.32000

Table A.3: Locations of study sites for *Gladiolus undulatus*. All sites are in Victoria, Australia, except Gifberg (GTZG01) and Paarl (GGZP02) in South Africa.

Population ID	**Population name**	**Latitude**	**Longitude**
GUAV01	Yan Yean	-37.52997	145.12596
GUAV02	Wandong	-37.36216	145.03613
GUAV03	Wallan	-37.42694	145.00292
GUAV04	Pearcedale	-38.19547	145.31133
GUAV05	Chirnside Park	-37.73458	145.30439
GUAV06	Buninyong Cemetery	-37.63983	143.85472
GUAV07	Mount Eliza Regional Park	-38.20641	145.11151
GUAV08	Lyons	-38.01389	141.46834
GUAV09	Gatum Gatum	-37.43891	141.95606
GUAV10	Gringegalgona	-37.41441	141.80457
GUAV11	Brit Brit	-37.45006	141.73803
GUAV12	Kanagulk	-37.14952	141.86337
GUAV13	Laharum	-36.92126	142.35826
GUAV14	Stawell	-37.10221	142.79472
GUAV15	Edenhope	-37.09671	141.17383
GUAV16	Linton	-37.69584	143.57120
GUAV17	Meereek	-37.18092	141.12745
GUAV18	Box Hill Cemetery	-37.82326	145.13649
GUAV19	Tyabb	-38.27258	145.07566
GUAV20	Panton Hill	-37.65115	145.25551
GUAV21	Wallan	-37.41512	144.99867
GUAV22	Anglesea Inlet	-38.40794	144.18785
GUAV23	Wantirna	-37.84800	145.21145
GUAV24	Caralulup	-37.21338	143.63354
GUAV25	Ben Nevis	-37.19111	143.12444
GUAV26	Bungalook Conservation Reserve	-37.82699	145.30704
GUAV27	JW Manson Reserve	-37.83827	145.24238
GUAV28	Wangoom	-38.32787	142.57730
GUAV29	Wannon	-37.62734	141.77749
GUAV30	Hamilton East	-37.75569	142.04961
GUAV31	Mount Duneed	-38.29015	144.35760
GUZG01	Gifberg	-30.89000	18.66000
GUZP01	Paarl	-33.74000	18.94000

Table A.4: Locations of study sites for *Gladiolus undulatus*. All sites are in Victoria, Australia. 'Aerial cormels' refers to the observation of aerial cormels in the population, and 'Seeds' refers to the observation of seeds in the population.

Population ID	**Population name**	**Latitude**	**Longitude**	**Aerial cormels**	**Seeds**
WMAV01	Pindari Rd	-37.59852	145.28220	Y	N
WMAV02	Tuerong Rd	-38.27181	145.07254	Y	N
WMAV03	Casteron	-37.59455	141.36854	N	Y
WMAV05	Lake Rd	-36.71398	141.95432	Y	N
WMAV06	Eramosa Rd West	-38.21331	145.11186	Y	N
WMAV07	Cape Clear	-37.79127	143.59742	Y	Y
WMAV08	Koomba Park	-37.85564	145.20880	Y	N
WMAV09	Chetwynd	-37.26095	141.39330	Y	Y
WMAV10	Broadford Cemetery	-37.19898	145.04606	Y	N
WMAV11	Tallarook Cemetery	-37.10020	145.09690	Y	N
WMAV12	Wallan–Whittlesea Rd	-37.42726	145.05776	Y	N
WMAV13	Ararat	-37.26777	142.91888	Y	N
WMAV14	Melville Forest	-37.50837	141.87019	Y	Y
WMAV15	Bulart	-37.58759	141.99925	Y	N
WMAV16	Coimadai (Gisborne–Bacchus Marsh)	-37.53257	144.47563	Y	N
WMAV17	Dunnetts Rd, Yan Yean	-37.53364	145.12712	Y	N
WMAV18	Linton Cemetery	-37.67207	143.56828	Y	N
WMAV19	Midland Highway, Springmount	-37.41373	143.92757	Y	N
WMAV20	Ballarat–Colac Rd	-37.75365	143.78574	Y	N
WMAV21	Meredith Cemetery	-37.83758	144.08221	Y	N
WMAV22	Franklinford–Yandroit Cemetery	-37.23575	144.10543	Y	N
WMAV23	Club Terrace	-37.55323	148.93765	Y	N
WMAV24	Cann River	-37.56401	149.16227	Y	N
WMAV25	Gapsted	-36.50606	146.67362	Y	N
WMAV26	Tooborac	-37.06396	144.81646	Y	N
WMAV27	Tereddan Drive	-37.83003	145.31627	Y	N
WMAV28	Dandenongs Ck Trail	-37.83986	145.23932	Y	N
WMAV29	Heathmont Rail Trail	-37.83383	145.25545	Y	N
WMAV30	Warrayatkin	-37.27685	142.99725	Y	Y
WMAV31	Ripon	-37.63060	143.58693	Y	N
WMAV32	Amherst Cemetery	-37.16414	143.67788	Y	N
WMAV33	Diamond Creek	-37.65947	145.14561	Y	N
WMAV34	Nutfield	-37.62112	145.19265	Y	N

Appendix B

Reproductive ecology of invasive Iridaceae in Australia: less of the same?

B.1 Manual hand-pollination

Table B.1: Results from hand-pollination trials on *Gladiolus tristis* by treatment and population. The mean and S.E. of fruit seed, mean and S.E. of seed set and mean and S.E. of proportion of viable seeds are reported here. For treatments, **B** = between-population cross-fertilisation, **C** = control (open pollination), **S** = self-pollen fertilisation and **W** = within-population cross-fertilisation. See the main text (Chapter 2) for details.

Treatment	Population	N	Mean no. fruit	S.E. no. fruit	Mean no. seed	S.E. no. seed	Mean seed viability	S.E. seed viability
B	GTAV01	27	0.00	0.00	0.00	0.00	NA	NA
B	GTAV03	16	0.00	0.00	0.00	0.00	NA	NA
B	GTAV09	16	0.00	0.00	0.00	0.00	NA	NA
B	GTAV15	10	0.00	0.00	0.00	0.00	NA	NA
C	GTAV01	23	0.00	0.00	0.00	0.00	NA	NA
C	GTAV03	18	0.00	0.00	0.00	0.00	NA	NA
C	GTAV09	16	0.00	0.00	0.00	0.00	NA	NA
C	GTAV15	10	0.00	0.00	0.00	0.00	NA	NA
S	GTAV01	10	0.00	0.00	0.00	0.00	NA	NA
S	GTAV03	12	0.00	0.00	0.00	0.00	NA	NA
S	GTAV09	16	0.00	0.00	0.00	0.00	NA	NA
S	GTAV15	10	0.00	0.00	0.00	0.00	NA	NA
W	GTAV01	14	0.00	0.00	0.00	0.00	NA	NA
W	GTAV03	15	0.00	0.00	0.00	0.00	NA	NA
W	GTAV09	15	0.00	0.00	0.00	0.00	NA	NA
W	GTAV15	9	0.00	0.00	0.00	0.00	NA	NA

Table B.2: Results from hand-pollination trials on *Gladiolus undulatus* by treatment and population. The mean and S.E. of fruit seed, mean and S.E. of seed set and mean and S.E. of proportion of viable seeds are reported here. For treatments, **B** = between-population cross-fertilisation, **C** = control (open pollination), **S** = self-pollen fertilisation and **W** = within-population cross-fertilisation. See the main text (Chapter 2) for details.

Treatment	Population	N	Mean no. fruit	S.E. no. fruit	Mean no. seed	S.E. no. seed	Mean seed viability	S.E. seed viability
B	GUAV09	8	0.00	0.00	0.00	0.00	NA	NA
B	GUAV16	6	0.00	0.00	0.00	0.00	NA	NA
B	GUAV21	10	0.00	0.00	0.00	0.00	NA	NA
C	GUAV09	8	0.00	0.00	0.00	0.00	NA	NA
C	GUAV16	6	0.00	0.00	0.00	0.00	NA	NA
C	GUAV21	10	0.00	0.00	0.00	0.00	NA	NA
S	GUAV09	8	0.00	0.00	0.00	0.00	NA	NA
S	GUAV16	5	0.00	0.00	0.00	0.00	NA	NA
S	GUAV21	10	0.00	0.00	0.00	0.00	NA	NA
W	GUAV09	8	0.00	0.00	0.00	0.00	NA	NA
W	GUAV16	6	0.00	0.00	0.00	0.00	NA	NA
W	GUAV21	10	0.00	0.00	0.00	0.00	NA	NA

Table B.3: Results from hand-pollination trials on *Watsonia meriana* var. *bulbillifera* by treatment and population. The mean and S.E. of fruit seed, mean and S.E. of seed set and mean and S.E. of proportion of viable seeds are reported here. For treatments, **B** = between-population cross-fertilisation, **C** = control (open pollination), **S** = self-pollen fertilisation, **V** = inter-varietal cross-ferilisation and **W** = within-population cross-fertilisation. See the main text (Chapter 2) for details.

Treatment	Population	N	Mean no. fruit	S.E. no. fruit	Mean no. seed	S.E. no. seed	Mean seed viability	S.E. seed viability
B	WMAV07	18	0.00	0.00	0.00	0.00	NA	NA
B	WMAV09	20	0.05	0.05	1.25	1.25	0.08	NA
B	WMAV13	8	0.00	0.00	0.00	0.00	NA	NA
C	WMAV07	15	0.00	0.00	0.00	0.00	NA	NA
C	WMAV09	20	0.00	0.00	0.00	0.00	NA	NA
C	WMAV13	8	0.00	0.00	0.00	0.00	NA	NA
S	WMAV07	17	0.00	0.00	0.00	0.00	NA	NA
S	WMAV09	20	0.00	0.00	0.00	0.00	NA	NA
S	WMAV13	8	0.00	0.00	0.00	0.00	NA	NA
V	WMAV07	18	0.00	0.00	0.00	0.00	NA	NA
V	WMAV09	10	0.10	0.10	2.80	2.80	0.14	NA
W	WMAV07	15	0.00	0.00	0.00	0.00	NA	NA
W	WMAV09	20	0.00	0.00	0.00	0.00	NA	NA
W	WMAV13	8	0.00	0.00	0.00	0.00	NA	NA

Table B.4: Results from hand-pollination trials on *Watsonia meriana* var. *meriana* by treatment and population. The mean and S.E. of fruit seed, mean and S.E. of seed set and mean and S.E. of proportion of viable seeds are reported here. For treatments, **B** = between-population cross-fertilisation, **C** = control (open pollination), **S** = self-pollen fertilisation, **V** = inter-varietal cross-ferilisation and **W** = within-population cross-fertilisation. See the main text (Chapter 2) for details.

Treatment	Population	N	Mean no. fruit	S.E. no. fruit	Mean no. seed	S.E. no. seed	Mean seed viability	S.E. seed viability
B	WMAV03	24	0.63	0.10	23.21	3.86	0.42	0.04
B	WMAV07	19	0.79	0.10	23.68	3.53	0.55	0.04
C	WMAV03	22	1.00	0.00	38.18	0.91	0.43	0.03
C	WMAV07	14	1.00	0.00	33.86	2.66	0.42	0.08
S	WMAV03	27	0.00	0.00	0.00	0.00	NA	NA
S	WMAV07	21	0.05	0.05	1.10	1.10	0.48	NA
V	WMAV07	18	0.11	0.08	3.61	2.53	0.37	0.03
W	WMAV03	14	1.00	0.00	38.21	1.49	0.55	0.03
W	WMAV07	17	1.00	0.00	27.29	1.43	0.43	0.05

B.2 Pollen viability

Table B.5: Results from pollen viability tests for *Gladiolus tristis* by medium and population. Methods **AS** = Alexander's stain and **BK** = Brewbaker–Kwack medium. See the text (Chapter 2) for details about scoring.

Method	Population	N	Mean no. viable pollen	S.E. viable pollen	Mean no. inviable pollen	S.E. inviable pollen
AS	GTAV09	3	2.00	2.00	13.33	1.20
AS	GTAV16	12	0.58	0.43	22.75	3.06
BK	GTAV02	25	2.96	1.29	417.36	217.04
BK	GTAV05	67	0.07	0.03	101.97	8.69
BK	GTAV15	74	0.51	0.34	102.35	41.33
BK	GTAV16	36	0.06	0.04	18.19	2.42
BK	GTAV20	5	27.00	15.44	1638.00	526.93

Table B.6: Results from pollen viability tests for *Gladiolus undulatus* by medium and population. Methods **AS** = Alexander's stain and **BK** = Brewbaker–Kwack medium. See the text (Chapter 2) for details about scoring.

Method	Population	N	Mean no. viable pollen	S.E. viable pollen	Mean no. inviable pollen	S.E. inviable pollen
AS	GUAV09	4	13.50	1.32	12.25	1.97
AS	GUAV16	2	13.00	2.00	50.00	3.00
AS	GUAV28	3	27.67	3.71	30.33	4.37
AS	GUAV29	6	12.17	3.64	41.17	11.05
BK	GUAV23	98	0.05	0.03	88.20	3.88

Table B.7: Results from pollen viability tests for *Watsonia meriana* var. *bulbillifera* by medium and population. Methods **AS** = Alexander's stain and **BK** = Brewbaker–Kwack medium. See the text (Chapter 2) for details about scoring.

Method	Population	N	Mean no. viable pollen	S.E. viable pollen	Mean no. inviable pollen	S.E. inviable pollen
AS	WMAV01	186	26.66	1.46	30.81	1.40
AS	WMAV02	5	10.40	3.96	10.00	2.30
AS	WMAV12	130	21.91	1.61	19.46	0.88
AS	WMAV33	206	10.54	0.47	49.00	1.99
BK	WMAV12	10	3.10	0.90	212.80	44.18

Table B.8: Results from pollen viability tests for *Watsonia meriana* var. *meriana* by medium and population. Methods **AS** = Alexander's stain. See the text (Chapter 2) for details about scoring.

Method	Population	N	Mean no. viable pollen	S.E. viable pollen	Mean no. inviable pollen	S.E. inviable pollen
AS	WMAV07	311	32.59	2.05	13.33	0.76

B.3 Reproductive output

Table B.9: Results from propagule output measurements for *Gladiolus tristis* by propagule type and population. Mean and S.E. of propagules are reported here per plant. Refer to Table A.1 for the population details.

Propagule type	Population	Mean no. of propagules	S.E. propagules
Cormel	GTAV02	6.80	NA
Cormel	GTAV05	43.35	NA
Aerial cormel	GTAV02	0.00	NA
Aerial cormel	GTAV05	0.00	NA
Seed	GTAV02	0.00	NA
Seed	GTAV05	0.00	NA

Table B.10: Results from propagule output measurements for *Gladiolus undulatus* by propagule type and population. Mean and S.E. of propagules are reported here per plant. Refer to Table A.1 for the population details.

Propagule type	Population	Mean no. of propagules	S.E. propagules
Cormel	GUAV10	338.83	27.40
Cormel	GUAV14	435.20	NA
Aerial cormel	GUAV10	0.00	0.00
Aerial cormel	GUAV14	0.00	NA
Seed	GUAV10	0.00	0.00
Seed	GUAV14	0.00	NA

Table B.11: Results from propagule output measurements for *Watsonia meriana* var. *bulbillifera* by propagule type and population. Mean and S.E. of propagules are reported here per plant. Refer to Table A.1 for the population details.

Propagule type	Population	Mean no. of propagules	S.E. propagules
Cormel	WMAV09	0.00	0.00
Cormel	WMAV12	0.00	0.00
Cormel	WMAV14	0.00	0.00
Aerial cormel	WMAV09	183.00	14.41
Aerial cormel	WMAV12	76.50	6.32
Aerial cormel	WMAV14	78.60	15.29
Seed	WMAV09	36.80	9.92
Seed	WMAV12	0.00	0.00
Seed	WMAV14	55.90	18.38

Table B.12: Results from propagule output measurements for *Watsonia meriana* var. *meriana* by propagule type and population. Mean and S.E. of propagules are reported here per plant. Refer to Table A.1 for the population details.

Propagule type	Population	Mean no. of propagules	S.E. propagules
Cormel	WMAV03	0.00	0.00
Cormel	WMAV07	0.00	0.00
Aerial cormel	WMAV03	0.00	0.00
Aerial cormel	WMAV07	0.00	0.00
Seed	WMAV03	475.17	94.24
Seed	WMAV07	362.00	62.79

Appendix C

Genetic patterns in introduced *Gladiolus* populations in southeastern Australia

C.1 Accessions for genetic analyses

Table C.1: Amplicon calling for *Gladiolus gueinzii*. '-9' signifies no amplification. Amplicon binning was performed by cumulative plot frequency plots of size distribution, and manually checked.

Sample.Name	Marker	Allele.1	Allele.2	Sample.Name	Marker	Allele.1	Allele.2
GGAN01001	GL07	-9		GGAN04025	GL21	184	
GGAN01002	GL07	209		GGAN04028	GL21	184	
GGAN01004	GL07	207		GGAN04035	GL21	184	
GGAN01011	GL07	-9		GGAN05003	GL21	184	
GGAN01028	GL07	207		GGAN05009	GL21	-9	
GGAN02002	GL07	207		GGAN05014	GL21	-9	
GGAN02015	GL07	-9		GGAN05029	GL21	-9	
GGAN02016	GL07	209		GGAN05030	GL21	184	
GGAN02021	GL07	209		GGAN06001	GL21	184	
GGAN02034	GL07	-9		GGAN06003	GL21	-9	
GGAN03001	GL07	209		GGAN06013	GL21	-9	
GGAN03017	GL07	-9		GGAN06025	GL21	184	
GGAN03031	GL07	207		GGAN06031	GL21	184	
GGAN03036	GL07	207		GGAN08005	GL21	-9	
GGAN03040	GL07	207		GGAN08011	GL21	-9	
GGAN04011	GL07	209		GGAN08018	GL21	-9	
GGAN04019	GL07	207		GGAN08023	GL21	-9	
GGAN04025	GL07	207		GGAN08029	GL21	184	
GGAN04028	GL07	207		GGAN01001	GL41	-9	
GGAN04035	GL07	207		GGAN01002	GL41	278	281
GGAN05003	GL07	207		GGAN01004	GL41	-9	
GGAN05009	GL07	207		GGAN01011	GL41	-9	
GGAN05014	GL07	-9		GGAN01028	GL41	-9	
GGAN05029	GL07	209		GGAN02002	GL41	-9	
GGAN05030	GL07	207		GGAN02015	GL41	-9	
GGAN06001	GL07	209		GGAN02016	GL41	-9	
GGAN06003	GL07	207		GGAN02021	GL41	-9	
GGAN06013	GL07	-9		GGAN02034	GL41	-9	
GGAN06025	GL07	-9		GGAN03001	GL41	-9	
GGAN06031	GL07	207		GGAN03017	GL41	-9	
GGAN08005	GL07	-9		GGAN03031	GL41	-9	
GGAN08011	GL07	207		GGAN03036	GL41	-9	
GGAN08018	GL07	207		GGAN03040	GL41	278	
GGAN08023	GL07	-9		GGAN04011	GL41	-9	
GGAN08029	GL07	207		GGAN04019	GL41	-9	
GGAN01001	GL21	-9		GGAN04025	GL41	-9	
GGAN01002	GL21	184		GGAN04028	GL41	-9	
GGAN01004	GL21	184		GGAN04035	GL41	278	
GGAN01011	GL21	184		GGAN05003	GL41	-9	
GGAN01028	GL21	184		GGAN05009	GL41	-9	
GGAN02002	GL21	184		GGAN05014	GL41	-9	
GGAN02015	GL21	-9		GGAN05029	GL41	278	281
GGAN02016	GL21	184		GGAN05030	GL41	278	
GGAN02021	GL21	-9		GGAN06001	GL41	278	281
GGAN02034	GL21	184		GGAN06003	GL41	-9	
GGAN03001	GL21	184		GGAN06013	GL41	-9	
GGAN03017	GL21	-9		GGAN06025	GL41	-9	
GGAN03031	GL21	184		GGAN06031	GL41	278	
GGAN03036	GL21	184		GGAN08005	GL41	-9	
GGAN03040	GL21	184		GGAN08011	GL41	-9	
GGAN04011	GL21	184		GGAN08018	GL41	-9	
GGAN04019	GL21	184		GGAN08023	GL41	278	281

Table C.2: Amplicon calling for *G. gueinzii* (continued).

Sample.Name	Marker	Allele.1	Allele.2	Sample.Name	Marker	Allele.1	Allele.2
GGAN08029	GL41	278		GGAN04019	GL63	238	
GGAN01001	GL45	167		GGAN04025	GL63	238	
GGAN01002	GL45	167		GGAN04028	GL63	238	
GGAN01004	GL45	167		GGAN04035	GL63	238	
GGAN01011	GL45	167		GGAN05003	GL63	238	
GGAN01028	GL45	167		GGAN05009	GL63	238	
GGAN02002	GL45	167		GGAN05014	GL63	238	
GGAN02015	GL45	167		GGAN05029	GL63	-9	
GGAN02016	GL45	167		GGAN05030	GL63	238	
GGAN02021	GL45	167		GGAN06001	GL63	238	
GGAN02034	GL45	167		GGAN06003	GL63	238	
GGAN03001	GL45	167		GGAN06013	GL63	238	
GGAN03017	GL45	167		GGAN06025	GL63	238	
GGAN03031	GL45	167		GGAN06031	GL63	238	
GGAN03036	GL45	167		GGAN08005	GL63	238	
GGAN03040	GL45	167		GGAN08011	GL63	238	
GGAN04011	GL45	167		GGAN08018	GL63	238	
GGAN04019	GL45	167		GGAN08023	GL63	238	
GGAN04025	GL45	167		GGAN08029	GL63	238	
GGAN04028	GL45	167		GGAN01001	GL84	-9	
GGAN04035	GL45	167		GGAN01002	GL84	-9	
GGAN05003	GL45	167		GGAN01004	GL84	-9	
GGAN05009	GL45	167		GGAN01011	GL84	-9	
GGAN05014	GL45	167		GGAN01028	GL84	95	
GGAN05029	GL45	167		GGAN02002	GL84	-9	
GGAN05030	GL45	167		GGAN02015	GL84	-9	
GGAN06001	GL45	167		GGAN02016	GL84	-9	
GGAN06003	GL45	167		GGAN02021	GL84	-9	
GGAN06013	GL45	167		GGAN02034	GL84	95	
GGAN06025	GL45	167		GGAN03001	GL84	-9	
GGAN06031	GL45	167		GGAN03017	GL84	-9	
GGAN08005	GL45	167		GGAN03031	GL84	-9	
GGAN08011	GL45	167		GGAN03036	GL84	-9	
GGAN08018	GL45	167		GGAN03040	GL84	95	
GGAN08023	GL45	167		GGAN04011	GL84	-9	
GGAN08029	GL45	167		GGAN04019	GL84	-9	
GGAN01001	GL63	238		GGAN04025	GL84	-9	
GGAN01002	GL63	238		GGAN04028	GL84	-9	
GGAN01004	GL63	238		GGAN04035	GL84	95	
GGAN01011	GL63	238		GGAN05003	GL84	-9	
GGAN01028	GL63	238		GGAN05009	GL84	-9	
GGAN02002	GL63	238		GGAN05014	GL84	-9	
GGAN02015	GL63	-9		GGAN05029	GL84	-9	
GGAN02016	GL63	236		GGAN05030	GL84	95	
GGAN02021	GL63	236		GGAN06001	GL84	-9	
GGAN02034	GL63	238		GGAN06003	GL84	-9	
GGAN03001	GL63	238		GGAN06013	GL84	-9	
GGAN03017	GL63	238		GGAN06025	GL84	-9	
GGAN03031	GL63	238		GGAN06031	GL84	95	
GGAN03036	GL63	238		GGAN08005	GL84	-9	
GGAN03040	GL63	238		GGAN08011	GL84	-9	
GGAN04011	GL63	238		GGAN08018	GL84	-9	
				GGAN08023	GL84	-9	
				GGAN08029	GL84	95	

Table C.3: Amplicon calling for *Gladiolus tristis*. '-9' signifies no amplification. Amplicon binning was performed by cumulative plot frequency plots of size distribution, and manually checked.

Sample.Name	Marker	Allele.1	Allele.2	Sample.Name	Marker	Allele.1	Allele.2
GTAV01002	GL07	-9		GTAV03035	GL21	183	195
GTAV01007	GL07	181		GTAV05001	GL21	183	195
GTAV01024	GL07	-9		GTAV05010	GL21	183	195
GTAV01031	GL07	-9		GTAV05015	GL21	-9	
GTAV01036	GL07	181		GTAV05018	GL21	183	195
GTAV02001	GL07	-9		GTAV05023	GL21	183	195
GTAV02003	GL07	200	204	GTAV07001	GL21	183	
GTAV02013	GL07	200	204	GTAV07002	GL21	183	
GTAV02077	GL07	-9		GTAV07006	GL21	-9	
GTAV02092	GL07	200	204	GTAV07023	GL21	183	
GTAV03002	GL07	-9		GTAV07025	GL21	183	195
GTAV03020	GL07	181		GTAV09004	GL21	-9	
GTAV03031	GL07	181		GTAV09023	GL21	-9	
GTAV03033	GL07	181		GTAV09036	GL21	183	
GTAV03035	GL07	181		GTAV09042	GL21	183	
GTAV05001	GL07	-9		GTAV09049	GL21	183	195
GTAV05010	GL07	-9		GTAV16001	GL21	183	
GTAV05015	GL07	-9		GTAV16013	GL21	-9	
GTAV05018	GL07	200	204	GTAV16015	GL21	183	
GTAV05023	GL07	200	204	GTAV16028	GL21	183	
GTAV07001	GL07	181		GTAV16030	GL21	183	195
GTAV07002	GL07	181		GTAV01002	GL35	163	
GTAV07006	GL07	-9		GTAV01007	GL35	163	
GTAV07023	GL07	181		GTAV01024	GL35	163	
GTAV07025	GL07	181		GTAV01031	GL35	163	
GTAV09004	GL07	-9		GTAV01036	GL35	-9	
GTAV09023	GL07	181		GTAV02001	GL35	166	
GTAV09036	GL07	181		GTAV02003	GL35	166	
GTAV09042	GL07	181		GTAV02013	GL35	166	
GTAV09049	GL07	181		GTAV02077	GL35	163	166
GTAV16001	GL07	181		GTAV02092	GL35	166	
GTAV16013	GL07	181		GTAV03002	GL35	163	173
GTAV16015	GL07	181		GTAV03020	GL35	161	173
GTAV16028	GL07	181		GTAV03031	GL35	163	173
GTAV16030	GL07	181		GTAV03033	GL35	163	173
GTAV01002	GL21	183		GTAV03035	GL35	163	173
GTAV01007	GL21	183	195	GTAV05001	GL35	166	
GTAV01024	GL21	-9		GTAV05010	GL35	166	
GTAV01031	GL21	-9		GTAV05015	GL35	166	
GTAV01036	GL21	183	195	GTAV05018	GL35	166	
GTAV02001	GL21	183	195	GTAV05023	GL35	166	
GTAV02003	GL21	183	195	GTAV07001	GL35	163	173
GTAV02013	GL21	183	195	GTAV07002	GL35	163	
GTAV02077	GL21	-9		GTAV07006	GL35	163	173
GTAV02092	GL21	183	195	GTAV07023	GL35	163	173
GTAV03002	GL21	183		GTAV07025	GL35	163	173
GTAV03020	GL21	-9		GTAV09004	GL35	163	173
GTAV03031	GL21	183		GTAV09023	GL35	-9	
GTAV03033	GL21	183		GTAV09036	GL35	163	
				GTAV09042	GL35	163	

Table C.4: Amplicon calling for *G. tristis* (continued).

Sample.Name	Marker	Allele.1	Allele.2	Sample.Name	Marker	Allele.1	Allele.2
GTAV09049	GL35	-9		GTAV02092	GL41	278	297
GTAV16001	GL35	163	173	GTAV03002	GL41	281	310
GTAV16013	GL35	163	173	GTAV03020	GL41	281	310
GTAV16015	GL35	163	173	GTAV03031	GL41	281	310
GTAV16028	GL35	166	173	GTAV03033	GL41	281	310
GTAV16030	GL35	176	180	GTAV03035	GL41	281	310
GTAV01002	GL40	176	180	GTAV05001	GL41	278	297
GTAV01007	GL40	176	180	GTAV05010	GL41	278	297
GTAV01024	GL40	176	180	GTAV05015	GL41	278	297
GTAV01031	GL40	176	180	GTAV05018	GL41	278	297
GTAV01036	GL40	176	180	GTAV05023	GL41	278	297
GTAV02001	GL40	172	190	GTAV07001	GL41	281	310
GTAV02003	GL40	172	190	GTAV07002	GL41	281	310
GTAV02013	GL40	172	190	GTAV07006	GL41	281	310
GTAV02077	GL40	172	190	GTAV07023	GL41	281	310
GTAV02092	GL40	172	190	GTAV07025	GL41	-9	
GTAV03002	GL40	176	180	GTAV09004	GL41	281	310
GTAV03020	GL40	176	180	GTAV09023	GL41	-9	
GTAV03031	GL40	176	180	GTAV09036	GL41	281	310
GTAV03033	GL40	176	180	GTAV09042	GL41	281	310
GTAV03035	GL40	176	180	GTAV09049	GL41	-9	
GTAV05001	GL40	172	190	GTAV16001	GL41	281	310
GTAV05010	GL40	172	190	GTAV16013	GL41	281	310
GTAV05015	GL40	172	190	GTAV16015	GL41	281	310
GTAV05018	GL40	172	190	GTAV16028	GL41	281	310
GTAV05023	GL40	172	190	GTAV16030	GL41	281	310
GTAV07001	GL40	176	180	GTAV01002	GL45	-9	
GTAV07002	GL40	176	180	GTAV01007	GL45	209	
GTAV07006	GL40	176	180	GTAV01024	GL45	-9	
GTAV07023	GL40	176	180	GTAV01031	GL45	-9	
GTAV07025	GL40	176	180	GTAV01036	GL45	209	
GTAV09004	GL40	176	180	GTAV02001	GL45	215	
GTAV09023	GL40	176	180	GTAV02003	GL45	215	
GTAV09036	GL40	176	180	GTAV02013	GL45	215	
GTAV09042	GL40	176	180	GTAV02077	GL45	215	
GTAV09049	GL40	176	180	GTAV02092	GL45	215	
GTAV16001	GL40	176	180	GTAV03002	GL45	209	
GTAV16013	GL40	176	180	GTAV03020	GL45	209	
GTAV16015	GL40	176	180	GTAV03031	GL45	209	
GTAV16028	GL40	176	180	GTAV03033	GL45	209	
GTAV16030	GL40	176	180	GTAV03035	GL45	209	
GTAV01002	GL41	281	310	GTAV05001	GL45	213	230
GTAV01007	GL41	281	310	GTAV05010	GL45	211	213
GTAV01024	GL41	281	310	GTAV05015	GL45	-9	
GTAV01031	GL41	281	310	GTAV05018	GL45	213	231
GTAV01036	GL41	-9		GTAV05023	GL45	213	230
GTAV02001	GL41	278	297	GTAV07001	GL45	209	
GTAV02003	GL41	278	297	GTAV07002	GL45	209	
GTAV02013	GL41	278	297	GTAV07006	GL45	-9	
GTAV02077	GL41	278	297	GTAV07023	GL45	209	

Table C.5: Amplicon calling for *G. tristis* (continued).

Sample.Name	Marker	Allele.1	Allele.2	Sample.Name	Marker	Allele.1	Allele.2	Allele.3
GTAV07025	GL45	209		GTAV01002	GL84	117	122	130
GTAV09004	GL45	-9		GTAV01007	GL84	117	122	130
GTAV09023	GL45	209		GTAV01024	GL84	-9		
GTAV09036	GL45	209		GTAV01031	GL84	117	122	130
GTAV09042	GL45	209		GTAV01036	GL84	117	122	130
GTAV09049	GL45	209		GTAV02001	GL84	126	130	133
GTAV16001	GL45	209		GTAV02003	GL84	126	130	133
GTAV16013	GL45	209		GTAV02013	GL84	126	130	133
GTAV16015	GL45	209		GTAV02077	GL84	126	130	133
GTAV16028	GL45	209		GTAV02092	GL84	126	130	133
GTAV16030	GL45	209		GTAV03002	GL84	117	122	130
GTAV01002	GL63	229		GTAV03020	GL84	117	122	130
GTAV01007	GL63	229	250	GTAV03031	GL84	117	122	130
GTAV01024	GL63	229	250	GTAV03033	GL84	117	122	130
GTAV01031	GL63	229	250	GTAV03035	GL84	117	122	130
GTAV01036	GL63	-9		GTAV05001	GL84	126	130	133
GTAV02001	GL63	239	245	GTAV05010	GL84	126	130	133
GTAV02003	GL63	239	245	GTAV05015	GL84	-9		
GTAV02013	GL63	239	245	GTAV05018	GL84	126	130	133
GTAV02077	GL63	239	245	GTAV05023	GL84	126	130	133
GTAV02092	GL63	-9		GTAV07001	GL84	117	122	130
GTAV03002	GL63	229	250	GTAV07002	GL84	117	122	130
GTAV03020	GL63	239	250	GTAV07006	GL84	117	122	130
GTAV03031	GL63	229	250	GTAV07023	GL84	117	122	130
GTAV03033	GL63	229	250	GTAV07025	GL84	117	122	130
GTAV03035	GL63	-9		GTAV09004	GL84	117	122	130
GTAV05001	GL63	245		GTAV09023	GL84	117	122	130
GTAV05010	GL63	245		GTAV09036	GL84	117	122	130
GTAV05015	GL63	245		GTAV09042	GL84	117	122	130
GTAV05018	GL63	245		GTAV09049	GL84	117	122	130
GTAV05023	GL63	245		GTAV16001	GL84	117	122	130
GTAV07001	GL63	239	250	GTAV16013	GL84	117	122	130
GTAV07002	GL63	239	250	GTAV16015	GL84	117	122	130
GTAV07006	GL63	239	250	GTAV16028	GL84	117	122	130
GTAV07023	GL63	239	250	GTAV16030	GL84	117	122	130
GTAV07025	GL63	-9						
GTAV09004	GL63	239						
GTAV09023	GL63	-9						
GTAV09036	GL63	239	250					
GTAV09042	GL63	239	252					
GTAV09049	GL63	-9						
GTAV16001	GL63	239	250					
GTAV16013	GL63	239	250					
GTAV16015	GL63	239	250					
GTAV16028	GL63	239	250					
GTAV16030	GL63	239						

Table C.6: Amplicon calling for *Gladiolus undulatus*. '-9' signifies no amplification. Amplicon binning was performed by cumulative plot frequency plots of size distribution, and manually checked.

Sample.Name	Marker	Allele.1	Allele.2	Sample.Name	Marker	Allele.1	Allele.2	Allele.3
GUAV03003	GL07	172		GUAV17001	GL35	-9		
GUAV03008	GL07	172		GUAV17010	GL35	-9		
GUAV03022	GL07	172		GUAV17018	GL35	225	261	
GUAV03035	GL07	172		GUAV17023	GL35	225	261	
GUAV03045	GL07	174		GUAV17030	GL35	225	261	
GUAV09001	GL07	172		GUAV19008	GL35	-9		
GUAV09008	GL07	174		GUAV19015	GL35	-9		
GUAV09018	GL07	172		GUAV19024	GL35	-9		
GUAV09025	GL07	172		GUAV19031	GL35	225	255	180
GUAV09031	GL07	172		GUAV19033	GL35	225	255	180
GUAV14051	GL07	172		GUAV23002	GL35	-9		180
GUAV14068	GL07	172		GUAV23020	GL35	-9		180
GUAV14072	GL07	172		GUAV23021	GL35	225	261	180
GUAV14073	GL07	174		GUAV23046	GL35	-9		180
GUAV14083	GL07	172		GUAV23050	GL35	225	261	180
GUAV16003	GL07	172		GUAV03003	GL40	161	177	180
GUAV16010	GL07	172		GUAV03008	GL40	161	177	180
GUAV16015	GL07	172		GUAV03022	GL40	161	177	180
GUAV16025	GL07	172		GUAV03035	GL40	161	177	180
GUAV16030	GL07	172		GUAV03045	GL40	161	177	180
GUAV17001	GL07	172		GUAV09001	GL40	161	177	180
GUAV17010	GL07	172		GUAV09008	GL40	161	177	180
GUAV17018	GL07	172		GUAV09018	GL40	161	177	180
GUAV17023	GL07	172		GUAV09025	GL40	161	177	180
GUAV17030	GL07	174		GUAV09031	GL40	161	177	180
GUAV19008	GL07	172		GUAV14051	GL40	161	177	180
GUAV19015	GL07	172		GUAV14068	GL40	161	177	180
GUAV19024	GL07	172		GUAV14072	GL40	161	177	180
GUAV19031	GL07	174		GUAV14073	GL40	161	177	
GUAV19033	GL07	172		GUAV14083	GL40	161	177	
GUAV23002	GL07	174		GUAV16003	GL40	161	177	180
GUAV23020	GL07	172		GUAV16010	GL40	161	177	180
GUAV23021	GL07	174		GUAV16015	GL40	161	177	180
GUAV23046	GL07	172		GUAV16025	GL40	161	177	
GUAV23050	GL07	172		GUAV16030	GL40	161	177	
GUAV03003	GL35	225	261	GUAV17001	GL40	-9		
GUAV03008	GL35	225	261	GUAV17010	GL40	-9		
GUAV03022	GL35	225	261	GUAV17018	GL40	161	177	180
GUAV03035	GL35	-9		GUAV17023	GL40	161	177	
GUAV03045	GL35	225	261	GUAV17030	GL40	161	177	
GUAV09001	GL35	225		GUAV19008	GL40	-9		
GUAV09008	GL35	225	255	GUAV19015	GL40	-9		
GUAV09018	GL35	225		GUAV19024	GL40	-9		180
GUAV09025	GL35	225		GUAV19031	GL40	161	180	
GUAV09031	GL35	225	255	GUAV19033	GL40	161	177	
GUAV14051	GL35	225	255	GUAV23002	GL40	-9		
GUAV14068	GL35	225	255	GUAV23020	GL40	-9		
GUAV14072	GL35	225	255	GUAV23021	GL40	161	180	
GUAV14073	GL35	225	255	GUAV23046	GL40	-9		
GUAV14083	GL35	225	255	GUAV23050	GL40	161	177	
GUAV16003	GL35	225	261					
GUAV16010	GL35	225	261					
GUAV16015	GL35	225	261					
GUAV16025	GL35	225	261					
GUAV16030	GL35	-9						

Table C.7: Amplicon calling for *G. undulatus* (continued).

Sample.Name	Marker	Allele.1	Allele.2	Sample.Name	Marker	Allele.1	Allele.2
GUAV03003	GL41	279	285	GUAV17001	GL45	-9	
GUAV03008	GL41	279	285	GUAV17010	GL45	-9	
GUAV03022	GL41	279	285	GUAV17018	GL45	-9	
GUAV03035	GL41	279	285	GUAV17023	GL45	184	193
GUAV03045	GL41	279	285	GUAV17030	GL45	-9	
GUAV09001	GL41	279	285	GUAV19008	GL45	-9	
GUAV09008	GL41	279	285	GUAV19015	GL45	-9	
GUAV09018	GL41	279	285	GUAV19024	GL45	-9	
GUAV09025	GL41	279	285	GUAV19031	GL45	-9	
GUAV09031	GL41	279	285	GUAV19033	GL45	184	193
GUAV14051	GL41	279	285	GUAV23002	GL45	-9	
GUAV14068	GL41	279	285	GUAV23020	GL45	-9	
GUAV14072	GL41	279	285	GUAV23021	GL45	-9	
GUAV14073	GL41	279	285	GUAV23046	GL45	-9	
GUAV14083	GL41	279	285	GUAV23050	GL45	184	193
GUAV16003	GL41	279	285	GUAV03003	GL63	229	241
GUAV16010	GL41	279	285	GUAV03008	GL63	229	241
GUAV16015	GL41	279	285	GUAV03022	GL63	229	241
GUAV16025	GL41	279	285	GUAV03035	GL63	229	241
GUAV16030	GL41	279	285	GUAV03045	GL63	229	241
GUAV17001	GL41	279	285	GUAV09001	GL63	229	243
GUAV17010	GL41	-9		GUAV09008	GL63	229	243
GUAV17018	GL41	279	285	GUAV09018	GL63	229	243
GUAV17023	GL41	279	285	GUAV09025	GL63	229	243
GUAV17030	GL41	279	285	GUAV09031	GL63	229	243
GUAV19008	GL41	279	285	GUAV14051	GL63	229	243
GUAV19015	GL41	279		GUAV14068	GL63	229	
GUAV19024	GL41	279	285	GUAV14072	GL63	229	
GUAV19031	GL41	279	285	GUAV14073	GL63	229	233
GUAV19033	GL41	279	285	GUAV14083	GL63	229	
GUAV23002	GL41	279	285	GUAV16003	GL63	229	241
GUAV23020	GL41	279	285	GUAV16010	GL63	229	241
GUAV23021	GL41	279	285	GUAV16015	GL63	229	241
GUAV23046	GL41	279	285	GUAV16025	GL63	229	241
GUAV23050	GL41	279	285	GUAV16030	GL63	229	241
GUAV03003	GL45	-9		GUAV17001	GL63	229	241
GUAV03008	GL45	-9		GUAV17010	GL63	-9	
GUAV03022	GL45	-9		GUAV17018	GL63	229	241
GUAV03035	GL45	184	193	GUAV17023	GL63	229	241
GUAV03045	GL45	-9		GUAV17030	GL63	229	241
GUAV09001	GL45	-9		GUAV19008	GL63	229	243
GUAV09008	GL45	-9		GUAV19015	GL63	-9	
GUAV09018	GL45	-9		GUAV19024	GL63	229	243
GUAV09025	GL45	-9		GUAV19031	GL63	229	243
GUAV09031	GL45	184	193	GUAV19033	GL63	229	243
GUAV14051	GL45	-9		GUAV23002	GL63	229	241
GUAV14068	GL45	-9		GUAV23020	GL63	229	241
GUAV14072	GL45	-9		GUAV23021	GL63	229	241
GUAV14073	GL45	-9		GUAV23046	GL63	229	241
GUAV14083	GL45	184	193	GUAV23050	GL63	229	241
GUAV16003	GL45	-9		GUAV03003	GL84	122	
GUAV16010	GL45	-9		GUAV03008	GL84	122	
GUAV16015	GL45	-9		GUAV03022	GL84	122	
GUAV16025	GL45	-9		GUAV03035	GL84	122	
GUAV16030	GL45	184	193	GUAV03045	GL84	122	

Table C.8: Amplicon calling for *G. undulatus* (continued).

Sample.Name	Marker	Allele.1	Allele.2
GUAV09001	GL84	122	
GUAV09008	GL84	122	
GUAV09018	GL84	122	
GUAV09025	GL84	122	
GUAV09031	GL84	122	
GUAV14051	GL84	122	
GUAV14068	GL84	122	
GUAV14072	GL84	122	
GUAV14073	GL84	122	
GUAV14083	GL84	122	
GUAV16003	GL84	122	
GUAV16010	GL84	122	
GUAV16015	GL84	122	
GUAV16025	GL84	122	
GUAV16030	GL84	122	
GUAV17001	GL84	122	
GUAV17010	GL84	122	
GUAV17018	GL84	122	
GUAV17023	GL84	122	
GUAV17030	GL84	122	
GUAV19008	GL84	122	
GUAV19015	GL84	122	
GUAV19024	GL84	122	
GUAV19031	GL84	122	
GUAV19033	GL84	122	
GUAV23002	GL84	122	
GUAV23020	GL84	122	
GUAV23021	GL84	122	
GUAV23046	GL84	122	
GUAV23050	GL84	122	

Appendix D

Multiply, and go forth: Potential distribution and niche dynamics of invasive Iridaceae in Australia

D.1 Model performance

Table D.1: Model performance for *Gladiolus gueinzii*. Refer to the text (Chapter 4) for details on the algorithm, predictor selection and test statistics used. **var_sel** = predictor selection method, **P** = number of predictors used, **AIC** = Akaike information criterion, **AICc** = Akaike information criterion (corrected), **BIC** = Bayesian information criterion, **Threshold** = threshold applied to generate binary occurrence surface from a probabilistic occurrence surface, **Occupancy** = % of study area occupied, **avi** = absolute validation index, **cvi** = contrast validation index, **auc** = area under the receiver operator curve, **sensitivity** = model sensitivity, **specificity** = model specificity, **TSS** = True Skill Statistic and **boyce** = Boyce's index. The model marked with an asterisk (*) is the optimum model.

Model	Species	Algorithm	var_sel	P	AIC	AICc	BIC	Threshold	Occupancy	avi	cvi	auc	sensitivity	specificity	TSS	boyce
GG20180603L	GG	GLM	None	46	827.830	287.330	904.354	0.852	3.130	0.470	0.030	0.958	0.940	0.980	0.920	NA
GG20180604A	GG	GLM	stepAIC	8	540.671	547.871	551.610	0.555	2.123	0.340	0.160	0.816	0.660	0.960	0.620	0.021
GG20180604B	GG	GLM	vifstep	24	783.855	869.570	823.781	0.852	3.131	0.470	0.030	0.947	0.940	0.960	0.900	NA
GG20180604C	GG	GLM	vifcor	21	777.830	832.183	812.765	0.852	3.130	0.470	0.030	0.970	0.940	1.000	0.940	NA
GG20180604H	GG	RF	RFE	41	584.490	153.990	647.071	0.617	0.405	0.390	0.110	0.900	0.740	1.000	0.740	0.967
GG20180604J*	GG	RF	varrank	11	602.795	612.949	620.809	0.758	0.521	0.440	0.060	0.940	0.880	1.000	0.880	0.930
GG20180604K	GG	RF	boruta	42	579.368	178.034	643.475	0.617	0.365	0.390	0.110	0.900	0.740	1.000	0.740	0.995
GG20180604L	GG	RF	None	46	536.165	265.915	602.128	0.602	0.323	0.360	0.140	0.880	0.720	1.000	0.720	0.990
GG20180605A	GG	Maxent	None	47	636.156	260.156	710.581	0.664	0.466	0.410	0.090	0.920	0.780	1.000	0.780	0.763
GG20180605B	GG	Maxent	ENMeval	7	411.111	417.333	419.918	0.523	0.519	0.300	0.200	0.850	0.600	1.000	0.600	0.600
GG20180605C	GG	Maxent	varrank	11	481.352	496.019	496.765	0.570	0.529	0.340	0.160	0.870	0.680	1.000	0.680	0.838
GG20180605D	GG	Maxent	HDMM	14	493.238	519.488	513.314	0.586	0.454	0.350	0.150	0.860	0.700	1.000	0.700	0.686

Table D.2: Model performance for *Gladiolus tristis*. Refer to the text (Chapter 4) for details on the algorithm, predictor selection and test statistics used. **var_sel** = predictor selection method, **P** = number of predictors used, **AIC** = Akaike information criterion, **AICc** = Akaike information criterion (corrected), **BIC** = Bayesian information criterion, **Threshold** = threshold applied to generate binary occurrence surface from a probabilistic occurrence surface, **Occupancy** = % of study area occupied, **avi** = absolute validation index, **cvi** = contrast validation index, **auc** = area under the receiver operator curve, **sensitivity** = model sensitivity, **specificity** = model specificity, **TSS** = True Skill Statistic and **boyce** = Boyce's index. The model marked with an asterisk (*) is the optimum model.

Model	Species	Algorithm	var_sel	P	AIC	AICc	BIC	Threshold	Occupancy	avi	cvi	auc	sensitivity	specificity	TSS	boyce
GT20180509A	GT	GLM	vifcor	16	1981.697	1989.149	2021.694	0.867	12.665	0.464	0.036	0.900	0.928	0.866	0.794	0.969
GT20180509B	GT	GLM	vifstep	15	1713.803	1721.075	1749.903	0.773	7.201	0.423	0.077	0.870	0.845	0.897	0.742	0.912
GT20180510A	GT	GLM	None	46	1728.936	1859.966	1838.509	0.742	6.945	0.412	0.088	0.880	0.825	0.938	0.763	1.000
GT20180510B	GT	GLM	BIOMOD	10	1831.690	1834.663	1856.117	0.836	4.537	0.467	0.033	0.910	0.935	0.978	0.913	0.748
GT20180510C	GT	GLM	stepAIC	6	1747.033	1748.124	1761.618	0.664	7.632	0.433	0.067	0.900	0.866	0.928	0.794	0.788
GT20180511B	GT	RF	RFE	29	1766.097	1797.734	1836.934	0.805	5.782	0.438	0.062	0.890	0.876	0.907	0.784	0.988
GT20180513A	GT	RF	boruta	45	1748.089	1863.089	1856.391	0.789	6.156	0.423	0.077	0.890	0.845	0.928	0.773	0.994
GT20180513B	GT	RF	BIOMOD	6	1886.553	1887.603	1901.349	0.836	11.944	0.448	0.052	0.900	0.897	0.897	0.794	0.898
GT20180513C	GT	RF	varrank	6	1758.552	1759.687	1772.918	0.758	12.038	0.418	0.082	0.880	0.835	0.928	0.763	0.234
GT20180513D*	GT	Maxent	HDMM	15	1831.611	1838.371	1868.599	0.836	7.854	0.448	0.052	0.940	0.897	0.979	0.876	0.958
GT20180515A	GT	Maxent	ENMeval	6	1819.838	1820.875	1834.702	0.867	7.233	0.454	0.046	0.930	0.907	0.959	0.866	0.663
GT20180515B	GT	Maxent	None	46	1662.309	1797.434	1771.303	0.742	5.185	0.407	0.093	0.880	0.814	0.938	0.753	0.787

Table D.3: Model performance for *Gladiolus undulatus*. Refer to the text (Chapter 4) for details on the algorithm, predictor selection and test statistics used. **var_sel** = predictor selection method, **P** = number of predictors used, **AIC** = Akaike information criterion, **AICc** = Akaike information criterion (corrected), **BIC** = Bayesian information criterion, **Threshold** = threshold applied to generate binary occurrence surface from a probabilistic occurrence surface, **Occupancy** = % of study area occupied, **avi** = absolute validation index, **cvi** = contrast validation index, **auc** = area under the receiver operator curve, **sensitivity** = model sensitivity, **specificity** = model specificity, **TSS** = True Skill Statistic and **boyce** = Boyce's index. The model marked with an asterisk (*) is the optimum model.

Model	Species	Algorithm	var_sel	P	AIC	AICc	BIC	Threshold	Occupancy	avi	cvi	auc	sensitivity	specificity	TSS	boyce
GU20180606C	GU	GLM	None	45	1969.794	2107.794	2074.677	0.398	58.806	0.213	0.287	0.583	0.442	0.448	-0.110	-0.568
GU20180606D	GU	GLM	vifstep	8	3315.802	3316.936	3339.103	0.711	46.470	0.376	0.124	0.591	0.729	0.541	0.271	-0.302
GU20180606E	GU	GLM	vifcor	10	2305.673	2308.293	2331.212	0.477	41.974	0.265	0.235	0.516	0.530	0.547	0.077	-0.115
GU20180606F	GU	GLM	stepAIC	8	2301.673	2303.348	2322.104	0.477	41.974	0.265	0.235	0.533	0.530	0.575	0.105	-0.115
GU20180606K	GU	RF	None	45	3137.174	3178.574	3271.436	0.727	8.522	0.398	0.102	0.882	0.796	0.945	0.740	0.941
GU20180606L*	GU	RF	boruta	43	3037.965	3076.577	3165.066	0.742	8.178	0.392	0.108	0.884	0.785	0.967	0.751	0.943
GU20180606M	GU	RF	RFE	13	3395.466	3397.994	3435.280	0.820	10.695	0.436	0.064	0.896	0.862	0.934	0.796	0.641
GU20180606N	GU	RF	varrank	13	3055.513	3058.292	3094.211	0.742	8.615	0.401	0.099	0.853	0.785	0.917	0.702	0.778
GU20180606Q	GU	Maxent	None	46	2248.286	2319.172	2371.664	0.570	5.419	0.304	0.196	0.806	0.602	0.945	0.547	0.916
GU20180606R	GU	Maxent	ENMeval	14	2046.726	2051.610	2083.338	0.523	5.477	0.282	0.218	0.781	0.564	0.945	0.508	0.884
GU20180606S	GU	Maxent	HDMM	13	2487.365	2490.704	2523.923	0.617	5.543	0.340	0.160	0.835	0.669	0.939	0.608	0.858
GU20180606T	GU	Maxent	varrank	18	2246.744	2254.179	2295.516	0.570	5.288	0.309	0.191	0.814	0.613	0.950	0.564	0.914

Table D.4: Model performance for *Watsonia meriana* var. *bulbillifera*. Refer to the text (Chapter 4) for details on the algorithm, predictor selection and test statistics used. **var_sel** = predictor selection method, **P** = number of predictors used, **AIC** = Akaike information criterion, **AICc** = Akaike information criterion (corrected), **BIC** = Bayesian information criterion, **Threshold** = threshold applied to generate binary occurrence surface from a probabilistic occurrence surface, **Occupancy** = % of study area occupied, **avi** = absolute validation index, **cvi** = contrast validation index, **auc** = area under the receiver operator curve, **sensitivity** = model sensitivity, **specificity** = model specificity, **TSS** = True Skill Statistic and **boyce** = Boyce's index. The model marked with an asterisk (*) is the optimum model.

Model	Species	Algorithm	var_sel	P	AIC	AICc	BIC	Threshold	Occupancy	avi	cvi	auc	sensitivity	specificity	TSS	boyce
WB20180522A	WB	GLM	None	45	4241.437	4274.824	4382.548	0.664	50.264	0.489	0.011	0.733	0.977	0.477	0.454	0.686
WB20180522C	WB	GLM	BIOMOD	5	4161.645	4162.011	4177.324	0.664	50.278	0.489	0.011	0.733	0.977	0.489	0.466	0.686
WB20180522D	WB	GLM	stepAIC	11	2639.160	2641.342	2670.954	0.727	4.683	0.382	0.118	0.874	0.764	0.983	0.747	0.877
WB20180522E	WB	GLM	vifcor	6	3635.072	3635.600	3653.744	0.914	13.732	0.477	0.023	0.911	0.954	0.868	0.822	0.702
WB20180522G	WB	RF	None	45	3192.438	3230.771	3329.101	0.836	5.930	0.443	0.057	0.914	0.885	0.943	0.828	0.994
WB20180522H	WB	RF	boruta	13	2911.797	2914.685	2950.038	0.742	7.488	0.402	0.098	0.871	0.805	0.937	0.741	0.917
WB20180523A*	WB	RF	BIOMOD	21	3341.738	3348.434	3406.317	0.867	7.530	0.460	0.040	0.937	0.920	0.954	0.874	0.990
WB20180523C	WB	Maxent	ENMeval	2	2602.660	2602.759	2608.317	0.664	8.179	0.359	0.141	0.819	0.718	0.920	0.638	0.844
WB20180523D	WB	Maxent	None	46	2486.958	2550.547	2613.225	0.586	8.331	0.330	0.170	0.796	0.661	0.931	0.592	0.793
WB20180524A	WB	Maxent	HDMM	18	2439.963	2447.088	2489.372	0.586	8.662	0.330	0.170	0.793	0.661	0.925	0.586	0.660
WB20180530E	WB	Maxent	varrank	12	2752.144	2754.955	2785.988	0.633	14.960	0.356	0.144	0.790	0.713	0.868	0.580	0.244

Table D.5: Model performance for *Watsonia meriana* var. *meriana*. Refer to the text (Chapter 4) for details on the algorithm, predictor selection and test statistics used. **var_sel** = predictor selection method, **P** = number of predictors used, **AIC** = Akaike information criterion, **AICc** = Akaike information criterion (corrected), **BIC** = Bayesian information criterion, **Threshold** = threshold applied to generate binary occurrence surface from a probabilistic occurrence surface, **Occupancy** = % of study area occupied, **avi** = absolute validation index, **cvi** = contrast validation index, **auc** = area under the receiver operator curve, **sensitivity** = model sensitivity, **specificity** = model specificity, **TSS** = True Skill Statistic and **boyce** = Boyce's index. The model marked with an asterisk (*) is the optimum model.

Model	Species	Algorithm	var_sel	P	AIC	AICc	BIC	Threshold	Occupancy	avi	cvi	auc	sensitivity	specificity	TSS	boyce
WM20180525A*	WM	GLM	None	45	3023.292	3065.111	3157.245	0.883	6.184	0.487	0.013	0.947	0.974	0.921	0.895	0.885
WM20180525B	WM	GLM	BIOMOD	7	2881.196	2882.032	2901.886	0.883	6.066	0.487	0.013	0.987	0.974	1.000	0.974	0.228
WM20180525C	WM	GLM	vifstep	14	2697.564	2701.417	2737.048	0.836	11.841	0.474	0.026	0.934	0.947	0.921	0.868	-0.410
WM20180525D	WM	GLM	vifcor	12	2503.829	2506.691	2537.477	0.805	6.491	0.461	0.039	0.908	0.921	0.895	0.816	0.863
WM20180525E	WM	GLM	stepAIC	9	2318.953	2321.046	2342.032	0.836	40.086	0.474	0.026	0.763	0.947	0.579	0.526	-0.517
WM20180526A	WM	RF	None	45	2028.872	2113.362	2143.797	0.773	6.765	0.447	0.053	0.921	0.895	0.947	0.842	0.417
WM20180526C	WM	RF	RFE	7	2067.885	2069.158	2085.835	0.805	11.070	0.447	0.053	0.895	0.895	0.895	0.789	-0.114
WM20180526E	WM	RF	BIOMOD	16	2289.035	2295.013	2331.949	0.836	8.638	0.474	0.026	0.908	0.947	0.868	0.816	-0.225
WM20180527E	WM	Maxent	ENMeval	5	1897.417	1898.077	1910.291	0.742	4.203	0.434	0.066	0.921	0.868	0.974	0.842	0.900
WM20180527F	WM	Maxent	None	46	1875.724	1973.996	1991.223	0.742	4.517	0.447	0.053	0.882	0.895	0.868	0.763	0.756
WM20180527H	WM	Maxent	HDMM	12	2074.524	2077.915	2106.371	0.773	4.355	0.447	0.053	0.934	0.895	0.974	0.868	0.943
WM20180530D	WM	Maxent	varrank	5	782.793	784.861	790.569	0.820	7.250	0.474	0.026	0.961	0.947	0.974	0.921	0.645

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