Population Dynamics at a Diploid-Polyploid Contact Zone in *Mercurialis annua* L.

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DECLARATION

Unless otherwise stated, the author conducted the work presented in this thesis. No part of this thesis has been submitted for another degree at this or any other university.

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ABSTRACT

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Hybrid zones offer ‘natural laboratories’ for studying the origin, maintenance and demise of species, serving as examples of evolution in action. Understanding the processes that mediate the simultaneous invasions and extinctions of a hybrid zone is therefore essential to understanding the range limits of species and the maintenance of their genetic identity.

Populations of diploid (2n = 16) and hexaploid (2n = 48) *Mercuralis annua* L. *s.l.* (Euphorbiaceae) have migrated from glacial refugia towards a zone of secondary contact that now exists in north-east Spain. Rapid movement of this zone in favour of the diploids over the past 50 years has been attributed to an interaction of endogenous and exogenous selection, and has lead to the formation of a narrow ‘tension hybrid zone’ that limits the spatial mixing of these lineages.

This work systematically disentangles the factors underlying the stability of the diploid-hexaploid contact zone in its current position in the north-east Spain to understand the fate of this hybrid zone if it follows its current trajectory into what I have characterised as a low-density trough. I examine the importance of demographic factors such as founding size, reproductive assurance in polyploids, and differences in the rate of seed bank formation for the persistence of both lineages in this area. This provides an estimate of ambient rates of extinction in this zone for each lineage, independent of their interactions with one another, and highlights their susceptibility to density-dependent processes at each life history stage.

I then examine asymmetries in hybridisation dynamics between lineages in experimental field populations of controlled density as well as in naturally occurring mixed populations in this zone, assessing the effect of biased gene flow of diploids on the outcome of these apparently rare contact events. I also refine an earlier form of the mass-action pollen pool model to account for the spatial context of individuals and their individual-based contribution to the pollen pool. Using this enables a prediction of hybridisation rates in the field for given mixed assemblages.

Results are interpreted in the context of tension zone dynamics and the long-term fate of sexual system variation in this species complex. The identification of long-run negative growth rates in both lineages, and the fact that a small fraction of sites remained by the end of a four-year time course of study, are both indicative of a region that represents a low-density trough into which the diploid-hexaploid contact zone will settle if movement continues along its density-dependent trajectory.

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CHAPTER 1. GENERAL INTRODUCTION

1.1 GENERAL BACKGROUND OF HYBRID ZONES

The mechanisms that shape species distributions are central to our understanding of the processes of character divergence and speciation. The origins of species ranges and their current dynamics can be disentangled to provide key insights into the long-term persistence or eventual demise of species. Within each species, the various ecotypes, subspecies and geographic races represent the genetic outcomes of these dynamics, shaped through evolution along divergent pathways. When these different forms come into contact and mate to produce offspring, a hybrid zone is formed. Hybridization is an important process in many plant taxa. It may lead to the formation of new species or to the collapse of existing taxa or affect levels of genetic variation, local adaptation, and the effectiveness of selection (Arnold 1992, Allendorf et al. 2001, Aldrich et al. 2003, Coyne and Orr 2004, Riesberg and Willis, 2007, Hipp and Weber 2008, Peterson et al. 2009, Moran et al. 2012).

Hybrid zones occur when closely related taxa come into contact and cross-fertilise to produce viable offspring (Barton and Hewitt 1985, 1989, Harrison 1993). These contact zones provide ‘natural laboratories’ for studying the origin (Hewitt 1988, Howard 1993, Moore and Price 1993, Cain et al. 1999, Marshall et al. 2002, Campbell 2004), maintenance (Rieseberg and Buerkle 2002, Lexer et al. 2004) and demise of species (Rhymer and Simberloff 1996, Haig 1998, Allendorf et al. 2001, Wolf et al. 2001, Allendorf and Lundquist 2003). They often represent a threat to biodiversity, either when hybrids displace other taxa or when the genes of one species swamp another across the contact zone (Wolf et al. 2001).

They also represent the unique opportunity to observe the process of 'evolution in action', representing stages of divergence along the evolutionary trajectories of the lineages in contact (Kirkpatrick and Ravigne 2002). Here we can assess the influence of the degree of gene flow between populations in preventing divergence, the role of extinction and colonisation dynamics in mediating the chance encounters of lineages, and the underlying role of the environment in governing the fitness consequences of contact events. Given the vast number of processes underpinning the behaviour of populations in secondary contact, hybrid zones may assume a variety of forms.

1.2 OUTCOMES OF CONTACT

The outcomes of contact for divergent lineages generally take on one of the four following patterns (cf. Moran et al. 2012): 1- complete reproductive isolation; 2- the formation of a hybrid zone wherein F1 hybrids are localised to a narrow geographic area; 3- introgression over a more widespread area, but where the parental lineages are still identifiable; or 4- the formation of a hybrid swarm, where most individuals exhibit intermediate morphologies and/or mixed genetic characteristics (e.g. Mexican populations of *Tigriopus californicus* (Hwang et al. 2012)). Hybrid zones are most often exhibited by species with differences in habitat requirements, where intermediate habitats exist between the parental ranges (Muller 1952). In contrast, introgression may be facilitated when species co-occur in an area with microsite-level habitat heterogeneity (Valbuena-Carabana et al. 2007).

1.2.1 Fitness of lineages and prior ecological adaptation

The location and stability of hybrid zones is often considered to be the result of either endogenous selection against hybrid genotypes, exogenous selection of genotypes along an ecological gradient, or a combination of both processes acting together in concert (Barton and Hewitt 1985, Arnold 1997). Under ecological selection, lineages are maintained in a hybrid zone because of differences in fitness between lineages across ecological gradients (or 'ecotones'), with selection against the parental genotypes in non-native habitats (e.g. Fritsche and Kaltz 2000). Ecotones are important areas for the maintenance and generation of biodiversity (Smith et al. 1997, Spector 2002) and for the study of processes that shape the distribution and abundance of organisms (reviewed in

Kark and van Rensburg 2006). Hybrid zones often coincide with ecotones (e.g. Barton and Hewitt 1985, Harrison 1993, Arnold 1997, Gee 2004, Leaché and Cole 2007).

The likelihood of contact of divergent lineages and their successful colonisation will depend on their habitat preferences, the amount of such suitable habitat available across a landscape, and the degree of overlap in preference between lineages. A suite of factors, in addition to ecophysiological adaptation, contributes to variation in plant population structure, including the distribution of habitat suitability and availability, variation in extinction and colonisation rates and the life-history (Freckleton et al. 2005, 2006). The precise nature of regional contact zone dynamics depends on the interaction of these factors. For instance, if rates of colonisation are high and suitable habitat is widely available, populations should be able to rapidly colonise new habitat and should also occupy most available habitat. In contrast, if rates of population turnover due to extinctions are high, not all suitable habitats will be occupied at a given point in time. Differential adaptation of competing lineages to certain ecological conditions is a commonly occurring phenomenon amongst hybrid zones (Barton 1979), however such differences in habitat preference are rarely the only conditioning factor for the location of a contact zone. Where the positioning of the contact zone is largely mediated by environmental factors, the area is referred to as an ecotonal hybrid zone. In the case where genetic incompatibilities between lineages are of greatest influence, the area is referred to as a tension zone.

The persistence of lineages across years will be subject to demographic and environmental stochasticity, and density dependence within and among age or stage classes in populations undergoing fluctuations around a stable equilibrium (Lande et al. 2006). Demographic stochasticity is caused by variation in fitness among individuals within years, usually attributed to independent chance events of individual survival and reproduction, producing random changes in population growth rate, with a variance inversely proportional to population size (Shaffer 1987). The susceptibility of a lineage to such chance fluctuations will ultimately be determined by its life-history and aspects of its sexual system. Additionally, as individual events tend to average out in large populations, demographic stochasticity is thought to be most important in small populations. Environmental stochasticity however and the conditions at a contact zone are thought to affect populations of all sizes, and affect the vital rates of individuals within populations in a similar way (May 1974, Lande et al. 2003). It has been shown that a population of modest size subject to environmental stochasticity or random catastrophes can persist for a long time, if population growth rate is substantially positive (Lande 1992). Contrasting the behaviour of interacting lineages under shared environmental conditions in terms of population size fluctuations is thus a necessary precursor to understanding their long-term persistence at such a zone.

1.2.2 Fitness of hybrids

Most hybrid zones occurring at temperate latitudes arise from the gradual expansion of divergent relictual populations from glacial refugia of the last glacial maximum, having accumulated sub-group specific genetic differences over the course of the past 10,000 years. The nature of these genetic differences largely determine the fitness consequences of hybridisation events occurring between contacting lineages. The fitness of hybrid offspring consequently dictates the nature of contact that may persist between lineages. For instance, when the fitness of hybrids is significantly reduced relative to its progenitors, the contact zone is expected to behave so as to minimize gene flow between lineages and the loss of gametes to hybrid production. When one lineage is more fit than the other, the lineage will quickly move as a front through the range of the competing lineage (cf. warfarin resistant rats, Greaves et al. 1977). It follows that the relative performance of either lineage will therefore be frequency dependent, where the rare form reduces in frequency each generation because it produces a higher proportion of unfit hybrids. A link between hybridisation and increased invasiveness has also frequently been observed (Ellstrand and Schierenbeck 2000, Vila et al. 2000, Schierenbeck and Ellstrand 2009, Hovick et al. 2012). Hybridization might increase invasiveness if hybrid populations harbour increased genetic variation (e.g., Lewontin and Birch 1966, Campbell et al. 2009), if they contain novel and beneficial allelic combinations (e.g., Whitney et al.

2006, Latta et al. 2007), or if they experience fixed heterosis (e.g., Moody and Les 2002, Facon et al. 2005).

Where selection against hybrids causes the area of contact to stabilise in regions of minimal dispersal between lineages, the hybrid area is referred to as a tension zone (Barton and Hewitt 1985). In a tension zone, the environment is thought to play a role only insomuch as it can affect the dispersal rates and population densities of the competing lineages (Barton 1979). For this reason, ecotonal and tension hybrid zones are often referred to as dispersal-independent and dispersal-dependent hybrid zones, respectively (Barton and Hewitt 1985). The presence of ecological divergence and tension zone dynamics are not mutually exclusive; both factors can affect the long-term stability of hybrid zones (e.g. Kruuk et al. 1999).

1.2.3 Frequency of contact

The population density of interacting lineages and their frequency across a landscape is another key determinant of their likelihood of contact. High population densities will provide an opportunity for greater connectivity between occupied habitat patches, and will support the recolonisation of unoccupied landscapes, and thus the long-term persistence of lineages at the regional scale. The prevailing disturbance conditions will affect the population densities that are attainable across a landscape through its effect on the rates of population extinction. Lineages may differ in their susceptibility to disturbance if they vary in their ability to persist under conditions of low density. The rate at which a species is able to produce offspring is often highly density dependent, influenced by the availability of conspecific mating partners, and their sexual system. An ability to self-fertilize at low density as is possible in hermaphrodites will enable populations to avoid possible Allee effects, and should be associated with an ability to persist under higher ambient rates of disturbance. In situations where population growth rates are found to be density-dependent, the relationship between initial population size and population persistence are no longer generalisable (Ewens et al. 1987). As with many species that are threatened with extinction, the long-run growth rates of populations are near zero or negative. Such populations with a negative long-run growth rate will be unlikely to benefit from large initial starting sizes, as has previously been noted by Ludwig (1976) and Brockwell (1985). This stresses the importance of assessing population densities across sufficient temporal scale in order to have reliable predictive power as to their long-term persistence (Lande 1992). For populations subject to demographic or environmental stochasticity, with positive long-run growth rates and a sufficiently large initial size, Richter-Dyn and Goel (1972) and Leigh (1981) showed that the average time to extinction is nearly independent of the initial population size. It is therefore important to characterise the density-dependence of the life-history stages responsible for population growth prior to understanding population persistence. Rates of extinction across a landscape, or considered oppositely the ability to colonise and persist in habitat patches will have a key influence on the composition of any mixed populations that are formed at a contact zone.

1.3 KEY EXAMPLES OF MOVING HYBRID ZONES

Movement of the tension zone is expected to occur when there are biases in dispersal, caused, for example by differences in fertility between lineages (Barton and Hewitt (1985). As tension zones are not maintained by an adaptive response to local environmental conditions of the competing lineages, they are often found moving from place to place (Barton and Hewitt 1985, Hewitt 1985, Buggs 2007). Although theoretical models predict that the structure of a hybrid zone can change under a variety of scenarios, only a few empirical studies of hybrid zones have unequivocally demonstrated zone movement. These studies are rare because few data sets exist that include repeated, temporally spaced, samples of the same hybrid zone (Carling and Zuckerberg 2011). Despite these logistical difficulties in documenting movement, several cases are known (e.g. Kohlmann and Shaw 1991, Paige et al. 1991, Martin and Cruzan 1999, Rohwer et al. 2001, Blum 2002, Dasmahapatra et al. 2002, Buggs and Pannell 2006). Rather than tension zones following gradients in habitat suitability, Hewitt (1975) and Endler (1977) suggested and Barton (1979) showed that the movement of tension zones might be influenced either by gradients of population density or the asymmetry of hybridisation between parental taxa.

Tension contact zones will move according to patterns of population density across a landscape, given the likelihood of contact, and the fitness of the resultant hybrid offspring. If there are more organisms on one side of the zone that on the other, they will push the zone forward by weight of numbers in a manner similar to asymmetric dispersal (Barton and Hewitt 1981). It follows that a tension zone will move so as to minimise the frequency of hybridisation events in the case of reduced hybrid fitness so as to preserve the fitness of parental lineages. Such movement will naturally allow lineages to drift to regions of low population density where inter-lineage interactions will be minimised, and their maximum fitness realised. Such regions, often taking the form of valleys or ridges of unsuitable habitat, have been referred to as 'low-density troughs' and are a documented obstruction to hybrid zone movement (Barton 1979). Within the hybrid zone literature, it had been realised early on that subspecific parapatric contacts and their hybrid zones could be remarkably stable in position over time (Hewitt 1975, 1988). They should be trapped in density troughs and unlikely to move far in a landscape of varying suitability, unless there are major ecological or climatic changes. The positions of hybrid zones in grasshoppers in the Alps, Kangaroo Island and the Pyrenees are congruent with this idea (Hewitt 2001).

Previous studies of hybrid zones in other species have focused on both environmental and genetic influences on movement. An ecotonal hybrid zone has been characterised in Madagascar where dry spiny forest and humid forest integrade (O’Connor et al. 1985). Here, mouse lemurs *Microcebus murinus* are associated with dry deciduous and humid littoral forests, whereas *M. griseorufus* are limited to southern dry spiny forests (Yoder et al. 2000). Recent morphometric and microsatellite analyses of Gligor et al. (2009) have identified intermediate morphotypes of the species, implicating environmental selection and an expansion of dry spiny forests due to aridification as a cause of habitat overlap, with the potential for movement of the hybrid zone.

Climatic variables are putative factors positioning the distributions of three salamander species in the genus *Plethodon* across the mountains of the Southern Appalachians (Hairston 1950, Highton 1970, Hairston et al. 1992). Mountain ridgelines provide corridors of suitable habitat between the distributions of these species, allowing for zones of hybridisation (Reagan 1992). However, a more recent analysis of differential mtDNA introgression hints at the importance of dispersal-selection balance in governing the positioning of these three species, in which case the zone may have settled into this region of overlapping habitats without actually being maintained by climate-associated selection (Chatfield et al. 2010). Differences in dispersal capabilities between lineages are thus seen as an important factor mediating the position of hybrid zones across topographical and ecological gradients and should be further explored in the *M. annua* northeastern contact zone.

1.4 *MERCURIALIS ANNUA* SPECIES COMPLEX AND ITS HYBRID ZONE

To determine the fundamental causes of the formation, distribution, and maintenance of a hybrid zone, one must consider whether interacting lineages differ in their affinity for particular environments, their ability to effect hybridisation in the opposite lineage, and the underlying density-dependence of their competitive interactions. The *Mercurialis annua* (Euphorbiaceae) species complex provides a unique platform for studying the issues surrounding density, the sexual system, and establishment/extinction dynamics on the maintenance of hybrid zones.

1.4.1 Sexual system and ploidy variation

In both the Iberian Peninsula and Morocco, the ruderal plant species complex of *Mercurialis annua* is a good example of a contact zone between divergent lineages. This species shows remarkable variation in its sexual systems, with populations ranging from dioecious through androdioecious to hermaphroditic (Pannell et al. 2008). The sexual system variation present in *M. annua* is also linked to variation in ploidy. Androdioecy is restricted to polyploid populations in this complex (Durand 1963, Pannell et al. 2004), and phylogentic reconstruction of the genus by Krahenbul et al (2002), based on ITS sequence analysis revealed that dioecy and perenniality are ancestral traits. There is also evidence that monoecy has evolved on at least two independent occasions, one of which has given rise to the annual polyploid complex in *M. annua*. Dioecy is confined to diploidy, whereas monoecy is found in ploidy levels ranging from tertraploidy to at least 12-ploid (Pannell et al. 2004). Tetraploids, octaploids and higher ploidy levels are exclusively monoecious, but the hexaploids range in sexual system from monoecy, to androdioecy and sub-dioecy (Durand and Durand 1992; summarised in Pannell et al. 2004). Studies of ITS paralogues have identified the annual dioecious *Mercurialis huetii* as a sister species to *M. annua*, leading to a model wherein hybridisation between tetraploid *M. annua* and *M. huetii* gave rise to triploids which then polyploidised to form the allopolyploid hexaploid lineage (Obbard et al. 2006).

Populations of diploid (2n = 16) and hexaploid (6n = 48) *M. annua* are thought to have migrated from separate Pleistocene glacial refugia towards the current zone of secondary contact occurring across the 20 km region between L'Hospitalet de l'Infant and Ametlla de Mar in Catalonia, Spain (Buggs and Pannell 2006) (and, additionally, in Galicia in northwestern Spain) where they hybridise to produce sterile tetraploid offspring (Buggs and Pannell 2006), supported by patterns of allelic richness and genetic diversity at several isozyme loci (Obbard et al. 2006). Detailed population surveys and meiotic cell squashes of Durand (1963) located the northeast contact zone to be approximately 80 km north of its current location. This represents 80 km of relatively rapid movement over the course of the past 50 years (Durand 1963, Buggs and Pannell 2006) into a current region of particularly low occupancy and smaller population sizes relative to other parts of the species range (Eppley and Pannell 2007). This movement has been attributed to both differential pollen swamping by the dioecious (diploid) lineage, leading to a biased production of sterile hybrids by the monoecious (hexaploid) lineage (Buggs and Pannell 2006), and to ecophysiological differentiation and diploid superiority across this region (Buggs and Pannell 2007).

1.4.2 Ecophysiological adaptation

Hexaploid *M. annua* were initially thought to be ecophysiologically adapted to the drier environments towards which the tension zone is currently moving (Durand 1963). The ability of monoecious hexaploids to self-fertilise may make them better suited to the drier environment than the dioecious diploids, where populations are smaller and rapidly turning over, thus supporting their persistence at lower densities than diploid populations (Buggs and Pannell 2007, Eppley and Pannell 2007). This theory is consistent with (a) the idea that reproductive assurance in self-fertile lineages promotes colonisation because only one individual is required to establish a new population (Baker 1955, Pannell and Barrett 1998) and (b) the expectation that regional variation in the occurrence of metapopulation dynamics regulates the maintenance of unisexual individuals, with females, and especially males occurring in areas where the rate of population turnover is not too high (Pannell 1997). The northward expansion of hexaploid populations along coastal corridors of the Iberian Peninsula from a north-African refugium is thought to have been facilitated by the ability to self-fertilise at low density, conferring a sort of reproductive assurance, and the production of a dormant seed bank that hedges against environmental uncertainty across years.

Eppley and Pannell (2007) studied two regions over which a distinct transition between monoecious hexaploid *M. annua* populations and dioecious diploid populations occur. They found an overall difference in population the size between monomorphic and dimorphic populations, with substantially larger numbers of plants in dimorphic populations. Polyploidisation is well known to elicit physiological changes given the increased DNA content of cells (Leitch and Bennett 1997), which may further produce differences in life history traits (Schranz and Osborn 2004). Additionally, the sexual dimorphism of the diploids and thereby obligate outcrossing limits the capability of population growth after a colonisation event at low density relative to the self-fertilising hexaploids (see simulation model in Dorken and Pannell 2007). However, reciprocal transplant experiments found that the diploids were fitter across all sites, both at the center and periphery of the contact zone, having 2.6 times greater biomass, higher photosynthetic rates and taller height (Buggs and Pannell 2007) than hexaploids. This finding may be attributed to a loss of sexual specialisation in the monoecious hexaploids, as opposed to the diploids, which are solely dioecious, and may therefore be less efficient in their growth. The current location of this contact zone was also identified as being considerably less hospitable to both lineages of *M. annua* than the more distant environments on either side of the contact zone (Buggs 2004, pg. 135). Seed germination rates did not differ between the lineages in this area (Buggs 2004, pp. 99, 107). Thus, a precise role for the differential ecophysiological adaptation of *M. annua* races to this area as a cause of contact zone movement is in need of further elucidation.

1.4.3 Metapopulation dynamics

Previous studies of *M. annua* have indicated that the stability of contact zones between diploid and polyploid populations will be maintained by a combination of metapopulation dynamics (Pannell 1997, Eppley and Pannell 2007) and biased gene flow between lineages (Buggs and Pannell 2006). Recently, Dorken and Pannell (2007) simulated a spatially explicit metapopulation model across a gradient in habitat disturbance in order to examine the relations between reproductive assurance, asymmetric mating, and extinction and dispersal rates on the dynamics of hybrid zones. They identified that local extinctions and seed dispersal were the key processes governing the maintenance of the contact zone through their effects on establishment dynamics. It was found that the amount of seed dispersal occurring in each lineage was a key determinant of their ability to occupy newly opened sites resulting from extinction events. That is, under lower rates of dispersal, the dioecious diploid lineage was often excluded from the metapopulation altogether, unless it occupied areas with low extinction rates. However, under higher dispersal rates, both males and females were more likely to disperse into populations together and thereby prevent the hermaphroditic lineage from establishing in the same site. Under conditions of low immigration (and thus, high extinction), reproductive assurance of hermaphrodites precluded occupation of the dioecious diploid lineage in the metapopulation. If however the *M. annua* contact zone follows its current southerly movement trajectory, the diploid populations will eventually come into contact with androdioecious hexaploid populations. The simulation identified that at this stage the rate of movement of the contact zone should accelerate because the high equilibrium frequency of males in the region counteracts the advantage of reproductive assurance that self-fertilising hermaphrodites maintain.

Simulation results were supported by the aforementioned regional surveys of Eppley and

Pannell (2007), which demonstrated that monoecious populations are smaller and occur at lower densities than respective dioecious populations. These patterns of occupancy and abundance found by Eppley and Pannell (2007) are consistent with their regulation by metapopulation dynamics. Thus, an assessment of population demography under field conditions is warranted if we are to be able to predict the fate of moving hybrid zones.

1.5 THE CURRENT STUDIES

In this thesis I describe a series of studies principally carried out at the diploid-hexaploid *M. annua* contact zone of the northeastern Iberian Peninsula. Common-garden experiments within a hybrid zone are a necessary precursor to understanding the both the endogenous factors mediating the positioning of such zones. Previous studies of interacting lineages and the fitness of their hybrid progeny have recognised the importance of environmental conditions in underpinning hybrid zone behaviour. Studies of *Carpobrotus* spp. for instance have found that although F1 hybrids are more resistant to herbivory than its parental taxa across three common gardens, the total biomass of hybrids was greater only in certain habitats (Vila and D'Antonio 1998). As a further example, with work on the narrow hybrid zone between two subspecies of big sagebrush *Artemisia tridentata,* the authors acknowledge that they unable to completely reject the dynamic equilibrium hypothesis for the maintenance of the hybrid zone given that the establishment and growth of hybrids and their parental taxa had not been assessed in the hybrid zone itself (Graham et al. 1995). Such genotype × environment interactions often impact the success of parental lineages and their F1 hybrids (Mercer et al. 2006, Arnold and Martin 2010), and thus experiments under environmental conditions representative of the contact zone are essential for generating biologically realistic interpretations of hybrid zone behaviour. One of the key aims of this thesis therefore is to corroborate earlier laboratory- or greenhouse-based work in this system in the diploid-hexaploid contact zone itself.

1.5.1 Experimental Metapopulation

Through a large-scale experimental setup in the northeastern diploid-hexaploid *M. annua* contact zone of the Iberian Peninsula, I follow the establishment, persistence and extinction dynamics for each lineage across a range of densities over a 4-year time-course (Chapter 2). Here I test the hypothesis that hermaphroditic hexaploid populations are better suited to low-density conditions through their ability to self-fertilise (cf. Baker's Law, Stebbins 1957), and determine whether either lineage experiences a greater propensity for persistence in the contact zone by assessing difference in life-history characters. This provides a refined assessment of ambient rates of landscape-level colonisation and extinction in this region, and an understanding of the population dynamics experienced independently by each lineage upon migrating into the area. I also characterise the role of a dormant seed bank in buffering populations from population size fluctuations and year-on-year extinctions.

1.5.2 Minority cytotype exclusion

In the absence of differential habitat preferences, the contact zone may be defined purely as a tension zone, where the position and maintenance are largely mediated by the direction of gene flow between lineages, and their rate of production of inviable hybrid offspring. The rate and direction of hybridisation between lineages was quantified in the northeastern contact zone across a range of densities and cytotype frequencies, using both naturally occurring mixed populations and experimental sowings (Chapter 3). In this study I directly looked for differential rates of hybrid production, thereby assessing the minority cytotype exclusion principle that posits higher rates of hybridisation in the cytotype that occurs in lowest frequency in a mixed populations. Given the advantages of sexual specialization of the diploids and their production of specialized pollen dispersal structures in the males, it is expected that for a population containing equal frequencies of diploids and hexaploids, hybridisation should still be biased in favour of the diploids. I directly quantified this bias using flow cytometry of individuals sampled from naturally occurring mixed populations in a spatially explicit manner that enabled me to relate hybridisation rates to the size and position of neighbouring plants.

This dissertation presents a detailed study of the processes governing the distribution of the diploid and hexaploid lineages of the *M. annua* species complex through a combination of laboratory- and field based studies conducted over the course of four years. I examine the population dynamics of experimentally established populations over this period to assess whether the likelihood of persistence or extinction differs between lineages, and aim to characterise the effects of variation in population density underpinning these differences in Chapter 2. In Chapter 3, I assess the rate of hybridisation between experimental populations at varying densities established at the northeastern contact zone to determine whether there are inherent differences between lineages that might affect that rate and direction of the hybrid zone movement. I interpret these results in the context of a ‘tension hybrid zone’ model wherein movement is mediated by landscape-level population density. Together these studies offer a comprehensive investigation of the role of population density and lineage interaction in structuring and maintaining the northeastern diploid-hexaploid hybrid zone, and an insight as to the future persistence or demise of these migrating lineages.

CHAPTER 2. EXPERIMENTAL INVESTIGATION OF ESTABLISHMENT DYNAMICS FOR CONTRASTING SEXUAL SYSTEMS IN *MERCURIALIS ANNUA* ACROSS A MOVING TENSION HYBRID ZONE

2.1 INTRODUCTION

2.1.1 Differential adaptation of lineages to the contact zone

Theoretical studies of tension zones have ascertained that population density and the dispersal rate are key determinants of the rate of movement of tension zones (Bazykin 1969, Fife and Peletier 1980, Key 1981). Density and dispersal rate differences push tension zones towards regions of low neighborhood size down a density gradient or in the preferred direction of dispersal (Fife and Peletier 1980, Key 1981, Barton and Hewitt 1985, Ruegg 2007). The lineage with the higher density or dispersal will then spread. If the suitable habitat is patchily distributed, this spread will occur through differential rates of extinction and recolonisation (Dorken and Pannell 2007, Dufkova et al. 2011). The *M. annua* contact zone clearly indicates a bias in dispersal in favour of the northerly diploid lineage. It remains to be identified whether this bias will be maintained in the area it is currently invading and whether or not the bias is facilitated or impeded by the possible existence of an underlying ecological gradient in habitat suitability. Environmental factors and the ecological differentiation of hybridising lineages will thus only influence tension zone movement to the extent that tension zones move towards low-density troughs.

2.1.2 Population turnover rates and the seed bank

Seed dormancy is another life-history trait likely to influence the persistence of plant populations occurring at a tension zone through its effect on the presence or absence of a seed bank. Germination from a seed bank may be thought of as colonisation in time, rather than space, and may allow populations to re-establish following the loss of all individuals in the vegetative state. Persistent seed banks may also increase the regional pool of available colonists for any given year, thereby reducing the advantage of reproductive assurance described above (Pannell and Barrett 1998). An additional effect of seed dormancy is to increase the effective population size, with implications for population genetic diversity (Templeton and Levin 1979). Kalisz and McPeek (1993) modeled the demographic implications of a seed bank and suggested that population growth rates should be enhanced by its presence under all environmental conditions, except when good years were very frequent – although this enhancement was minimal. In their model, the presence of the seed bank decreased the probability of extinction, while increasing the time to extinction, which was longest when environmental conditions were unpredictable.

Notwithstanding the predictions of Kalisz and McPeek (1993), a seed bank may be demographically costly during years where survival and reproduction are high because seeds in a gene bank must first wait before they can contribute to population growth (Cohen 1966, Venable and Brown 1988). At the regional level, this observation suggests that populations containing genotypes that produce dormant seeds should be buffered from extinction in bad years, but will contribute fewer progeny in good years (Lewontin 1965, Mertz 1971). Despite the extensive theoretical work on the importance of seed banks in population dynamics (MacDonald and Watkinson 1981, Pacala 1986, Maron and Gardner 2000), relatively few studies have assessed the population-level importance of the seed bank, or the relative population size of recently produced seeds versus those buried in the soil, and the density-dependence of their formation and germination – no doubt because of the difficulty of measuring seed longevity and seed-bank decay *in situ* (Alexander and Schrag 2003). MacDonald (1976) has noted that a population with non-overlapping generations may undergo a significant change in behaviour when even a small fraction of each generation is held over to the next, be it through seed dormancy or the perennation of individual plants. Using a simple deterministic model, he found that a seed bank would act to reduce the amplitude of size fluctuations about an equilibrium state, thus damping the effect on the dynamical behaviour of populations. This effect would enable annual plant populations to return to an equilibrium state after a disturbance, and to consequently avoid possible chaotic oscillations. MacDonald and Watkinson (1981) outline that previous models of annual plant population dynamics that have accounted for a seed bank (e.g. Cohen 1966) have not taken into account density-dependent processes, which are known to be significant in population seed production and self-thinning.

2.1.3 Density-dependence and the Allee effect on growth rates

The density-dependence of tension zone dynamics may be constrained by two opposing forces: (i) reduced individual survivorship and fecundity through competition for resources and microsites for germination (negative density-dependence) (Freckleton and Watkinson 2001), and in contrast (ii) an increase in growth rate with density if, for example, mating opportunities are limited below a certain density (positive density-dependence, or the Allee effect) (Pannell and Barrett 1998, Stephens et al. 1999, Morgan et al. 2005). Negative density-dependence is expected to regulate population growth as it approaches a local equilibrium, whereas positive density-dependent reproduction prohibits the survival of populations that are too small and may thus limit colonisation success (Turnbull et al. 2000).

Positively density dependent seed production may be considered as a ‘component Allee effect’ because although one component of individual fitness may be positively related to fitness, it may not necessarily translate into a positive relationship between the population level growth rate and density (‘demographic Allee effect’) (Stephens et al. 1999). This distinction is necessary, because for positive density-dependence to lead to demographic Allee effects, and to thus have a discernible impact on population establishment at a tension zone, it must override the opposing influence of negative density-dependence. In plants, such a distinction is likely to be observed below densities at which competition for resources and microsites for germination occurs (Berec et al. 2007). For example, as density increases, the seed production per individual declines to the point where the number of seeds produced balances the mortality experienced with increased competition. While the importance of this dynamic process in ecology has been under-appreciated, recent evidence (Kramer and Drake 2010, Hoffman et al. 2010) suggests that it might have an impact on the population dynamics of many plant and animal species. Differences in fertility and seed production between lineages that are maintained by density gradients can thus be linked to movement of the tension zone through its influence on colonisation success (Barton and Hewitt 1985).

Studies of the causal mechanisms generating Allee effects in small populations could provide a key to understanding their dynamics and subsequent influence on tension zone movement (Courchamp et al. 1999). For example, decreased sexual reproduction owing to a lower probability of finding a mate at very low densities might generate a lower rate of recruitment, which in turn lowers the probability of finding mates in the next generation. This effect may ultimately lead to the collapse of the population of one of the competing lineages (Fagan et al. 2010). Richter-Dyn and Goel (1972) showed that colonisation success is a threshold function of population size, with small populations tending to go rapidly extinct unless they exist in an especially resource-rich area. Further, Amarasekare (1998) and Dennis (1989) both showed that strong Allee effects would lead to patch extinction. Studies of invasive hybrid cordgrass in the genus *Spartina* have identified a role for self-fertility in overcoming the problems of pollen limitation that occur in low-density stands (Davis et al. 2004, Taylor et al. 2004). The pollen limitation of isolated plants greatly slowed hybrid invasion (Davis et al. 2004, Taylor et al. 2004), whereas the few self-compatible hybrids, having adapted to environmentally challenging conditions, maximised their reproductive fitness by producing large numbers of self-fertilised seeds in isolation (Daehler 1998, Sloop et al. 2009). This manner of reproduction greatly accelerated the colonisation of new and challenging environments for the cordgrass and is instrumental in its long-term persistence (Sloop et al. 2009).

Multiple causes evidently mediate the persistence of populations in areas of low density, and thus, the movement of hybrid zones towards such regions. Few studies have thus far systematically disentangled these causes however, and to my knowledge there have been only few detailed case studies of all these issues around the role of density, the sexual system (and ploidy) and differential population turnover in mediating tension zone movement (e.g. *Empetrum spp.*, Richards 1997, *Chamerion angustifolium,* Husband et al. 2002, *Senecio spp.*, Prentis et al. 2007). An analysis of the factors influencing population establishment and persistence at a contact zone, and the role that density plays in mediating these patterns, can provide a foundation to understanding the factors mediating contact zone movement.

Elucidating patterns in population dynamics will necessarily involve documenting and understanding the underlying sources of variation in the density-dependence of demographic parameters. Density-dependence affects plants at many different life-history stages. Previous studies have suggested that the importance and intensity of competition may shift predictably through the life cycle of a plant (Schiffers and Tielborger 2006). However, most experiments on plant-plant interactions have looked at survival and growth as the major response variables and few studies have investigated the degree to which competition may be detected already at the very beginning of a plant's life. This is surprising since it is known that the time at which a plant germinates relative to its neighbours has large consequences for its lifetime fitness (reviewed by Verdu and Traveset 2005).

Since Allee et al. (1949), standard textbooks (e.g. Southwood 1978) categorise a given factor in terms of its effect (at the rate of birth, death or migration) being correlated (dependent on) or uncorrelated with (independent of) population density. For instance, if a certain type of mortality is correlated with population density, the factor causing it is called a density-dependent factor. Conversely a density-independent factor is one whose effect is uncorrelated with density. Relatively few studies have simultaneously considered density-dependence in more than one stage in plants (see Howard and Goldberg 2001 for review). Considering the different life-history stages, the germination behaviour of seeds may depend upon the density of its neighbouring seeds or seedlings (Miller et al. 1994, Lortie and Turkington 2002, Grundy et al. 2003, Dyer 2004, Kluth and Bruelheide 2005, Li et al. 2005, Turkington et al. 2005). Despite this, very few studies have examined the effects of sowing density on timing of emergence across seasons (Bergelson and Perry 1989, Dyer et al. 2000, Turkington et al. 2005). The persistence of plant populations and their regional occupancy should be enhanced when individuals produce and disperse large numbers of seeds, in addition to when colonists of new habitat are able to overcome the problem of finding mating partners when population densities are low (Pannell and Barrett 1998).

A range of studies has shown that density may have a negative impact on seed and seedling survival (Augspurger and Kelly 1984, Clark and Clark 1984), juvenile growth and survival (Hubbell et al. 1990, Condit et al. 1992), and adult growth, survival and fecundity (Hubbell et al. 1990, Alvarez-Buylla 1994, Gilbert et al. 1994). Given that density-dependence may differ considerably (even in direction) among demographic parameters (e.g., De Steven 1991, Howard and Goldberg 2001), generalising about patterns of density-dependence and extrapolating to lifetime fitness and population dynamics requires comparisons to be made among life-history stages, as well as among types of species and physical environments. By understanding the biological and environmental sources of variation in the magnitude and mechanism of density dependence at each life-history stage, we may begin to construct a framework for understanding the demographic processes underpinning population dynamics and thus, hybrid zone movement.

2.1.4 Low-density troughs and the hybrid sink effect

The shape of the chromosomal hybrid zone of the grasshopper *Podisma pedestris* provides a nice illustration of the effects of dispersal and density on the structure of a tension zone. In *P. pedestris*, the contact zone is held in place by two inhospitable slopes of low density. It then bulges out under the pressure of higher population density on one side, counterbalanced by the tension of the zone on the other side (Nichols 1984). This zone has been pushed towards the area of lowest density, trapped by local barriers in a "hybrid sink" (Barton 1980). Barton and Hewitt (1985) argued that tension zones will be stable in such hybrid sinks of low population density, and that they would be frequent enough to counteract movement due to fitness differences or asymmetry of hybridisation. The population density at the centre of a tension zone will usually be lower than that of the surrounding regions if the cline is maintained by hybrid unfitness or processes of minority cytotype exclusion (Levin 1975, Barton 1980). The action of differential gene flow from source regions towards a central hybrid sink has also been implicated in the movement of the *Mus musculus* and *M. m. domesticus* contact zone towards troughs of low population density and barriers in the environment (Macholan et al. 2008). Similarly, the narrow hybrid zone between Northern and California spotted owls (*Strix occidentalis*) has been constrained by a low-density trough (Barrowclough et al. 2005). The loss of hybrids should depress population sizes in this area, thereby tightening the interface between the two clines, as more organisms will migrate into the area than migrate out (Moran 1979). Understanding the role that population density plays in mediating the simultaneous invasions and extinctions of a tension zone is thus of central importance to understanding their movement and their long-term fate (Ellstrand 1992, Rhymer and Simberloff 1996).

While density-independent models using transition matrices have been useful in identifying population growth rates at low density, it is clear that for many applications, the incorporation of density dependence is also important (Law and Watkinson 1987). There is now little doubt that density dependence operates in most plant and animal populations, failure to detect it may be largely attributed to inadequacies of data sets or techniques (Hassell et al. 1989, Turchin 1990, Holyoak and Lawton 1992). The majority of demographic studies that attempt to quantify density-dependence in the field are non-experimental (but see Fowler (1995) and Watkinson (1997)). Thus, the effects of density variation (within species or overall) on individual fitness may often be confounded by underlying environmental factors that may in turn be associated with this density variation. Previous studies on density effects obtained by plant population mapping are at best circumstantial, since the effects of spacing may be confounded with microenvironmental differences. Plants growing in poor conditions may be smaller and have lower survivorship, yet be far apart, while those growing in good conditions may be close together, but large and with a high survivorship, as was observed in naturally occurring stands of *Vulpia fasiculata* (Watkinson and Harper 1978).

This confounding influence of density-independent factors should be avoided, or at least be controlled for by using an experimental approach. The study of Alvarez-Buylla (1994) on the role of density-dependence in mediating the population dynamics of a tropical palm, *Cecropia obtusifolia,* identified a negative relationship between population density and plant survival, however, this connection was attributed to site quality, rather than resource-competition. Similarly, the work of Piñero et al. (1984) attributed the higher transition rates between life stages of larger plants to more favourable light conditions and consequentially, higher rates of reproduction and growth. Density-independent effects were also used to explain transition rates from seedling to the juvenile stage in *Rhopalostylis sapida*, wherein variation in light conditions and interference from lianas confounded patterns of growth (Enright and Watson 1992). Antonovics and Levin (1980) also point out that in Symonides' (1974) study of *Spergula vernalis*, while one can invoke density-dependence in more crowded sites and micro-environmental differences in the less-dense sites, one cannot eliminate either alternative. This observation has left some authors to conclude that density-dependence is not always operating, and is often circumvented by other forces, depending on both the organism and local environmental circumstances (Strong 1983). Evidence from site differences must therefore be regarded with a fair degree of skepticism. Nevertheless, when numerous sites are compared, particularly when support for density effects comes from additional lines of evidence, demonstrations of negative relations between density and plant size or reproductive output can be more convincing (cf. Watkinson and Harper 1978). A recent study on the effects of predation of understory bamboo on the regeneration of neighbouring tree species (Caccia et al. 2009) revealed that although earlier studies did not identify any density dependence of granivory rates (Caccia et al. 2006), density may still have the potential to influence bamboo–seedling interactions through consumer-mediated effects (Janzen 1970, Clark and Clark 1985). The authors further realised the importance of extensively characterising microenvironmental variation between sites to disentangle its influence from density (Caccia et al. 2009). A study of the effects of understory density on tree seedling establishment (Royo and Carson 2008) found that density mediated establishment in an indirect manner, by influencing the foraging behaviour of seedling predators of the plant species of that niche. Their study therefore necessitated a factorial manipulation of both plant cover and predation in order to accurately disentangle the effects of density on recruitment.

2.1.5 The current study

To further dissect various components that contribute to large-scale population dynamics underlying tension zone movement, it is necessary to develop an understanding of the relative roles and effects of (i) rates of population extinction, (ii) rates of emergence and germination from a dormant seed bank and (iii) interactions with local processes that regulate local population sizes and densities. Using the northeast *M. annua* contact zone where diploid, dioecious populations of *M. annua* are rapidly displacing hexaploid, monoecious populations, this current study assesses the susceptibility of each lineage to density-dependent processes, and the suitability for each lineage to the area in which hybridisation is currently occurring, irrespective of dispersal between lineages. Here, a large-scale hierarchically designed field survey is used to explore the consequences of life-history variation and differences in the susceptibility to density-dependence for the population ecology of *M. annua*, analysing how life-history variation affects population establishment and persistence. Replicate roadside surveys, such as this one, are an efficient way to evaluate geographic variation in dynamics, the landscape matrix and habitat characteristics across a broad geographical area (Alexander et al. 2009). Such unidirectional grids along roadsides have been effectively used to study range limits (Prince et al. 1985), invasion and spread (Wilcox 1989, Crawley and Brown 1995, 2004), scaling-down of colonisation and extinction rates (Moody-Weis et al. 2008), and host-pathogen interactions (Antonovics et al. 1994, Antonovics 2004).

This study examines rates of population turnover over three years of survey in an area within the region towards which the contact zone is moving. Specifically, in order to assess the potential cascade impacts of density-dependent processes on tension zone dynamics, the following questions will be addressed: (i) How will negative and positive density-dependence influence the establishment and growth of each lineage in regions of low neighbourhood size? (ii) To what extent are establishment and extinction rates in these low-density regions affected by density-dependence in individual seed production, germination, or recruitment from the seed bank? (iii) Does the strength of positive and negative density-dependence vary between lineages? That is, are monoecious hexaploid populations pre-adapted to low-density conditions as may be encountered during tension zone movement via reproductive assurance? Comparing the effects of density on seed production with growth rates will reveal whether component Allee effects are manifest as demographic effects, and how population sizes are predicted to change with both sexual systems in the contact zone.

2.2 MATERIALS AND METHODS

2.2.1 Experimental setup and habitat

M. annua is a wind-pollinated annual herb, largely confined to ruderal habitats, including roadsides and disturbed waste grounds both around the Mediterranean Basin and across Europe (Durand 1963, Tutin et al. 1964). It is a winter annual in northeastern Spain, with peak flowering occurring in March (pers. obs.). Primary seed dispersal is ballistic (up to ~1m), while secondary dispersal occurs via ants (Lisci and Pacini 1997), wind and possibly, through anthropogenic activity (pers. obs.).

A unidirectional roadside experiment was setup 10 km south of the northeast hybrid zone (ahead of the current movement trajectory) assessing initial establishment and extinction/persistence rates at a range of different densities for each lineage and was monitored over the course of three subsequent years. The experimental site ran across a region of spatial transition in the sexual system, with populations north of the region being predominantly dioecious, while populations south of the region were monoecious. A low density of naturally occurring populations exists within this transition. In March

2008, large amounts of seed were obtained from populations at opposite sides of a sexual system transition zone in Eastern Spain from Cambrils (dioecious, diploid) and Santa Carles de la Rapita (monoecious, hexaploid), approximately 40 km north and south of the contact zone, respectively.

Germination rates of seed sourced from the Cambrils and Santa Carles de la Rapita populations were assessed in the greenhouse. Such an assessment was conducted in order to estimate inherent differences in seed viability between the lineages in a controlled environment that might influence field establishment rates. Precisely 300 seeds per population were sown onto germination trays containing Emerald Green Pro-Mix soil-based compost (100 seeds evenly distributed per tray). Trays were watered regularly and randomised once per week within the greenhouse. Germinants were counted and removed daily, so as to prevent competition with other seeds.

Seeds from each of the two lineages were sown at varying densities along the abandoned Xerta-Calig canal at the southern tip of the transition zone. The area was selected on the basis of its consistency of substrate, and the ease with which experimental populations could be demarcated and relocated with reference to clear landmarks. Several naturally occurring populations were identified in this vicinity prior to setting up the experiment, as were other ruderal species commonly associated with suitable *M. annua* habitat.

Seeds from both lineages were sown at a range of different densities at replicated sites along the experimental transect, which was 25 km in length. Over three subsequent growing seasons, I observed the establishment and eventual dynamics in each of the experimental populations. All replicate sites were established along the transect in a randomised block design, wherein each of the 20 blocks contained an equal number of sowing density treatments and sites of each sexual system (42 individual sowing sites in a straight line per block). Each experimental sowing site was a 4 m long straight line, 2 cm in width; seeds were sown along this line and buried by 5 cm of soil (Fig. 2.1), and separated from the next such sowing site by 16 m. For each sexual system the following sowing treatments were applied to the sites: either of 1 seed (N = 200 independent replicates), 10 seeds (N=140), 100 seeds (N=60), or 1000 seeds (N=20); seed numbers were estimated by weighing for the 100 and 1000 seed treatments. Seeds were sown in September 2008, between the end of the spring and beginning of the summer, when *M. annua* populations in the area had subsided. This practice of sowing at regular 16 m intervals in a straight line along the canal prevented the necessity of an *a priori* interpretation of suitable habitat within the study area, in a manner similar to the assessment of regional occupancy and abundance conducted by Eppley and Pannell (2007). Although seeds and pollen may occasionally disperse over greater distances, such gene flow or dispersal was expected to be minimal throughout the two years of the study, at least relative to the amount of within-site movement (though see Discussion). In order to locate sites the following year, they were both photographed and precisely mapped out using a GPS locator, with directions from permanent landmarks to the corners of each site.

2.2.2 Colonisation and extinction measurements

The experimental area was re-visited to assess establishment rates during peak flowering in March 2009 and extinction/growth rates in March 2010. Sites were identified using a combination of GPS, maps and photograph references, searched for seedlings for two minutes each. All of the plants were counted as a measure of the proportionate establishment success at each experimental site. Plant size variation was estimated among treatment combinations by taking measurements of height, diameter at the widest point, number of nodes and number of seeds on the two lowest branches on a single plant per site (i.e., replicating over sites). The randomly selected single plant of median height within each population was chosen after having briefly screened all plants for size and seed production, and selecting from the line of best height fit that transected the tips of most plants. The sex ratio for dioecious sites (number of males and females, which are easily distinguishable) was also determined.

2.2.3 Dispersal measurements

In year one, the distribution of plants along each sowing line was noted, with counts of plants in each meter of the 4 m sowing line. Plant gender was also recorded. Coordinates describing the spatial extent of plants at each of the experimental sites in year two were obtained by creating a polygon that encapsulated each of the plants at a site, subtended by the 4 m sowing line (see Figure 2.1). This permitted measurement of the area and maximum dispersal distance of each site that had established.

2.2.4 Seed bank assessment

Colonisation from the dormant seed bank was assessed by noting which plants emerged on the initial September 2008 sowing line at each site in March 2010, compared to plants "off the line" which were the progeny of fertilisation events that occurred in the 2009 growing season. The sowing line arrangement allowed for the identification of emergence from the seed bank at all sites in March 2010, regardless of whether they were occupied the previous year. Emergence from the seed bank in March 2011 could only be inferred at sites that were unoccupied in all preceding years.

2.2.5 Statistical analyses

Effects of density and the other treatments on demography could be assessed either on population size or individual performance at each stage. These two approaches have quite different null hypotheses and require quite different statistical analyses. For population size analyses, the biological null hypotheses is that the number of emerging seedlings per site increases linearly with number of seeds planted per site, number of surviving plants increases linearly with number of emergents, while final biomass per site increases linearly with number of survivors. For individual performance analyses, the biological null hypotheses are that per-seed probability of emergence, survival probability, and mass per plant are each independent of density, i.e., have slopes of zero when regressed against the appropriate measure of density. Significant negative slopes indicate negative density-dependence (competition) and significant positive slopes indicate positive density-dependence (facilitation). The linear regression methods employed to detect density-dependent effects on the population level growth rate were modifications of the standard Ricker equation regression (ln (N_{t+1} / N_t) against N_t), in which the degree of density dependence was given by the gradient of the regression (Turchin 1990). Such difference equations have been used extensively in studies of plant population dynamics in species with discrete generations (e.g. Watkinson and Harper (1978) study of *Vulpia fasciculata*).

Ecophysiological adaptation to low density in each of the lineages was assessed using logistic regression, with extinction, establishment and persistence rates for each lineage (i.e. characterising sites containing plants as '1' and sites without plants as '0') modeled as a response to density, plant size, seed production and the presence of a seed bank. This was achieved using generalised linear mixed models (GLMM) with the lme4 implementation in R, treating site as a random block effect. Each block of 42 sites contained all combinations of lineage and sowing density treatment in equal proportions. The mixed effects models allow for a hierarchical structure in experimental design, providing an intuitive way of accounting for within block dependency in nested experiments (Pinheiro and Bates 2000, McCulloch and Searle 2001). Models included sexual system (fixed factor), density (covariate/fixed factor), seed production (covariate), plant height/diameter/nodes (covariate), number of plants emerging at a site in year one (covariate) and sexual system × covariate interaction terms, with site fitted as a random factor.

Alternative models were compared using Akaike's information criterion (AIC), which requires the calculation of the maximum log-likelihood of each model (Akaike 1973, Burnham and Anderson 2004). A model with a low AIC is more parsimonious than a model with a high AIC. AIC allows for the evaluation of multiple models simultaneously, regardless of nesting structure. A decrease in the AIC value between models less than -2 is considered significant, according to Burnham and Anderson (2004). Models were tested for evidence of non-linearity using quadratic terms, and variables were transformed as necessary in order to maintain constant variance on the original scale of measurement. GLMMs with Poisson errors and log link functions were used for count data (e.g. seed production). The residual deviance was compared to the residual degrees of freedom to assess overdispersion, in which case quasi-Poisson was used instead of Poisson error structure. Quantile-quantile plots and partial residual plots (Landwehr et al. 1984) were also used to assess whether or not the error distribution of the data was modeled correctly.

To examine geographical patterns of spatial autocorrelation in seed germination and site quality values of Moran's *I* were calculated across a range of spatial scales (Fortin and Dale 2005). The procedure involved calculating a matrix of paired distances between all sites and the difference in emergence rates between all the sites in the distance matrix. Moran's *I* is analogous to the product-moment correlation coefficient, with positive spatial autocorrelation indicated by values of *I* between 0 and 1 (although values can exceed 1, especially with smaller sample sizes).

To ascertain how meteorologically usual the years over which the experiment took place were, weather station measurements for the study period were compared with measurements taken from 1970-2000. Data were obtained from Agencia Estatal de Meteorologia for Tortosa.

2.3 RESULTS

2.3.1 Seedling emergence

Seedling emergence and thus population size declined systematically across years. The diploid lineage was better adapted to the region it is currently invading as evidenced by higher germination rates relative to the hexaploid lineage. Greenhouse germination rates were greater for the diploid Cambrils population (0.65 ± 0.046) than for the hexaploid Santa Carles de la Rapita population (0.47 ± 0.06), ($\chi^2_1 = 20.6$, $p < 0.001$), and this advantage was maintained in the field experiment.

Across hexaploid treatments, 70 of the 420 sites established in year one, with a combined total of 892 plants (Fig. 2.2a). Of these 70 sites, 29 went extinct by year two, 41 persisted into year two, and only 4 exhibited germination from the sowing line in years two or three, indicating likely emergence from the seed bank (29-4 = 25 apparent extinctions). Additionally, 26 hexaploid sites that were unoccupied in year one were occupied in year two, presumably due to emergence from a dormant seed bank. Altogether, 66 hexaploid sites were occupied in year two (including 26 from the seed bank alone), with a combined total of 1039 plants (433 of which were from the supposed seed bank).

Across diploid treatments, 87 of the 420 sites established in year one, 47 of which went extinct by year two, and the remaining 40 persisted (Fig. 2.2a). Three of the 47 extinct sites had emergents from the seed bank (47-3 = 44 apparent extinctions). Additionally, 40 diploid sites that were unoccupied in year one were occupied in year two due to emergence from the initial sowing line alone. Altogether, 85 of the 420 diploid sites were occupied in year two (including 40 due to emergence from the seed bank alone). Across all diploid sites, there were 2103 plants in year one and 1768 plants in year two (Fig. 2.2b) (including 583 from the supposed seed bank). At one site, a single female present in year one (from a single seed sowing treatment) lead to a cluster of 6 females at that site in year two, suggesting possible self-fertilisation by a lone female. Population size was generally more variable across years and sowing density treatments in the diploid lineage than the hexaploids (Table 2.11).

The proportion of plants establishing across all sites was marginally higher in the diploid than in the hexaploid lineage ($\chi^2_1 = 605.76$, $p < 0.001$; Table 2.1) and was marked by greater variability (Table 2.12). Establishment success at all sites in year one was negatively related to initial seed sowing density in year one (negative density-dependence) ($\chi^2_1 = 123.89$, $p < 0.001$; Table 2.1), but this effect of negative density-dependence differed between lineages and was weaker in the diploid populations ($\chi^2 = 605.76$, $p < 0.01$; Table 2.1).

In year three, seedlings at 59 of the 840 sites emerged, with a combined total of 951 plants (Fig. 2.2c). 17 of these sites were unoccupied the previous year, probably due to emergence from a dormant seed bank. An example of perennation was apparent in a site of 6 female plants into year three, with all 6 plants surviving the winter period, and increasing in size.

The emergence of plants at a specific site was not influenced by the presence of a bank or mound at that site ($\chi^2_1 = 0.608$, $p = 0.436$), but was positively related to the logarithm of the number of seeds sown at the beginning of the study ($\chi^2_1 = 28.7$, $p < 0.001$; Tables 2.1, 2.10a). The rate of seedling emergence in year three was significantly different between lineages, once again being slightly higher in the diploid lineage ($\chi^2_1 = 5.36$, $p = 0.0206$; Table 2.12), indicating that the ecophysiological adaptation of the diploid lineage to the southerly region it is currently invading was maintained across all years. Seedling emergence in year three was positively related to the number of plants occurring at a site in the preceding year ($\chi^2_1 = 97.9$, $p < 0.001$; Table 2.11), and increased with the amount of cover at a site ($\chi^2_1 = 4.36$, $p = 0.037$). A Moran's *I* test on the residuals of the fitted model did not identify any significant spatial autocorrelation across study sites in the establishment rate (Fig. 2.7) (Moran *I* statistic standard deviate = 1.515, $p = 0.065$). A statistically significant positive result would have indicated that similar germination rates cluster spatially, after accounting for the variation explained by lineage differences and sowing density treatments. Randomisation of treatments across the 840 study sites has sufficiently enabled unbiased characterisation of the spatial processes underlying habitat suitability.

2.3.2 Plant size

Diploid plants (21.1 ± 1.11 cm) were on average larger than hexaploid plants (15.8 ± 0.832 cm; $\chi^2_1 = 23.5$, $p < 0.001$; Table 2.2), offering further evidence of the diploid lineage being pre-adapted to the area it is currently invading. Similarly, diploid plants were wider in crown diameter (11.8 ± 0.736 cm) than hexaploid plants (9.30 ± 0.554 cm; $\chi^2_1 = 11.3$, $p < 0.001$; Table 2.3). Neither plant height nor crown diameter was influenced by initial seed-sowing density (Table 2.3, 2.4).

2.3.3 Seed production

There was a significant interaction between lineage and the number of plants at a site on the seed production per plant of that site ($\chi^2_1 = 8.23$, $p = 0.00413$; Table 2.4). Accounting for this interaction, at sites containing hexaploid plants, there was no relationship between the number of plants present at a site (population density) and the seed production at that site ($\chi^2_1 = 1.50$, $p = 0.221$; Table 2.4). At sites containing diploid plants however, seed production was positively related to the number of plants present at that site ($\chi^2_1 = 27.6$, $p < 0.001$; Table 2.4; Figure 2.8).

2.3.4 Population growth rate

Average population density declined drastically across populations over the course of the three-year study (2009-2011) (Table 2.16a,b). Higher variability was generally observed in the diploid populations across all years (Table 2.11).

Among established sites, the average population growth rate (calculated by dividing the number of plants present in year $t + 1$ by t) was greatest in the hexaploid lineage at the lowest sowing densities (Table 2.16a,b). In the hexaploid lineage, by the end of the study, only 3 of the 13 sites that emerged in the first year remained, whereas in the diploids only 1 of 9 sites remained (Table 2.16a,b). Growth rates were highly variable across sites and density treatments, but were all less than 1 (indicating decreasing population size) by the final year of the study. Across all sowing density treatments and both lineages, less than one-third of sites that established in the first year remained by the final year of study. When excluding sites in which emergence from the seed bank led to population re-establishment, only the 10 seed sowing treatment hexaploid sites exhibited an increase in population size. However, it needs to be recognised that only 3 of the 21 sites that emerged in year 1 remained (Table 2.16a,b).

The population-level growth rate from year one to year two, defined as the number of plants present at a site in year two, divided by the number of plants present at the same site in year one, did not differ between the lineages according the linear mixed effects model analysis ($\chi^2_1 = 2.78$, $p = 0.0952$; Table 2.5a; Fig. 2.3a).

The growth rate was not related to the number of seeds sown in year one ($\chi^2_1 = 0.0693$, $p = 0.792$; Table 2.5a), but there was a non-significant decline with the number of plants present at a site in year one ($\chi^2_1 = 2.78$, $p = 0.0952$; Table 2.5a). Growth rate at a given site was not explained by the total number of seeds produced by all plants at that site in the preceding year ($\chi^2_1 = 0.50$, $p = 0.48$; Table 2.5a).

After excluding sites that went extinct in year two and re-analysing the growth rate as log (N_t / N_{t-1}), where N_t refers to the number of plants at a site in year t, it was found that the growth rate was positively influenced by both the log number of seeds sown in year one ($\chi^2_1 = 21.1$, $p < 0.001$; Table 2.5b) and the number of seeds produced per plant ($\chi^2_1 = 12.0$, $p < 0.001$; Table 2.5b). Measured in this way, log population growth rate was negatively influenced by the log number of plants emerging in year one ($\chi^2_1 = 10.9$, $p < 0.001$; Table 2.5b). Log population growth rate was not significantly related to plant size as measured by height or diameter, the total number of seeds produced by a population, or the sexual system/lineage.

The log population growth rate from year two to three (Fig. 2.3b) was neither related to the number of seeds sown in year one ($\chi^2_1 = 0.344$, $p = 0.557$), nor to the number of plants at a site in year two ($\chi^2_1 = 2.06$, $p = 0.151$). It also did not differ between lineages ($\chi^2_1 = 0.0625$, $p = 0.803$). Furthermore, growth rate from year two to three was not influenced by the amount of cover at a site at year three ($\chi^2_1 = 1.55$, $p = 0.213$) or the presence of a bank or soil mound ($\chi^2_1 = 0.856$, $p = 0.355$). As with the growth rate measures from year one to two, neither plant height nor diameter the preceding year had any influence on growth rates from year two to three ($\chi^2_1 = 0.831$, $p = 0.362$, and $\chi^2_1 = 0.857$, $p = 0.355$ respectively).

2.3.5 Seed dormancy

In year one, all plants that emerged did so from the sowing line that had been experimentally established in the previous year. In year two, it was observed that many plants emerged from the same sowing line. This emergence could have been due to the emergence of seeds produced by their parents and scattered along the same line, and it is indeed reasonably likely that some of them were. However, emergence also occurred along this same line in year two in a similar fashion to that observed in year one, at sites that were unoccupied in year one.

Seedlings were detected on the initial year zero sowing line in year two at 51 hexaploid sites and 56 diploid sites (107 sites altogether). This finding stands as evidence for a seed bank that persists over at least one year. No difference was observed between hexaploid (0.034 ± 0.0080) and diploid sites (0.039 ± 0.011) in terms of the proportion of plants emerging from the seed bank ($Z = 1.07$, $p = 0.29$; Table 2.6). The proportion of plants emerging from the seed bank was negatively related to the density at which seeds were initially sown, such that sites of higher density sowing treatments were less likely to be exhibit re-emergence after apparent extinction ($Z = -8.48$, $p < 0.001$; Table 2.6, 2.13). The significant sexual system × sowing density interaction indicates that the diploid lineage is less susceptible to the negative density dependence on emergence from the seed bank (Table 2.6). This relationship translated into a higher proportion of diploid than hexaploid seeds emerging from the seed bank and should thus act to support the diploid lineage at low densities in subsequent years (Table 2.13). Variability between lineages in terms of the proportion of seedlings delaying germination to year two was low (Table 2.13).

2.3.6 Dispersal areas and distances

The area over which individual populations expanded into their second year was positively influenced by both the number of seeds sown in year one ($F = 6.10$, $p = 0.0161$; Table 2.7) and the number of plants present in the population at the time of area measurement in year two ($F = 5.44$, $p = 0.0229$; Table 2.7).

Hexaploid populations reached a maximum spread of 55.9 m^2 at a site containing 3 plants, with a 1000-seed sowing treatment. The maximum spread distance obtained by a hexaploid population from one year to the next was 17.9 m from the initial sowing line with a 10-seed sowing treatment containing 23 plants. On average, spread area was 10.4 ± 2.5 m^2, while maximum distance was 3.5 ± 0.7 m across the hexaploid populations.

Diploid populations reached a maximum spread of 142.8 m^2 at a site containing 5 plants, with a 100-seed sowing treatment. The maximum dispersal distance obtained by a diploid population from one year to the next was 22.0 m from the initial sowing line with a 10-seed sowing treatment containing 3 plants. On average, dispersal area was 14.6 ± 5.2 m^2 and maximum distance was 3.6 ± 0.8 m across the diploid populations.

The spread that a population obtained in year two was not influenced by the suitability of that site in the preceding year, as defined by the proportion of plants germinating per site in year one ($F = 3.81$, $p = 0.0551$), the number of seeds produced per plant in year one ($F = 0.818$, $p = 0.369$) or the seeds produced per population in year one ($F = 1.63$, $p = 0.205$).

The dispersal distance, or movement, obtained by populations from year one to year two did not differ between lineages ($F = 0.0014$; $p = 0.971$; Fig. 2.4). Neither did the seed production of sites ($F = 0.674$; $p = 0.415$) or the number of plants germinating ($F = 1.37$, $p = 0.240$) in the preceding year differ between lineages. There was a significant positive relationship between the number of seeds initially sown at a site in year zero and the dispersal distance obtained by that site in year two ($F = 5.83$, $p = 0.0186$; Table 2.8, 2.14).

2.3.7 Extinction rate

Of the 157 sites that established in year one in both lineages, only 85 persisted into year two (not counting sites that were re-established from the seed bank). Of these 85 sites, only 31 emerged in the final year of study (Table 2.16a). Including sites that were re-established from a dormant seed bank in the second year of study (155 total sites), only 42 persisted into (re-emerged) in the final year (Table 2.16b). The analyses of factors influencing the extinction rate from years one to two and from years two to three is presented in Tables 2.9a-c and 2.10b, respectively.

There was a strong inverse relationship between the number of seeds produced by individual plants at a site in year one and the extinction rate at those sites in the second year ($\chi^2_1 = 33.7$, $p < 0.001$; Table 2.9c). The same relationship was observed between the total number of seeds produced by all plants at a given site and the extinction rate ($\chi^2_1 = 15.2$, $p < 0.001$; Table 2.9a; Figure 2.6), but this model did not have a high predictive power as demonstrated by a higher AIC than the per plant seed production model. This relationship was corroborated by a significantly positive Pearson's product-moment correlation ($r = 0.4973$; 95% CI 0.36 – 0.61) between the logarithm of the number of seeds produced by a population in 2009 and the logarithm of the number of plants emerging the following year (Figure 2.6) ($t_{138} = 6.7343$; $p < 0.001$), in addition to a significantly positive Spearman's rank correlation ($\rho = 0.444$; $p < 0.001$).

The probability of extinction at a site also decreased with the number of plants that emerged at that site in the preceding year ($\chi^2_1 = 10.8$, $p = 0.0010$; Table 2.9b), but the extinction rate did not differ between lineages ($\chi^2_1 = 0.486$, $p = 0.486$).

The extinction rate at a site from years two to year three significantly decreased with the log number of seeds sown at that site at the beginning of the study ($\chi^2_1 = 4.74$, $p < 0.001$). The extinction rate into year three did not differ between lineages ($\chi^2_1 = 0.480$, $p = 0.489$) and was unrelated to the number of plants occurring at a site in year one ($\chi^2_1 = 0.758$, $p = 0.384$). However, there was a highly significant negative relationship with the log of the number of plants occurring at a site in year two ($\chi^2_1 = 16.5$, $p < 0.001$). The extinction rate was not related to the amount of plant cover occurring at a site ($\chi^2_1 = 1.63$, $p = 0.202$), but had a non-significant positive association with the presence of a bank or soil mound at that site ($\chi^2_1 = 3.79$, $p = 0.0516$) and the size of plants in the preceding year ($\chi^2_1 = 3.20$, $p = 0.0735$).

2.3.8 Weather

Generally, precipitation was lower over the course of the experiment than the 30-year average (INM 2002), except in 2008 where precipitation was 11% higher. The mean temperature over the course of the experiment was 8% higher than the 30-year average for Tortosa (17.3ºC). The mean temperature was higher in each year, and the maximum temperature attained in each of the years of the study was higher than the average 30 year maximum (22.6ºC). The average temperature was not lower the 30-year average minimum temperature in any of the years (12.1ºC) (Table 2.12).

2.4 DISCUSSION

The purpose of this study was to assess the population dynamics of *M. annua* in northeastern Spain towards which the diploid-hexaploid contact zone is currently rapidly moving, i.e., whether it will continue to move, and why (what demographic factors will be most important). Tension zone movement, as a density-dependent process, is governed by the ability of populations to colonise and persist in new habitat. This density-dependence varies in its action at different life history stages. This study characterised the strength of the action of density-dependence and linked this to spatiotemporal variation in population demography.

In the simulation work of Dorken and Pannell (2007), at equilibrium, the frequency of the two lineages was determined by the balance between (i) reproductive assurance, which promoted the spread of the hermaphroditic lineage and (ii) biased dispersal between lineages, which promoted the spread of the diploid lineage. This study has quantified the density-dependence of seed production and draws attention to the importance of reproductive assurance at low-densities, as may be encountered by the moving contact zone in the area under study. While there was no significant difference in the dispersal abilities or spread of either lineage as might have been expected (cf. Heilbuth 2001), lineages did differ in their susceptibility to action of density-dependence at different stages in their growth.

This study examined demographic rates at various stages in the life cycle and has decomposed the population growth rate into its constituent parts, testing for different forms of density dependence. The first stage considered was germination and population establishment, and it was identified that the germinating fraction was negatively density-dependent. This study has also tested whether the strength of positive or negative density dependence varies between sexual systems. The strength of negatively density-dependent germination was not as severe in the diploid lineage relative to the hexaploids.

Most of the experimental populations in this study area are apparently destined for extinction (Fig. 5a-d), but the relative rates at which the two sexual systems disappear might offer insights as to what the fate of each lineage is in more suitable habitats, as may be found on either side of the contact zone under study (Buggs 2004). Population growth was largely negative for both lineages across most sites, and was linked to seed production the previous year, which was in turn related to initial founding density. Overall, the establishment rates of both lineages were quite poor, relative to previous studies conducted in surrounding regions (Buggs 2004); the habitat was found to be generally unsuitable for *M. annua* across the 30 km transect under study, as reflected by the low percentage of sites that emerged each year in both lineages. A clear influence of the seed productivity of a site on its probability of persistence/occupation in the following year was identified, as was the existence of a persistent soil seed bank that buffers populations from extinction.

The following discussion examines what aspects of the species biology are responsible for the population declines at this contact zone area, including, but not limited to: the density-dependence of germination rates, seed production, seed dormancy, plant sizes, and dispersal distances, and their influence on the extinction, growth and colonisation rates of both lineages in the northeastern low-density tension zone. The slightly higher temperature in which the experiment took place, and the marginally lower precipitation as compared to the preceding 30 years of climate data at the site indicates that caution should be exercised in interpreting these results.

2.4.1 Density dependence at different life-history stages

2.4.1.1 Seedling establishment

This study has identified negative density-dependence acting at the establishment stage approaching the highest experimental sowing density treatments. It is unlikely that such high-density seed rain would be experienced in the field (pers. obs.); whether density-dependent effects would be realised in natural populations requires further examination. However, under the conditions of this study, negative density-dependence has significantly depressed population sizes and seedling establishment with the most noticeable effects occurring at sites that received 1000 seeds at the beginning of the study.

In the simulation work of Dorken and Pannell (2007), colony establishment rates governed the dynamics of the contact zone by allowing each lineage to colonise unoccupied areas of the metapopulation. Thus, local extinctions and seed dispersal were the key processes governing the maintenance of the contact zone through their effects on establishment dynamics. In the northeastern contact zone considered in this study, seedling emergence was equally negatively density-dependent in both lineages of *M. annua* (Table 2.1), while the diploid lineage exhibited greater establishment overall, which will be an important determinant of the invasion success of this lineage. Hence, for diploid and hexaploid populations at the contact zone delivering high amounts of seed to suitable empty habitat, the diploids overall would exhibit greater establishment success into the following generation, and should further promote the southerly movement of this contact zone.

These patterns were realised in spite of higher than average temperatures in the region. Durand (1963) initially posited that the hexaploid lineage would be better adapted to the supposed higher temperature of the study region relative to the native diploid range further north. However, it was later shown that the diploid lineage performed better in reciprocal transplant experiments throughout this area (Buggs 2007). This study provides evidence that this fitness advantage of the diploid lineage will be maintained regardless of temperature fluctuations that may be associated with climate change. Density-independent factors (e.g. weather conditions or disturbance) have often been implicated in determining population sizes. The area under study on the periphery of the northeast contact zone seems to limit the persistence of species by offering habitat that is largely unsuitable for the persistence of either *M. annua* lineage, and precluding dispersal into the region. The presence of ecological divergence and tension zone dynamics are not mutually exclusive; both can affect the long-term stability of hybrid zones (e.g. Kruuk et al. 1999).

It was observed that population sizes declined into year three (Table 2.12), with less than 10% of sites emerging across all sowing treatments (Table 2.16a). Patterns of emergence across sites in year three were positively related to the number of plants emerging in year two and thus, to the initial sowing density treatments. Both initial year zero sowing density and year two population size had similar predictive power on year three population size. This result shows that although high levels of seed received at a site may sustain large population sizes up to at least 3 years into the future, patterns of population decline across a landscape will be realised across all population starting sizes. This effect can be likened to the asymptotic population density described in Yoda et al. (1963) that occurs after population thinning at high densities, which itself falls as time passes.

Given previous observations of *M. annua* population densities in this region and per capita rates of seed production (Eppley and Pannell 2007), it is unlikely that the amount of seed rain received in the vicinity of this study area will be sufficient to sustain long-term population persistence. Projecting from Table 2.12, it emerges that seed rain greater than the experimental densities in this study will experience significant negative density-dependence that will limit establishment success, yielding negative returns for population growth. Furthermore, such high densities of seed rain (in excess of 1000 seeds for $0.08m^2$) have not been observed in natural populations (pers. obs.). Previous work has identified strong negative density-dependence in experimental field populations of *M. annua* (Moore and Pannell unpubl. ms.). However, in the region under study, it is unlikely that densities much higher than those obtained in this study would occur naturally. Thus, it is unlikely that the conditions for negatively density dependent growth will be realised.

In the final year of study, *M. annua* seedling emergence tended to be higher at sites that had greater vegetation cover. This contrasts to the work of Rabinowitz (1985), which found no influence of the environment/competitors on colonisation ability or establishment potential, thereby substantiating a non-interactive portrayal of colonisation. In this study, the apparent facultative effect of other plant species (which varied largely in their composition between sites) on the emergence of *M. annua* seedlings needs further characterisation in terms of the mechanisms of action. Possibilities include, but are not limited to, effects on soil moisture retention (Breshears and Barnes 1999) and hormonal mechanisms (Baskin and Baskin 2004). The percentage of cover occurring at a site may also stand as an indication of general site quality. However, percentage cover did not explain whether plants were likely to occur at a site at all (extinction), but rather, only accounted for variation in numbers of plants occurring at a site. Neither was any significant spatial autocorrelation in site quality observed in the study that may have been used to explain variation in cover.

Negative density-dependence in establishment has been demonstrated in several previous studies that have similarly focused on plants that were capable of postponing establishment to the following season (Palmblad 1968, Ross and Harper 1972, Linhart 1976, Bergelson and Perry 1989, Murray 1998, Goldberg et al. 2001, Lortie and Turkington 2002, Grundy et al. 2003, Kluth and Bruelheide 2005, Li et al. 2005, Turkington et al. 2005). For these plants, there is an advantage of not germinating under competitive conditions in the first year, because they may possibly exploit more favourable conditions in later years. A means by which severe competition can be avoided both at the establishment stage and throughout the life of a plant is irrefutably of great selective value (see discussion section entitled "Seed bank emergence" below).

The presence of seedlings at high densities was found to inhibit subsequent germination of desert annuals in a manner similar to that of *M. annua* (Inouye 1980). Dyer et al. (2000) found similar density-dependent inhibition in five of seven species of annual grasses native to the Mediterranean. Other studies have reported varying effects of high seed densities on germination (negative, Palmblad 1968, Murray 1998; positive, Linhart 1976) while some have reported no effect (McMurray et al. 1997, Murray 1998). However, these studies have been largely conducted under highly controlled laboratory conditions. Based on his own tests of 11 species and on tests of 11 other species reported in the literature, Linhart (1976) found that positive density-dependent responses were often a characteristic of species of closed habitats, while neutral or negative responses occurred primarily in weedy species of open habitats. This observation is in line with the open disturbed habitat requirements of *M. annua,* in which negative density-dependent responses have now been observed.

In this case, it is difficult to discern the specific mechanism responsible for negative density-dependent establishment, and whether or not a response is elicited by the presence of seedlings or the presence of other seeds. The mechanism(s) of density dependence are unclear from several previous studies conducted in other species (e.g. Matos et al. 1999 study of *Euterpe edulis*), but it is thought to result from both the presence of high densities of seedlings and adults. A number of factors result in seedlings being suppressed in the immediate vicinity of adult plants (Hutchings 1997), including shade cast by the adult plant, root competition, specialist insect herbivores and pathogens associated with the canopy overhead and intraspecific competition from high densities of seedlings. In this analysis, the effects of high densities of seeds on the potential for seedling establishment, and their subsequent survival and growth, have been disentangled. Earlier work in desert annuals has suggested that decreased levels of germination were a response by seeds to the presence of seedlings, rather than an active inhibition of germination by seedlings. When in direct competition for water or nutrients, newly germinated seedlings are likely to suffer much greater reductions in fitness than are established individuals (Ford 1975). It is also unlikely that annual seedlings, faced with extremes of temperature and moisture and a potentially short growing season (e.g., Tevis 1958), would divert metabolic energy to production of germination inhibitors. The implications of this behaviour are such that population size may be regulated at a maximum density, beyond which subsequent seed rain will have diminishing returns in terms of population size.

2.4.1.2 Plant size and seed production

Plant size was not influenced by the seed sowing density, but decreased with the number of plants occurring at that site. In contrast to the supposed facultative effects of neighbouring plants on population germination rates, this observation is indicative of resource-based competition acting at the seedling-adult stage. This finding was in common with both lineages of *M. annua*. The fact that plant sizes were only marginally dependent on population density indicates either that the densities at which resource competition manifests itself as reduced plant growth were not entirely reached in this study, and/or the effects of competition are realised only during the germination phase of plant growth.

This study corroborates earlier work in our lab (Hesse unpubl. ms.) that has identified density-independent seed production in the monoecious hexaploid lineage, and positively density-dependent seed production in the diploid lineage. Several other studies have also shown that populations of plants are regulated via density-dependent effects on seed production (Jarry et al. 1995, Buckley et al. 2001). In this study, it was expected that positive density-dependence would limit seed set only for diploid populations because females cannot self-fertilise, while hermaphrodite hexaploids can. Accordingly, it was observed that plants of the hexaploid lineage maintained higher seed set than diploids at low densities, with the component Allee effect being stronger in the diploid lineage (Figure 2.8; Table 2.4). Furthermore, seed production was not associated with plant size in either lineage. In outcrossing species, mating success is often positively density-dependent (Ashman 2004, Stehlik 2006). Table 2.11 shows that population establishment by the obligate outcrossing diploid lineage has a tendency to fail at the low-density 1- and 10-seed sowings (i.e., density will decrease and populations will be driven to extinction (cf. Pannell and Barrett 1998)). This threshold does not apply to self-fertile hexaploids, however, as putative selfing has allowed seed production in the absence of mates. This result supports the theoretical analysis of Pannell and Barrett (1998), in which the regional persistence of outcrossing species requires greater levels of seed dispersal than does that of self-fertile hermaphrodites. Additionally, this difference in dispersal requirements increases rapidly as regional habitat occupancy rates fall. In the dynamic landscape of this northeastern contact zone, it may be posited that the hexaploid selfers would better persist at lower densities and occupancy rates than the diploid obligate outcrossers. As the contact zone moves towards this low-density study area, the threshold occupancy rates required to maintain the diploid lineage may not be available (cf. Pannell and Barrett 1998).

These results are consistent with the simulation results of Dorken and Pannell (2008) where the self-fertilisation of hermaphrodites led to the exclusion of unisexuals under both low dispersal rates and low population densities (i.e. two or fewer seed immigrants per generation). This trend occurred because low dispersal and population densities reduced the probability that females would acquire compatible mates in newly colonised populations. The low-density sites in this study revealed greater initial population growth of the hexaploid lineage, presumably due to their reproductive assurance as evidenced by greater seed set. This result further corroborates the work of Pannell and Barrett (1998), which showed that the advantage of reproductive assurance increased with the extinction rate and decreased with the density of occupied sites. However, the benefits of reproductive assurance at low density need to be weighed against the degree of inbreeding depression that accompanies self-fertilisation. Levels of inbreeding depression in hexaploid populations of *M. annua* however have been identified as being relatively low at the latitude under study and should not outweigh the benefits of reproductive assurance (Pujol et al. 2009).

In Dorken and Pannell (2008), dispersal via pollen promoted the maintenance of the diploid lineage within their simulations by allowing lone females to produce seeds, thereby reducing the advantage of reproductive assurance possessed by self-fertile hermaphrodites. However, this study reveals that it is unlikely for diploid populations to reach high occupancy rates in this area, given the negative annual growth rates and general tendency towards decline. Under these circumstances, it is unlikely that diploid dispersal via pollen will be sufficiently strong to preclude hermaphrodite persistence in the area via pollen swamping.

In only a few earlier studies has it been possible to relate the density of seeds sown to the number of seeds produced per individual. While positive density-dependence has received less attention than negative density-dependence, it is increasingly being recognised as an important force in plant population dynamics (Courchamp et al. 1999, Stephens et al. 1999, Goldberg et al. 2001, Blundell and Peart 2004, Morgan et al. 2005). For positive density-dependence, as this study has documented in the seed production of diploid individuals, to lead to demographic Allee effects, it must be strong enough to override any effects of negative density-dependence. There was no manifestation of negative density-dependence in seed production in either of the lineages, and thus, annual growth of a population will be limited by the availability of suitable microsites for germination than by the ability to produce seeds at high density. At low-density pioneer sites of this contact zone, the observed advantage of reproductive assurance in the hexaploid lineage should support northward population expansion.

2.4.1.3 Seed bank emergence

Across the 840 study sites, 8% of sites delayed emergence into year two, and 1.5% delayed emergence into year three. Of the 72 sites that went extinct from year one into year two, 17 were re-established from the seed bank in year three. These recoveries point to the likely importance of a seed bank for the persistence of *M. annua* populations in the northeastern contact zone.

Disturbance at a site leading to extinction in one year can be reversed by emergence from the dormant seed bank in the next year after, assuming the disturbance event was aboveground only. It was identified that the probability of regeneration at such a site will be positively related to the number of plants occurring at that site prior to the disturbance event, but negatively related to the density at which seeds occur in the seed bank because of negatively density-dependent establishment.

The proportion of plants emerging from the seed bank was negatively related to the density at which seeds were initially sown. That is, experimental sites receiving a higher number seeds in year zero were likely to have a greater proportion of their seeds delaying germination by one year. This finding is consistent with the negative density-dependence of year one establishment, and also indicates that seeds set aside in the seed bank are not necessarily programmed for emergence the following year. However, this observation is at odds with earlier studies where dormancy in the first season was followed by germination in the following season (Lortie and Turkington 2002, Kluth and Bruelheide 2005) or where plants have exhibited accelerated seedling emergence in competitive neighbourhoods, i.e. positive density-dependence (Dyer et al. 2000). In both the region towards which the contact zone is moving and the heavily disturbed ruderal habitat with which *M. annua* is typically associated, germination from the seed bank will be an important force in buffering the long-term persistence of populations through the recolonisation of sites that are apparently extinct aboveground.

Seed dormancy is expected to act in a manner similar to density-dependent germination to limit population growth and buffer against annual variation in population size. Dormant seeds that remain viable in the soil for more than one season are a common feature of plant life cycles (Leck et al. 1989) that are favoured by natural selection when conditions for survival and reproduction are variable (Cohen 1966). The inhibition of germination has the effect of spreading germination of seeds in the seed bank over a greater period of time, thereby reducing the potential loss of seeds in the event of drastic environmental deterioration. Under metapopulation conditions of frequency disturbance and population turnover, such an ability would be very important in ensuring the persistence of populations over the long term, as has been observed in this study.

Theoretical models have shown that a major possibility to spread extinction risk in unpredictably varying environments is to postpone germination to more favourable years

(Cohen 1966, Venable and Lawlor 1980, Valleriani 2005). However, remaining dormant in seasons that would have been suitable for reproduction also entails a cost in terms of decreased reproductive potential in terms of lost opportunities. Likewise, seeds that delay germination are subject to predation by ant and rodent granivores; these predators have been shown to have noticeable effects on the density and composition of a community of annuals in the Sonoran desert (Inouye 2005).

Seed dormancy is expected to occur in annual ruderal species, such as *M. annua*, that frequently colonise disturbed habitats, where dormancy will offer a higher tolerance of populations to habitat deterioration. The observation of viable soil seed populations that persist over at least one season in this study of *M. annua* confirms the limitations of the definition of population extinction and patch occupancy in plants (Husband and Barrett 1996, Freckleton and Watkinson 2002). Rates of extinction and colonisation are a central part of most metapopulation models (Harrison 1991) and have been instrumental in accounting for ploidy variation in *M. annua* (Pannell 1997). The existence of a seed bank in *M. annua* indicates that apparent local extinctions cannot be classified with certainty as complete local extinctions, or whether apparent recolonisation events involve recolonisation per se or simply regeneration from the seed bank. However, in this study, both lineages of *M. annua* were equally likely to develop dormant seeds, and as such, a temporary seed bank does not skew the applicability of the metapopulation model to understanding sexual system variation in this species. However, the possibility of persistent and sizable banks of hexaploid seeds occupying the northern limit of the hexaploid range could well impede the progress of diploids into this area, through increased rates of hybridisation upon their emergence. This effect would depend on the frequency of seed rain into the area, in addition to the density at which diploid populations are able to obtain, as this would dictate the potential for diploid pollen swamping and hybridisation to occur at the contact zone.

If a sufficient density of individuals has already established on a site, it may be advantageous for seeds to remain dormant, rather than germinate and risk a reduction in growth, survival and fecundity through competition with larger, older plants. Inouye

(1980) describes two criteria that must be met for this type of response to be selected: 1- dormancy must be a viable alternative and 2- the cost of delaying growth and reproduction must be less than the probable reduction in fitness resulting from a high density of competitors. The potential for germination to be delayed across seasons was identified in *M. annua* in this study. For annual species without a persistent bank of dormant seeds (see Watkinson 1981) a deterministic model of the type described by Watkinson (1980) may be sufficient in describing population growth (see Jefferieset al. 1981, Watkinson and Harper 1978). However, these models must be modified for species with a seed bank to account for any buffering effect on population extinction rates. Furthermore, previous models for annual plant populations, which have allowed for a seed bank (Cohen 1966), have not taken into account density dependent processes that are known to be significant both in seed production and seedling self thinning (Macdonald and Watkinson 1981). Such density-dependent recruitment should buffer populations against year-to-year variations in emergence rates by imposing an annual maximum limit on emergence. The presence of a seed bank should further reduce the amplitude of fluctuations about an equilibrium population size by exhibiting a similar damping (stabilising) effect.

Stabilising effects on plant population size from one year to the next were also observed through the effects of perennation. There were two sites in the study where individuals persisted through the winter period and increased substantially in size and biomass. MacDonald (1976) has noted that a population with non-overlapping generations may undergo a significant change in behaviour when a small fraction of each generation is held over to the next. Using a simple deterministic model, he found that a small carry-over of individuals from generation to generation had a tendency to stabilise the equilibrium point. Therefore, the action of a seed bank, density-dependence at the germination stage and the instances of perennation observed in *M. annua* should stabilise the gradual decline of populations towards extinction in this contact zone area, thus preventing chaotic oscillatory population size fluctuations and damping them back to equilibrium following a disturbance.

2.4.1.4 Effects of density on growth and extinction rates

A demographic Allee effect was observed in the relationship between both the seed production of a site and the number of seeds sown, on the population growth rate the subsequent year (Table 2.5b). Both of these components were themselves observed to be density-dependent, with germination rates decreasing and seed production increasing with density (Tables 2.1 and 2.4 respectively). These results further demonstrate that for component Allee effects to lead to demographic Allee effects (density-dependent changes in population level growth rate), they must occur at densities lower than that where competitive interactions occur. Population densities were consistently low across the experimental sites in this study, therefore offering scope to observe density-dependent regulation under field conditions.

Models for population growth in a limited environment are based on two fundamental premises: that populations have the potential to increase exponentially and that density dependent feedback progressively reduces the actual rate of increase (Hassell 1975). These requirements were met by the *M. annua* populations of this study, with population growth rates initially being positively related to sowing density treatment and seed production the preceding year, followed by negatively density-dependent growth (in related to population size the preceding year). The growth rates observed in the final year of the study were unrelated to both the initial year zero sowing density treatments and the population sizes of the preceding year, thus indicating a low predictive power for long-term population persistence in this study area. However, apparent extinctions were negatively related to population size in preceding years.

The analyses reveal a clear positive influence of the seed productivity of a site (Table 2.9a) and a similar influence of the number of seeds produced by individual plants (Table 2.9c) on the persistence (inverse of extinction) of a population into the following year. However, the results also indicate that a high amount of seed rain arriving at a site does not automatically lead sustained year-on-year population growth and is subject to the control of density-dependent processes acting at different directions at different life-history stages. Although population growth rate was positively influenced by the log number of seeds sown in year one, sites with greater numbers of plants emerging in year one had lower growth rates into year two, than sites with less plants emerging. These processes acted independently of site quality, as assessed using the surrogate of plant size. This finding is consistent with the work of Rabinowitz (1985) that found remarkably few differences in population levels or survival between the disturbance/resources and the undisturbed control, and the origins and disappearances of the plants seemed largely independent of resource availability. This study found that the long-term persistence of populations will be favoured if sites receive/produce amounts of seed at a density that is sufficiently high to allow successful establishment (and the induction of dormancy at sites likely to receive disturbance), but not so high as to preclude establishment in future years via negative density dependent effects on seedling survival and seed production.

The extinction rate of populations into year two was inversely related to the number of seeds produced per plant the preceding year, and the number of plants emerging in year one. That is, larger populations that produced more seeds were more likely to persist into the next year. However, this relationship between population size and extinction rate was not maintained into the following year. The extinction rates did not differ between the two lineages, regardless of whether seed bank emergence was accounted for. Although negative density dependent processes impacted the growth rate of populations, in terms of the numbers of plants emerging year-on-year, these same processes did not cause population crashes or apparent extinction at the densities considered in this study.

The observations of population dynamics at these densities are consistent with models of short-lived plants that have ephemeral, pulsed dynamics lasting only a single generation, with recruitment determined almost entirely by germination biology and by the frequency and intensity of disturbance (see Crawley and Ross 1990 for review). The degree of competition between conspecifics in the highest density sites of this study was not sufficiently detrimental to seed production so as to prevent population establishment in the following year. Neither was it observed that mortality in the highest density sites led to proportionally fewer survivors than from intermediate sowing densities, e.g. *Glycine* *soja* (Yoda et al. 1963). The scenario in this *M. annua* experimental metapopulation resembles contest competition, wherein resources are partitioned unequally between individuals, such that a maximum population density may be attained and will thus not lead to a population crash the following year. Extinction rates were not the result of population crashes observed in scramble competition occurring at highest densities where resources are equally partitioned.

The collapse of populations of widely spread plant species has been often observed but rarely explained. The review of Simberloff and Gibbons (2004) documents the most well-known cases of non-indigenous plant species that are initially in the 'increase phase' of an invasion and then begin to undergo apparently spontaneous decline, and their meta-analyses reveals few documented explanations for these declines, the few available of which were ad hoc suggestions lacking supporting evidence. A well-known example of a population crash is found in that of *Elodea canadensis* that was widespread across Europe as a problematic weed in the mid 20th century, but has since declined to a point of rarity for no understood reason (Simpson 1984, Andersson and Willén 1999, Pysek et al. 2002). Earlier work in our lab has implicated the rust *Melosperma pulcherrima* as a commonly occurring detriment to the health of *M. annua* plants (Buggs 2004). However, its occurrence was rare at field site of this study. The observed pattern of decline overarching the density-dependent effects on population persistence in this study supports the view of Buggs (2004) that this specific area represents a low-density trough, generally inhospitable for populations of *M. annua*, and is likely to impede movement of the tension hybrid zone.

2.4.2 Implications for contact zone movement

2.4.2.1 Differential adaptation to low-density trough

Higher germination rates for the diploid lineage were observed in both the greenhouse and field, with the diploid plants consistently larger than the hexaploid lineage (cf. Harris and Pannell 2008). These size differences may be attributed to sexual specialisation of the diploid lineage that allows for more efficient allocation of resources to plant growth (e.g. Charnov et al. 1976, Bawa 1980, Brunet 1992). As a comparable example, the reciprocal transplant experiments of Costich (1995) found dioecious *Ecballium dioicum* to be better suited to the environment under study than *E. elaterium*, and suggested that this was due to a greater control of resources by gender-differentiated plants. Lower fitness in the hexaploid *M. annua* lineage could also be an indirect effect of their sexual system if it is causing them to be adapted to a metapopulation structure with rapid turnover of local populations, where the lineage experiences a trade-off between colonising ability and its potential to compete with the diploids (cf. Jakobsson and Eriksson 2003). Perhaps in the dynamics of natural populations, the superior colonising ability of the hexaploid lineage outweighs the reduced vegetative growth (Buggs 2004, pg. 134).

It was found that the diploid lineage was pre-adapted to the region it is currently invading in terms of higher initial establishment success relative to the performance in the field of the hexaploid lineage. However, such superiority was maintained only above a threshold density, which was higher than that required by the hexaploids. This observation is in contrast to earlier work (Buggs 2004), which determined that germination rates should not differ between the diploid and hexaploid lineages found close to the northeast contact zone, where the samples in this study were obtained (unlike the northwest contact zone). This is indicative of a broader tolerance to environmental conditions of the diploid populations sampled here, suggesting that for the same amount seeds arriving at a site, diploids would have a higher probability of establishment than hexaploids. It is expected that the higher initial germination rates of the obligate outcrossing diploid lineage in this zone should compensate for their reduced seed production at low densities. Despite the fact that seed production in hexaploid populations is virtually density-independent, their advantage of reproductive assurance will be offset by the ecological suitability of diploid seeds to this area. However, both lineages declined throughout the duration of the study, with similar growth rates year-on-year, and without differences in terms of the proportion of seeds allocated to the seed bank, or in the rate of emergence from the seed bank. This result suggests that the rate of decline of the diploid populations should be offset by their greater germination rates in the region that they are currently invading.

Higher population densities within a region should promote the local maintenance of dimorphic sexual systems by increasing the probability of successful immigration by unisexual plants (Pannell 1997, Dorken and Pannell 2007, Eppley and Pannell 2007). The previously observed larger population sizes of diploids (see Eppley and Pannell 2007) should theoretically lead to larger amounts of seed arriving at sites in the south of the diploid range towards the northeast tension zone, and initially denser establishment of diploid populations in this region than the hexaploids. It is expected that this will be followed by population decline in a fashion similar to that observed in this experimental study.

In order to determine whether the *M. annua* tension hybrid zone will continue its current movement trajectory through the hexaploid range, the amount of diploid seed rain requires quantification. It must be determined whether diploid populations will be able to establish at a density that is sufficiently high to enable them to pass through the unsuitable habitat of this low-density trough of the study area. However, in order for this to happen, the seed rain should not be so dense as to be opposed by negative density dependent effects at the germination stage. Considering the low frequency at which populations were observed in this region (pers. obs.), it is likely that diploid populations will pass through this region unobstructed by hexaploids. However the rate of movement may decrease due to the low establishment rates. Upon reaching the androdioecious populations south of this low-density trough, the rate of movement should return to at least its current accelerated rate. The presence of androdioecy should accelerate movement because of the decreased advantage of reproductive assurance relative to the monoecious hexaploids (Buggs 2007). However, it remains to be seen whether androdioecious populations exhibit similar density-dependent behaviour at different stages in the population life cycle relative to monoecious hexaploids.

A clear influence of the seed productivity of a site on its probability of persistence/occupation in the following year was identified at the *M. annua* hybrid zone, as was the existence of a persistent soil seed bank that buffers populations from extinction. The ecophysiological adaptation of the diploids to the region combined with the higher plant sizes obtained by this lineage, should facilitate its displacement of the hexaploids and allow diploids to reach higher population densities, assuming dispersed seeds reach suitable microsites for germination. In general, however, the low suitability of the area will prevent populations from reaching large sizes and will thus minimise contact between the lineages, acting as a barrier to gene flow and trapping the contact zone in the low-density trough it currently occupies. It would thus seem that the hybrid sink effect is not the single most important factor in reinforcing the barrier to gene flow between the diploid and hexaploid lineages of *M. annua*, as gene flow into the less fit population may be counteracted by flow into less dense and unsuitable regions.

2.4.2.2 Dispersal areas and distances

The absence of a significant relationship between lineage, or sowing density on dispersal area and distance, indicate that dispersal dynamics are highly stochastic in *M. annua* and are primarily influenced by exogenous factors. Dorken and Pannell (2008) found in a simulation that under lower rates of seed dispersal (production), the dioecious lineage was often excluded from the metapopulation altogether unless it occupied areas with low extinction rates. However, under higher dispersal rates, males and females were more likely to disperse into populations together and thereby prevent the hermaphroditic lineage from establishing in the same site. The results of this study indicate that dispersal in both lineages will be influenced by similar abiotic processes, although the diploid lineage will experience a higher rate of establishment or germination upon arriving at a site.

Dispersal area was positively influenced by the number of plants present at a site at time of area measurement (year two). Dispersal area was not influenced by habitat suitability the preceding year, or seed production (per plant or per population) the preceding year. Given that diploid populations currently reside north of the contact zone at higher densities than hexaploids south of the zone (Eppley and Pannell 2008), we can anticipate a higher influx of diploid seed into the area, biasing hybridisation rates in favour of the diploids. The disturbance gradient in the simulation of Dorken and Pannell (2008) affected the strength of selection against dispersers into a site by moderating the probability that they would hybridise. The low establishment rates observed in this study would indicate that the formation of mixed populations, and thus the occurrence of hybridisation, should be relatively infrequent.

The dispersal distance did not differ according to the seed production of sites nor the number of plants germinating in the previous year. There was a significant influence of the logarithm of the number of seeds initially sown at a site on the dispersal distance obtained in year two (coefficient ± s.e. = 0.14 ± 2.41). This finding implies that sites that receive a greater number of seeds will obtain larger spreads the following growing season, irrespective of lineage. These results contrast with the seed shadow hypothesis (Heilbuth 2001) that predicts dimorphic species to have a smaller spatial spread after seed dispersal, given that they have half the number of individuals dispersing seeds as monoecious lineages. The diploid lineage is expected to have a colonisation disadvantage for access to unoccupied sites at the landscape level relative to the self-fertile hexaploids. However this should be offset by their higher germination rates, at least considering the populations near the northeastern contact zone from which seed collections were obtained. This advantage has not previously been taken into account when modeling the ability of diploid lineages to compete with hermaphrodites.

The effects of density on gene flow by seed dispersal are not well understood. Where seed movements are influenced by wind gusts, as might occur in *M. annua* (pers. obs.), general principles similar to those for pollen dispersal probably apply: increased density may result in decreased dispersal because of reduced wind-speed, increased probability of impaction, or decreased plant size (Watkinson 1980). Little is known about the quantitative aspects of seed dispersal in plants, and even less about how such dispersal distances are related to density (Antonovics and Levin 1980). If seedlings were to be more widely dispersed by the diploids, such dispersal could compound the problem of scarcity of mating partners at low density. The lack of a difference between lineages in terms of maximum population spread or dispersal distance indicates that selection for increased dispersal capabilities for the benefits of occupying new habitat and avoiding competition is not opposed by selection for increased population density and availability of conspecific mating partners.

Differences in plant stature are expected to have implications for seed flow because the distance traveled by these units is a function of the height of reproductive structures. The greater the elevation, the greater will be the mean and variance of the dispersal distance, and the longer will be the tail of the distribution (Gregory 1973). Despite this, the significant differences between lineages in plant size did not translate into differences in the maximum dispersal distances attained from the initial sowing lines at the sites under study.

The dispersal distances obtained in the study sites do not approach the average 1.6 km of movement per year concluded in the Buggs (2007) study, thus implicating a possible role for anthropogenically-mediated chance dispersal events that facilitate a more rapid rate of colonisation. Yet, while it is difficult to quantify such chance events occurring at the study site given their stochastic nature, *M. annua* emerged from what appeared to be newly deposited rubbish and construction heaps. Thus, such anthropogenically-mediated dispersal events are likely to be an important factor in positioning this hybrid zone.

2.4.3 Conclusion

The central aim of this project was to identify the factors regulating tension zone dynamics in *M. annua* by quantifying the underlying influences of density-dependence and the sexual system at different stages of the population life cycle. Population dynamics in the diploid and hexaploid lineages are regulated by density dependence in individual germination, seed production, and emergence from the seed bank, with component Allee effects translating to observable changes in population growth rates.

The population declines observed at the northeast *M. annua* diploid-hexaploid contact zone are similar in both lineages, and are influenced by both density-dependent processes acting at different life-history stages and the density-independent influences of habitat suitability. It is apparent that populations entering this area will be subjected to the concomitant pressures of a low-density trough in which neither lineage is likely to persist at a high density. The low frequency of establishment has implications for the landscape-level colonisation ability of the lineages, and thus the frequency of contact/interaction of lineages. The low suitability of the area is likely to be more important in its influence in mediating contact zone movement than the rare hybridisation events that have been described (Buggs 2007). If the seed rain of the diploid populations is sufficiently high to allow the lineage to pass through this low-density trough, a greater influence of hybridisation related processes in mediating further movement will be observed as the populations reach higher density hexaploid populations towards the south of the Iberian Peninsula.

Considering the simulation of Dorken and Pannell (2007), selection was reduced at higher extinction rates because of the greater probability that neighbouring sites were unoccupied. Closer to the boundary between lineages, and thus at lower extinction rates, selection against dispersers was thought to increase because of the greater probability that they would potentially hybridise with the occupants of the site. However, over the course of this three-year study at the northeast contact zone, extinction rates were high, while growth rates were low in an area that is soon expected to be the interface of both lineages. This result indicates a low probability of populations from opposing lineages encountering each other in this low-density trough, and should thus serve to facilitate the spread of the diploid populations along their current southwards movement trajectory.

The *M. annua* reciprocal transplant experiments conducted in this region (Buggs 2004) indicate that the area in the vicinity of the contact zone was considerably less hospitable to the species than more distant environments on either side. This finding was evidenced by lower photosynthetic rates, shorter height and lower biomass in the contact zone (Buggs 2004). The low establishment rates in this study also fit with surveys of the frequency of natural populations of *M. annua*, which are rare in this area (Eppley and Pannell 2007), likely due to the elevated topology of the region and narrower coastal strip that supports more schlerophyllous vegetation (CORINE 1993). This region of poor habitat for both lineages is likely one location where the tension zone may become trapped, and may accordingly counteract cline movement (Barton 1979, Barton and Hewitt 1985).

As Strong (1983) has illustrated, density dependence is sometimes operating and sometimes not, and is often circumvented by other forces, depending on the organism and local circumstances. The experimental manipulation of density in this study has allowed for a precise assessment of density-dependent regulation, although Royama (1977) has emphasised that statistical density-dependence does not imply causal density-dependence. Thus, the role of density-independent environmental effects of habitat suitability on population persistence cannot be neglected. Density-dependent processes are easily displayed in experimental artificial plant populations but have rarely been demonstrated in natural populations (Watkinson and Harper 1978). Antonovics and Levin (1980) further point out that one can invoke density-dependence in more crowded sites and micro-environmental differences in the less-dense sites, but one cannot eliminate either alternative. By replicating the study across a large landscape and high number of sites and by incorporating random site effects into the statistical models, it has been possible to account for microenvironmental variation in the interpretation density-dependent effects, thereby linking the clearly identified density-dependent effects of laboratory experiments (Harper 1977) to the field.

While a range of studies have explored the consequences of spatial structure for the dynamics of plant species (see Clark and Ji 1995), few studies has examined in detail how such processes affect predictions of population structure and population numbers under field conditions. Silvertown and colleagues (Silvertown et al. 1993, 1996, Franco and Silvertown 1996) have made a start on using existing studies of population dynamics to assess the importance of different life-history stages, however otherwise studies have rarely taken into explicit account different kinds of density-dependence at each stage. Empirical work on this problem is probably the most critical gap in our understanding for the further development of a comprehensive theory of community structure in plants.

2.5 TABLES AND FIGURES

Table 2.1 Minimum adequate model GLMM on year one germination rates of experimental sowings of diploid and hexaploid *M. annua* at the northeastern Spain contact zone.

Minimum Adequate Model Parameters	Coefficients ± s.e.	χ^2	AIC
Intercept	-3.984±4.755e-1		Null = 6010
Sowing Density	-4.999e-04 ±4.402e-5	$\chi^2 = 123.891$, $p < 0.001$	Sowing Density = 4080
Sexual System	9.828e-01±4.167e-2	$\chi^2 = 605.760$, $p < 0.001$	Sexual System = 3598
			Full Model = 3476
Random effect of site: Var. = 4.131, Res. Var. = 48.210			

Table 2.2 Minimum adequate model LMM on plant height of experimental populations of diploid and hexaploid *M. annua* at the northeastern contact zone.

Minimum Adequate Model Parameters	Coefficients ± s.e.	χ^2	AIC
Intercept	16.203±0.850		Null = 1077
Sexual System	5.570±1.116	$\chi^2 = 23.525$, $p < 0.001$	Sexual System = 1056
No. Plants per Site	-0.028±0.016	$\chi^2 = 3.289$, $p = 0.070$	No. Plants per Site = 1057
Sowing Density		$\chi^2 = 1.603$, $p = 0.070$	Full Model = 1057
Random effect of site: Var. = 0.000, Res. Var. = 54.321			

Table 2.3 Minimum adequate model LMM on plant diameter measured in experimental populations of diploid and hexaploid *M. annua* at the northeastern Spain contact zone.

Minimum Adequate Model Parameters	Coefficients ± s.e.	χ^2	AIC
Intercept	9.319 ±0.552		Null = 939
Sexual System	2.511±0.737	$\chi^2 = 11.338$, $p < 0.001$	Sexual System = 929
No. Plants per Site		$\chi^2 = 2.042$, $p = 0.153$	Full Model = 929
Random effect of site: Var. = 0.310, Res. Var. = 20.695			

Table 2.4 Minimum adequate model LMM on number of seeds produced by diploid and hexaploid *M. annua* in experimental populations at the northeastern Spain contact zone.

Minimum Adequate Model Parameters	Coefficients ± s.e.	*Z*	AIC
Intercept	2.533±0.059		Null = 1281
Sexual System (MS)	-0.795±0.079	$Z = -10.092$, $p < 0.001$	Sexual System = 1262
log No. of Plants per Site	0.066±0.023	$Z = 2.933$, $p < 0.010$	No. Plants per Site = 1240
(NP)	0.230±0.030	$Z = 7.711$, $p < 0.001$	Full Model = 1204
Random effect of site: Var. = 4.666, Res. Var. = 33.504			

Table 2.5a Minimum adequate model GLMM on population growth rate of experimental populations of diploid and hexaploid *M. annua* at the northeastern Spain contact zone, as defined by (N_t / N_{t-1}) where N_t refers to the number of plants at a site in year *t.*

Minimum Adequate Model Parameters	Coefficients ± s.e.	χ^2	AIC
Intercept	1.571±0.274		Null = 768
No. of Plants per Site	-0.010±0.006	$\chi^2 = 2.784$, $p = 0.095$	No. Plants per Site = 766
Sexual System		$\chi^2 = 2.648$, $p = 0.103$	Full Model = 766
No. Seeds per Plant		$\chi^2 = 0.500$, $p = 0.480$	
Random effect of site: Var. = 0.231, Res. Var. = 7.291			

Table 2.5b Minimum adequate model LMM on population growth rate of experimental populations of diploid and hexaploid *M. annua* at the northeastern Spain contact zone, as defined by log (N_t / N_{t-1}) where N_t refers to the number of plants at a site in year *t.*

Minimum Adequate Model Parameters	Coefficients ± s.e.	χ^2	AIC
Intercept	0.266±0.130		Null = 140
log No. Seeds Sown	0.023±0.048	$\chi^2 = 21.143$, $p < 0.001$	log No. seeds sown = 120
No. Seeds per Plant	0.017 ± 0.005	$\chi^2 = 12.006$, $p < 0.001$	No. seeds per plant = 110
log. No. Plants per Site	-0.267±0.064	$\chi^2 = 10.944$, $p < 0.001$	Full Model = 95
Sexual System		$\chi^2 = 2.061$ $p = 0.151$	
Random effect of site: Var. = 1.087, Res. Var. = 6.299			

Table 2.6 Minimum adequate model GLMM on emergence from the seed bank in experimental sowings of diploid and hexaploid *M. annua* at the northeastern Spain contact zone.

Minimum Adequate Model Parameters	Coefficients ± s.e.	*Z*	AIC
Intercept	-4.034±0.300		
Sexual System (MS)	0.119±0.111	$Z = 1.069$, $p = 0.28514$	Null = 2531
No. Seeds Sown (NS)	-0.000± 0.000	$Z = -8.484$, $p < 0.001$	
MS*NS	0.000±0.000	$Z = 2.720$, $p < 0.001$	MS*NS = 2412
Random effect of site: Var. = 1.613, Res. Var. = 48.887			

Table 2.7 Minimum adequate model LM on population area (spread) in year two of experimental populations of diploid and hexaploid *M. annua* at the northeastern Spain contact zone.

Minimum Adequate Model Parameters	Coefficients ± s.e.	*F*	Adjusted R-squared
Intercept	11.429 ±0.681		
Height (H)	-0.051±0.053	$F = 2.283$, $p = 0.136$	
Diameter (D)	-0.144±0.091	$F = 2.691$, $p = 0.106$	
No. Seeds Sown	0.001±0.000	$F = 6.097$, $p < 0.05$	No. seeds sown = 0.07069
No. Plants in Year 2	0.010±0.004	$F = 5.442$, $p < 0.05$	No. plants in year 2 = 0.07
(H*D)	0.006±0.003	$F = 5.381$, $p < 0.05$	(H*D) = 0.07216
Sexual System		$F = 0.126$, $p = 0.724$	Full model = 0.2227
No. Plants in Year 1		$F = 0.039$, $p = 0.336$	Adj. R-sq = 0.223

Table 2.8 Minimum adequate LM on population dispersal distance in year two of experimental populations of diploid and hexaploid *M. annua* at the northeastern Spain contact zone.

Minimum Adequate Model Parameters	Coefficients ± s.e.	*F*	Adjusted R-squared
Intercept	4.821±0.251		
log No. Seeds Sown	0.137±2.414	$F = 5.828$, $p < 0.05$	Adj. R-sq = 0.067
No. Seeds per Site		$F = 0.727$, $p = 0.397$	
No. Seeds per Plant		$F = 0.584$, $p = 0.240$	
No. Plants per Site		$F = 1.404$, $p = 0.240$	

Table 2.9a Minimum adequate GLMM on year two extinction probability.

Minimum Adequate Model Parameters	Coefficients ± s.e.	χ^2	AIC
Intercept	0.479±0.229		Null = 222
Total Seeds per Site	-0.002±0.000	$\chi^2 = 15.255$, $p < 0.001$	Total seeds per site = 208
No. Plants per Site		$\chi^2 = 0.133$, $p = 0.715$	Full Model = 208
Sexual System		$\chi^2 = 1.871$, $p = 0.171$	

Random effect of site: Var. = 0.339, Res. Var. = 31.843

Table 2.9b Minimum adequate GLMM on year two extinction probability.

Minimum Adequate Model Parameters	Coefficients ± s.e.	χ^2	AIC
Intercept	0.410±0.223		Null = 222
No. of Plants per Site	-0.020±0.008	$\chi^2 = 10.813$, $p < 0.001$	No. Plants per Site = 213
No. Seeds per Site		$\chi^2 = 0.133$, $p = 0.715$	Full Model = 213
Sexual System		$\chi^2 = 1.871$, $p = 0.171$	

Random effect of site: Var. = 0.154, Res. Var. = 30.980

Table 2.9c Minimum adequate GLMM on year two extinction probability.

Minimum Adequate Model Parameters	Coefficients ± s.e.	χ^2	AIC
Intercept	2.025±0.416		Null = 222
Seeds per Plant	-0.162±0.030	$\chi^2 = 33.723$, $p < 0.001$	Total seeds per site = 175
Sexual System		$\chi^2 = 0.002$, $p = 0.965$	Full Model = 175

Random effect of site: Var. = 0.154, Res. Var. = 30.980

Table 2.10a Minimum adequate GLMM on year three population size.

Minimum Adequate Model Parameters	Coefficients ± s.e.	χ^2	AIC
Intercept	-0.742 ± 0.471		Null = 5843
log Sowing Density	0.004 ± 0.001	$\chi^2 = 78.7$, $p < 0.001$	Sowing Density = 5770
Sexual System	0.820 ± 0.484	$\chi^2 = 5.36$, $p < 0.050$	Sexual System = 5765
Year 2 Pop. Size	0.179 ± 0.018	$\chi^2 = 97.9$, $p < 0.001$	No. Plants in Year 2 = 5669
% Cover	0.016 ± 0.008	$\chi^2 = 4.3635$, $p < 0.001$	% Cover = 5667
Presence of Bank		$\chi^2 = 0.608$, $p = 0.965$	

Random effect of site: Var. 1.220, Res. Var. = 60.160

Table 2.10b Minimum adequate GLMM on year two to three extinction probability.

Minimum Adequate Model Parameters	Coefficients ± s.e.	χ^2	AIC
Intercept	2.670 ± 0.661		Null = 185
log Sowing Density	0.111 ± 0.139	$\chi^2 = 4.74$, $p < 0.001$	log Sowing Density = 178
log Year 2 Pop. Size	-0.821 ± 0.215	$\chi^2 = 16.5$, $p < 0.001$	log Year 2 Pop. Size = 160
Presence of Bank	0.783 ± 0.418	$\chi^2 = 3.79$, $p = 0.052$	Presence of Bank = 158
Year 2 Plant Height	-0.040 ± 0.022	$\chi^2 = 3.20$, $p = 0.070$	Year 2 Plant Height = 157
Sexual System		$\chi^2 = 0.480$, $p = 0.489$	
Random effect of site: Var. 0.0478, Res. Var. = 39.012			

Table 2.11 Mean population size (variance) across years and initial sowing densities (H = hexaploid, D = diploid) in experimental populations of *M. annua* at the diploid-hexaploid contact zone of northeastern Spain.

Population Size	1 H	1 D	10 H	10 D	100 H	100 D	1000 H	1000 D
2009	1	1	2.62 (3.84)	2.75 (4.83)	10.04 (54.32)	19.54 (174.7)	49.33 (1222.61)	110.25 (4044.6)
2010	1.86 (6.64)	0.67 (4)	4.90 (35.59)	3.09 (28.09)	9.35 (154.6)	17.28 (813.2)	40.33 (3922.24)	62.33 (4610)
2011	0	0	1.10 (11.1)	0.0313 (0.0313)	2.13 (104.39)	0.657 (4.76)	1.25 (13.84)	0.25 (0.75)

Table 2.12 Number of plants emerging per year, as a fraction of initial sowing density treatment in experimental populations of *M. annua* at the diploid-hexaploid contact zone of northeastern Spain.

Germination	1 H	1 D	10 H	10 D	100 H	100 D	1000 H	1000 D
2009	0.065 (0.061)	0.045 (0.043)	0.039 (0.014)	0.063 (0.024)	0.039 (0.005)	0.114 (0.020)	0.030 (0.001)	0.066 (0.005)
2010	0.160 (0.638)	0.080 (0.224)	0.087 (0.083)	0.101 (0.084)	0.050 (0.010)	0.121 (0.053)	0.024 (0.003)	0.044 (0.003)
2011	0.745 (30.391)	0.550 (9.485)	0.069 (0.366)	0.211 (1.757)	0.009 (0.004)	0.016 (0.005)	0.001 (9.73e-6)	0.006 (3.97e-4)

Table 2.13 Mean proportion of seeds delaying germination to year two (variance) in experimental populations of *M. annua* at the diploid-hexaploid contact zone of northeastern Spain.

Seed Bank	1	10	100	1000
Hexaploid	0.380 (0.247)	0.472 (0.183)	0.360 (0.185)	0.242 (0.116)
Diploid	0.501 (0.265)	0.384 (0.234)	0.260 (0.170)	0.289 (0.148)

Table 2.14 Mean population surface area (i.e. spread) per seed in m^2 (variance) in experimental populations of *M. annua* at the diploid-hexaploid contact zone of northeastern Spain.

Per Seed Spread	1	10	100	1000
Hexaploid	4.570 (38344)	0.891 (26885)	766 (48)	260 (7)
Diploid	1.197	1.193 (103991)	0.180 (1056)	86 (0)

Table 2.15 Climate data for the period under study, and the preceding 30-year period for the city of Tortosa, located 10km south east of the study populations of *M. annua* at the diploid-hexaploid contact zone of northeastern Spain

	Mean Temperature (ºC)	Maximum Temperature (ºC)	Minimum Temperature (ºC)	Precipitation (mm)	Humidity (%)
1970 – 2010	17.3	22.6	12.1	43.7	63
2008 – 2010	18.6	23.4	12.7	36.4	59.5
2008	18.4	23.0	12.8	48.6	61.6
2009	19.2	24.2	13.2	27.8	58.6
2010	18.1	22.9	12.3	32.8	58.3

Table 2.16a Mean population growth rates for established sites calculated as population size in year *t* + 1 / *t* (range); with n = sample size showing the number of sites that emerged in year *t* + 1 as a fraction of the number of that emerged in year *t*, ignoring colonization from the seed bank.

Growth rate	1 H	1 D	10 H	10 D	100 H	100 D	1000 H	1000 D
2010 / 2009	2 (3-7); n=5/13	0.7 (0.7); n=1/9	2 (0.5-16); n=15/21	1.5 (0.5-13); n=14/32	1.8 (0.3-11.3); n=11/22	1.2 (0.13-10); n=21/35	1.7 (0.1-8.1); n=9/13	0.7 (0.2-3.8); n=9/12
2011 / 2010	0.7 (0.3-3); n=3/5	1 (1); n=1/1	1.6 (4.8-12); n=3/15	0.3 (0.3-1.4); n=3/14	0.3 (0.1-1.2); n=4/11	0.2 (0.1-1.2); n=9/21	0.2(0-1.4); n=3/9	0.6 (0.2-2.4); n=5/9

Table 2.16b Mean population growth rates for established sites calculated as population size in year *t* + 1 / *t* (range); with n = sample size showing the number of sites that emerged in year *t* + 1 as a fraction of the number of that emerged in year *t*, including sites that were re-established from the seed bank.

Growth rate	1 H	1 D	10 H	10 D	100 H	100 D	1000 H	1000 D
2010 / 2009	2 (3-7); n=5/13	0.7 (0.7); n=1/9	2 (0.5-16); n=15/21	1.5 (0.5-13), n=14/32	1.8 (0.3-11.3); n=11/22	1.2 (0.13-10); n=21/35	1.7 (0.1-8.1); n=9/13	0.7 (0.2-3.8); n=9/12
2011 / 2010 incl. bank	0.3 (0.3-3); n=3/13	0.1(1); n=1/11	0.9 (4.8-12); n=3/25	0.5 (0.3-13.8); n=6/33	0.3 (0.1-1.5); n=8/20	0.2 (0.1-1.3); n=11/30	0.13 (0-1.4); n=3/11	0.8 (0.1-2.4); n=7/12

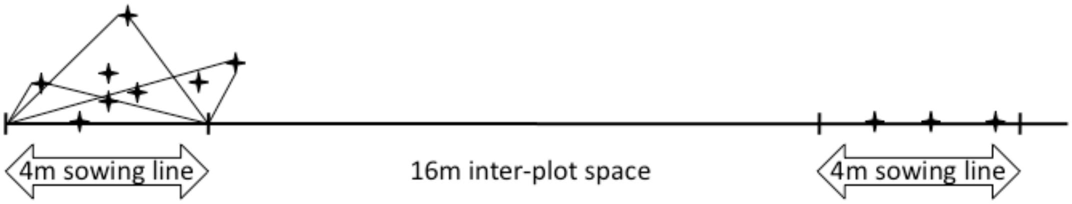

Figure 2.1 Experimental sowing setup, detailing dispersal distance polygon measurements that encapsulate all plants at a site. Stars represent plants. The sowing line on the right is representative of emergence from the seed bank.

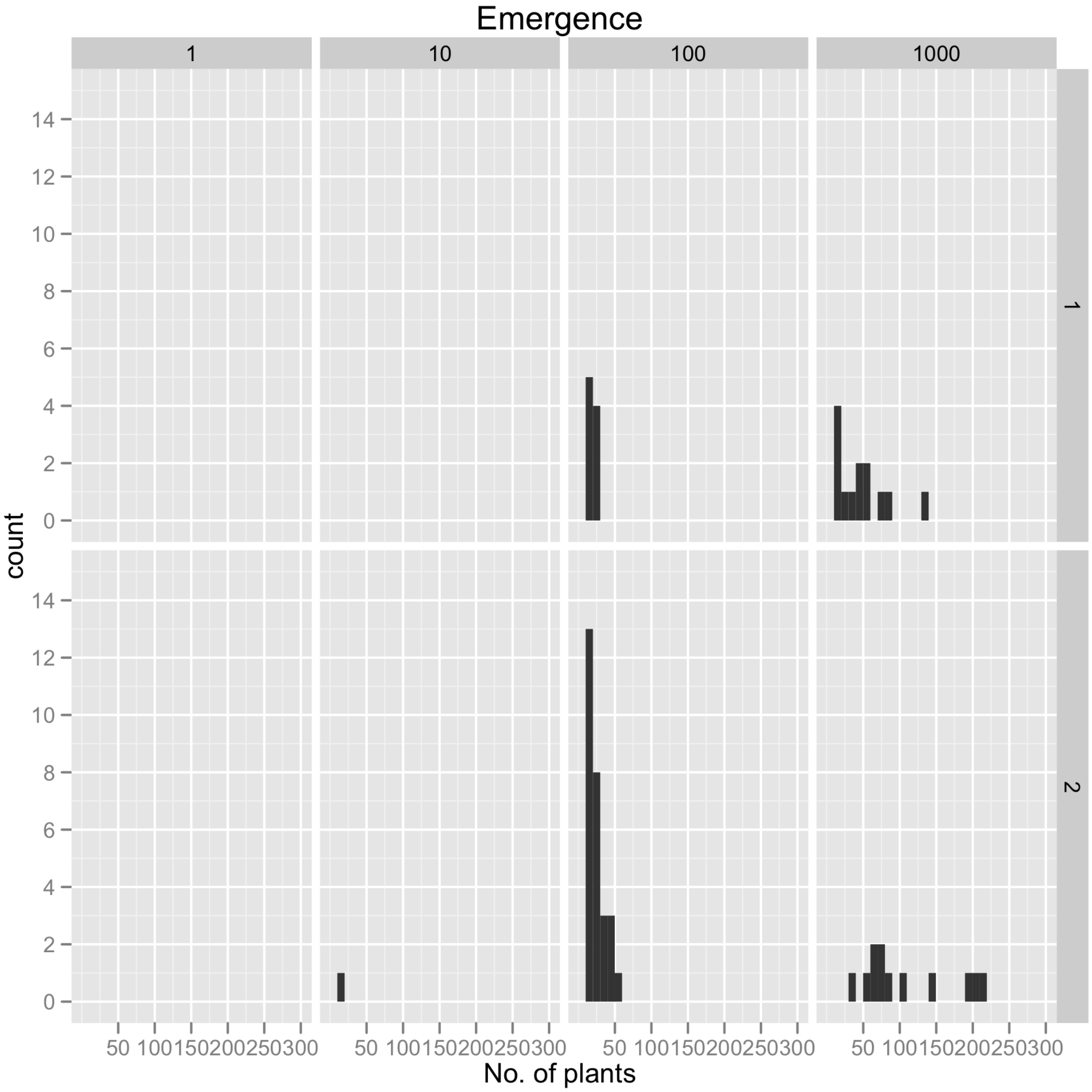

Figure 2.2a Frequency histogram of seedling emergence in year one. Partitions along the x-axis separate year zero sowing density treatments (1, 10, 100, 1000 seeds), and y-axis partitions separate lineages (1 = monoecious-hexaploid, 2 = dioecious-diploid).

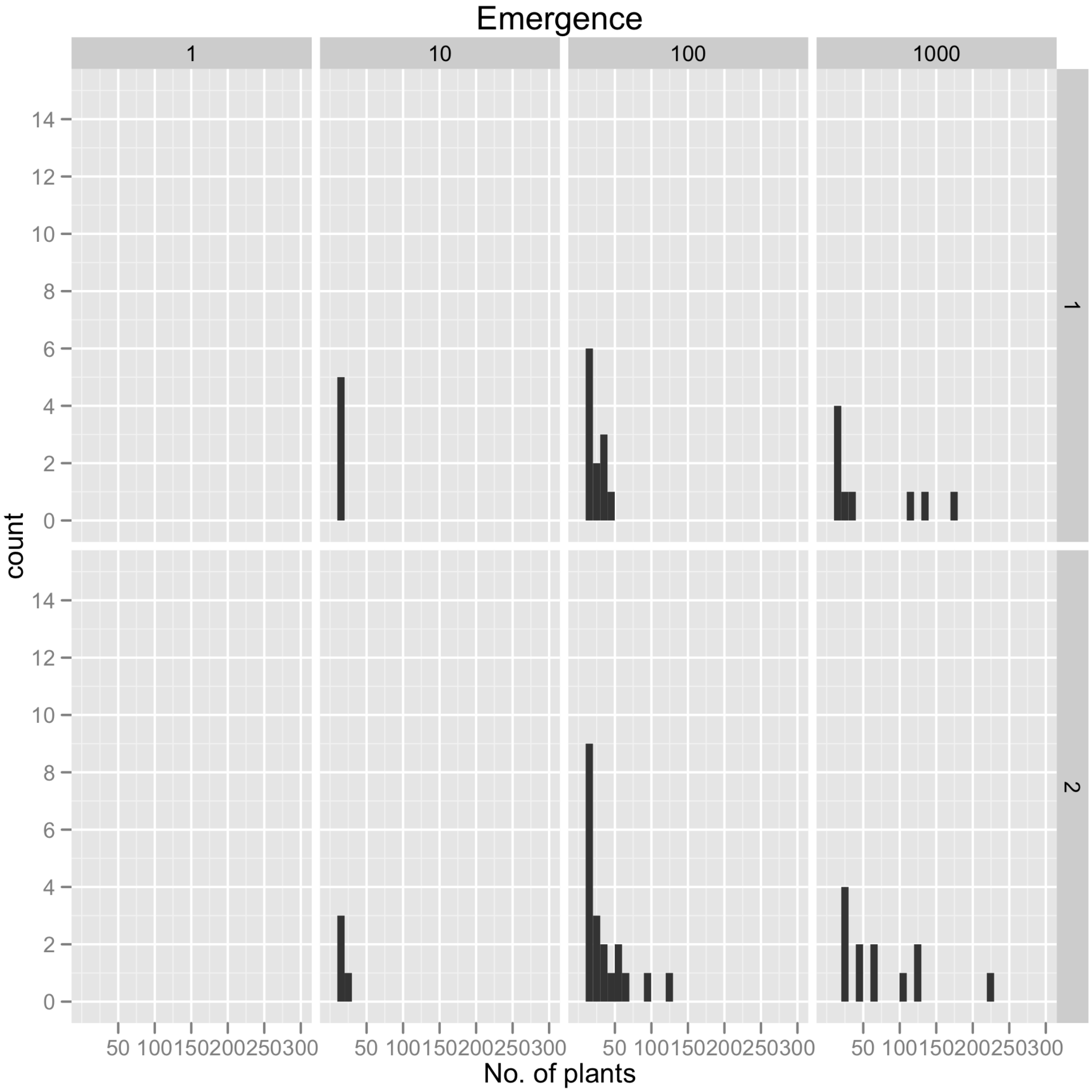

Figure 2.2b Frequency histogram of seedling emergence in year two. Partitions along the x-axis separate year zero sowing density treatments (1, 10, 100, 1000 seeds), and y-axis partitions separate lineages (1 = monoecious-hexaploid, 2 = dioecious-diploid).

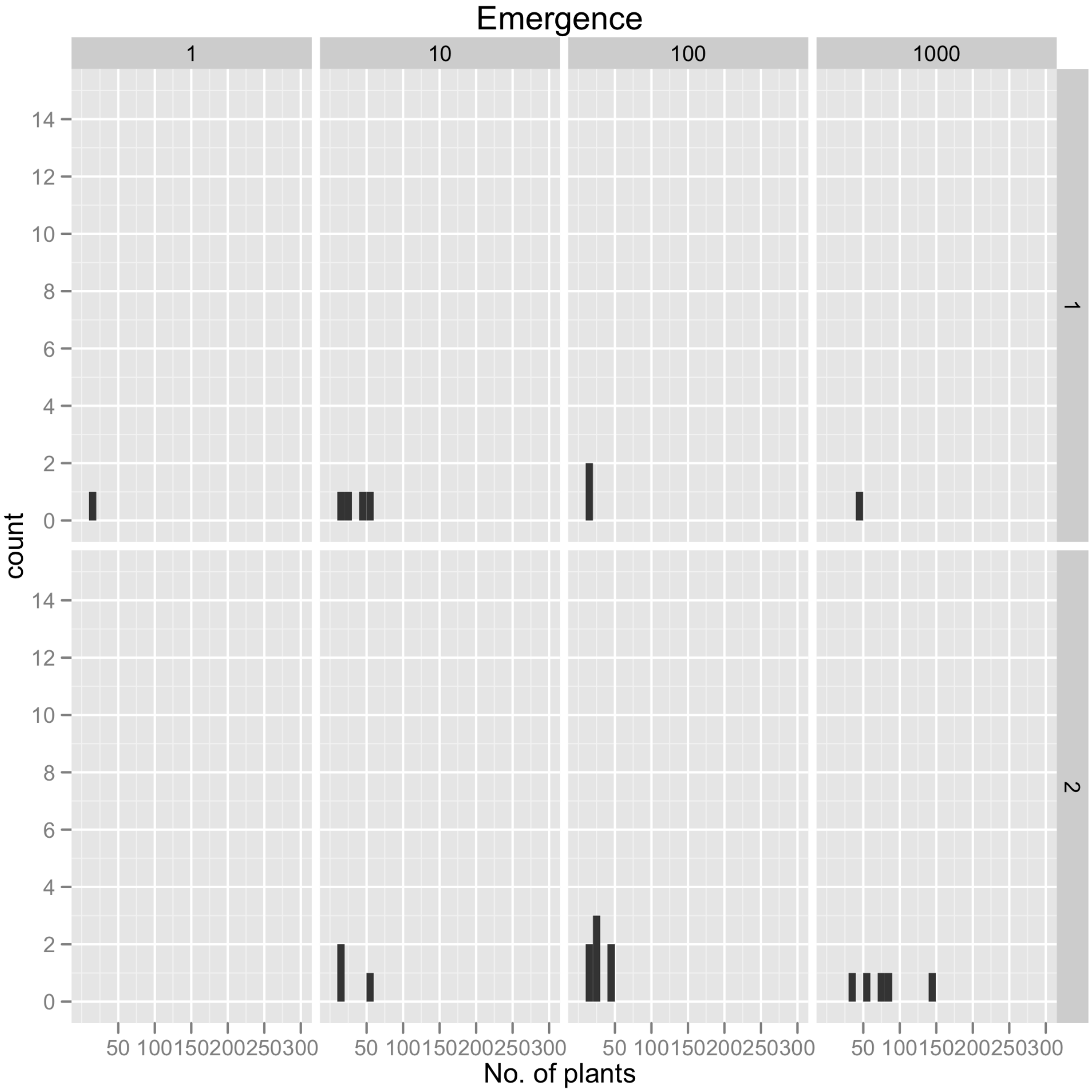

Figure 2.2c Frequency histogram of seedling emergence in year three. Partitions along the x-axis separate year zero sowing density treatments (1, 10, 100, 1000 seeds), and y-axis partitions separate lineages (1 = monoecious-hexaploid, 2 = dioecious-diploid

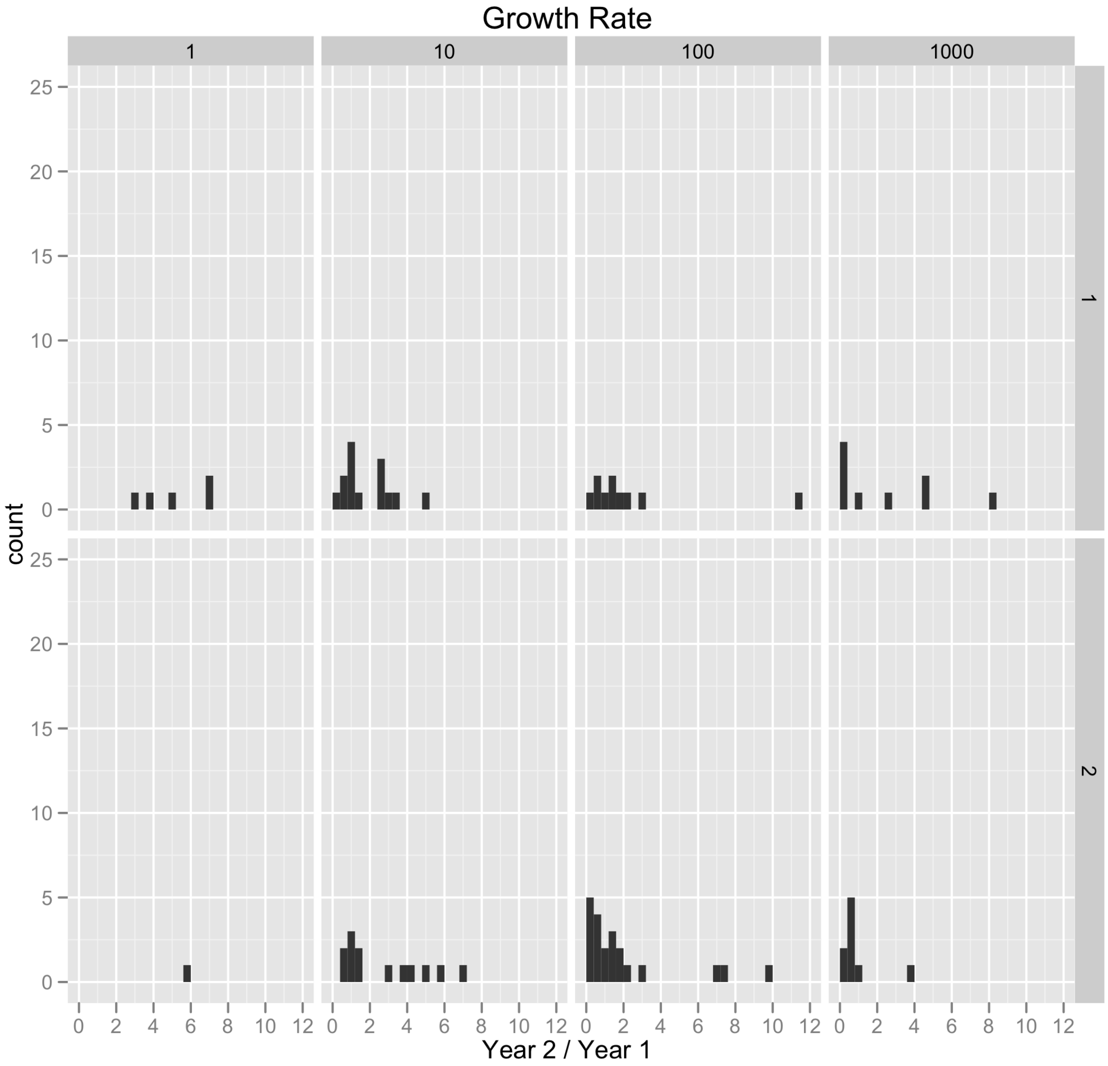

Figure 2.3a Frequency histogram of population growth rates from year one to two. Partitions along the x-axis separate year zero sowing density treatments (1, 10, 100, 1000 seeds), and y-axis partitions separate lineages (1 = monoecious-hexaploid, 2 = dioecious-diploid).

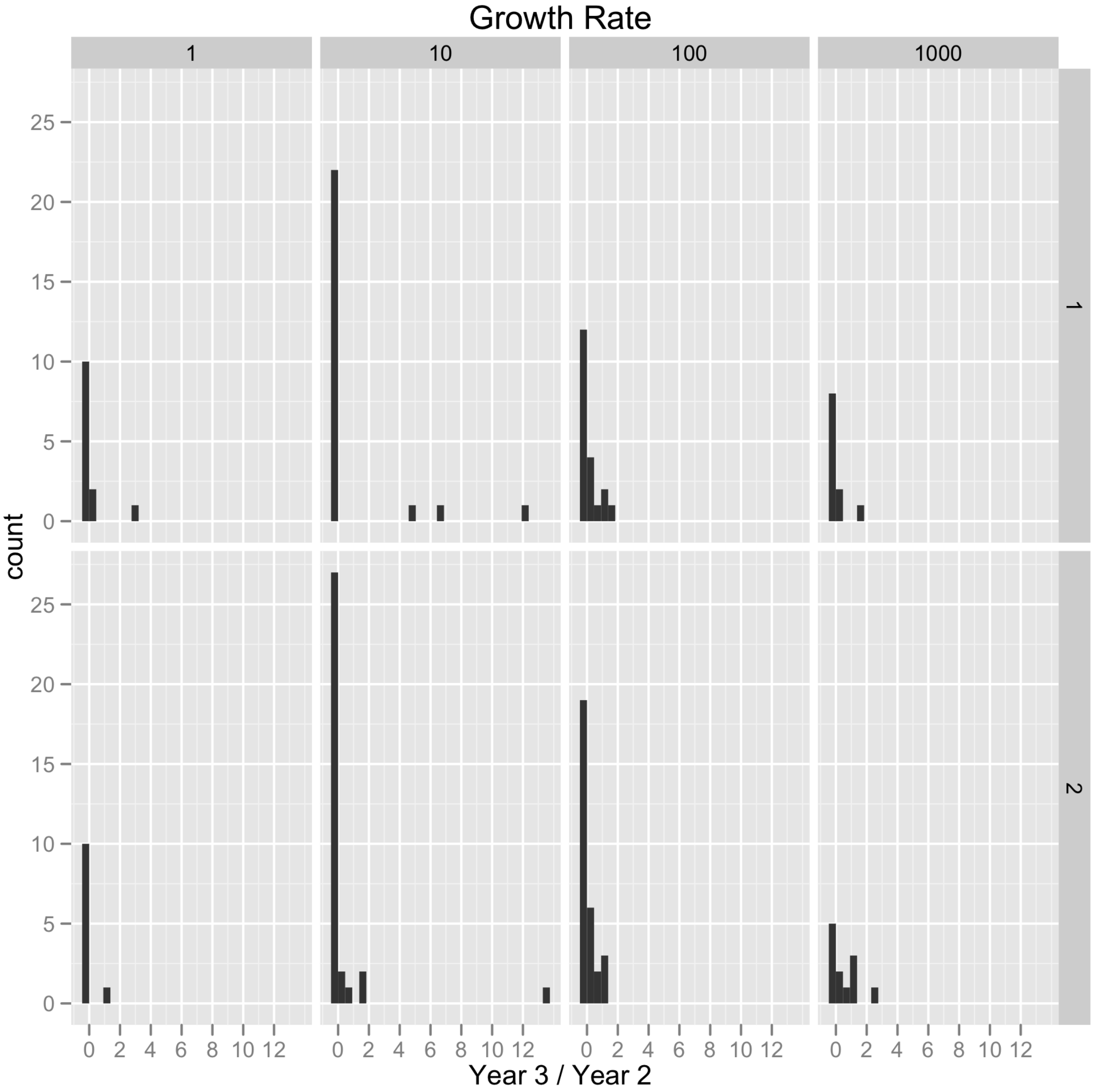

Figure 2.3b Frequency histogram of population growth rates from year two to three. Partitions along the x-axis separate year zero sowing density treatments (1, 10, 100, 1000 seeds), and y-axis partitions separate lineages (1 = monoecious-hexaploid, 2 = dioecious-diploid).

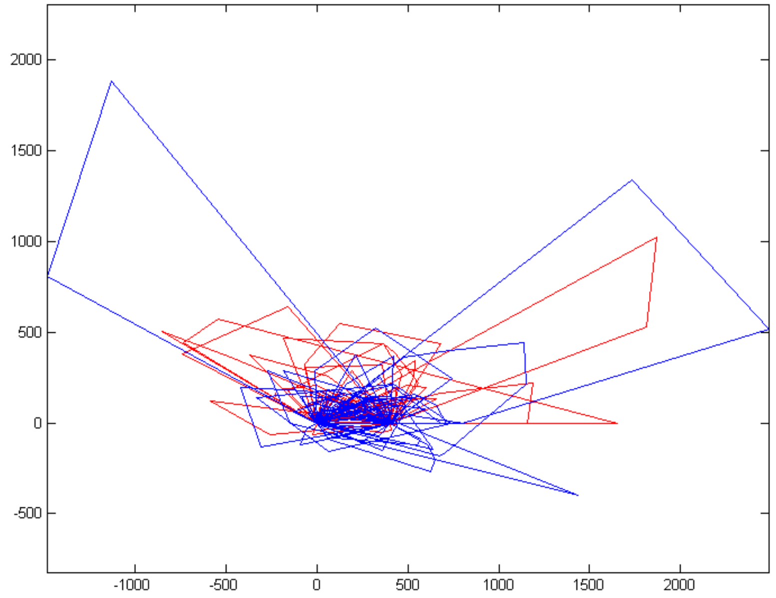

Figure 2.4 Dispersal polygons for all sites in year two, encapsulating all of the plants at each site (cm) subtended by a 4m sowing line. Red = hexaploid, blue = diploid

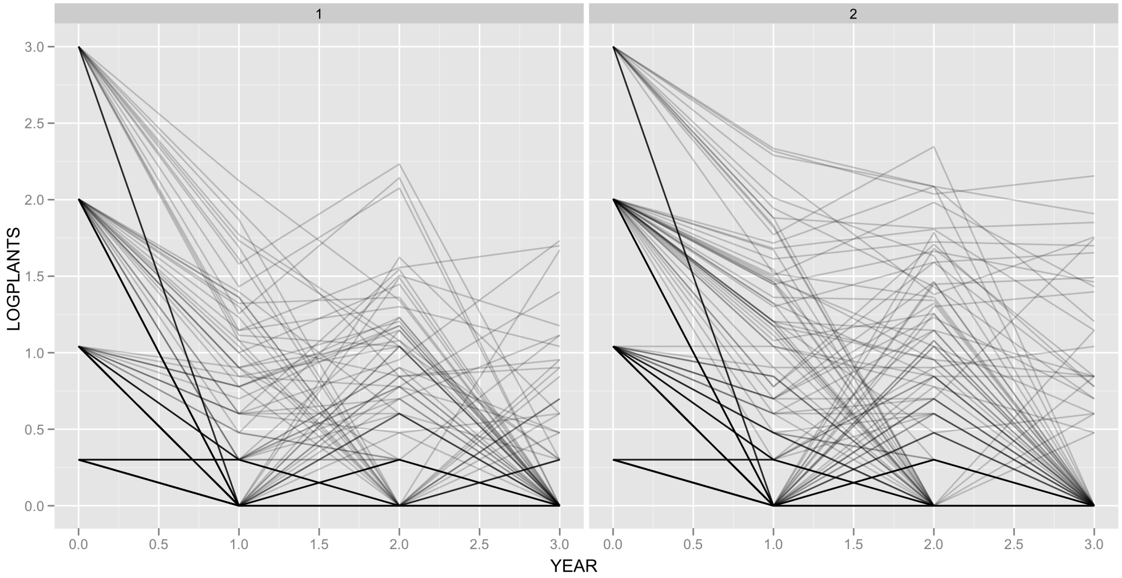

Figure 2.5a Time series plots of log (no. of plants emerging at a site +1) against year. Year zero indicates the number of seeds sown. Plot 1 is for monoecious-hexaploid sites, and plot 2 for dioecious-diploid sites

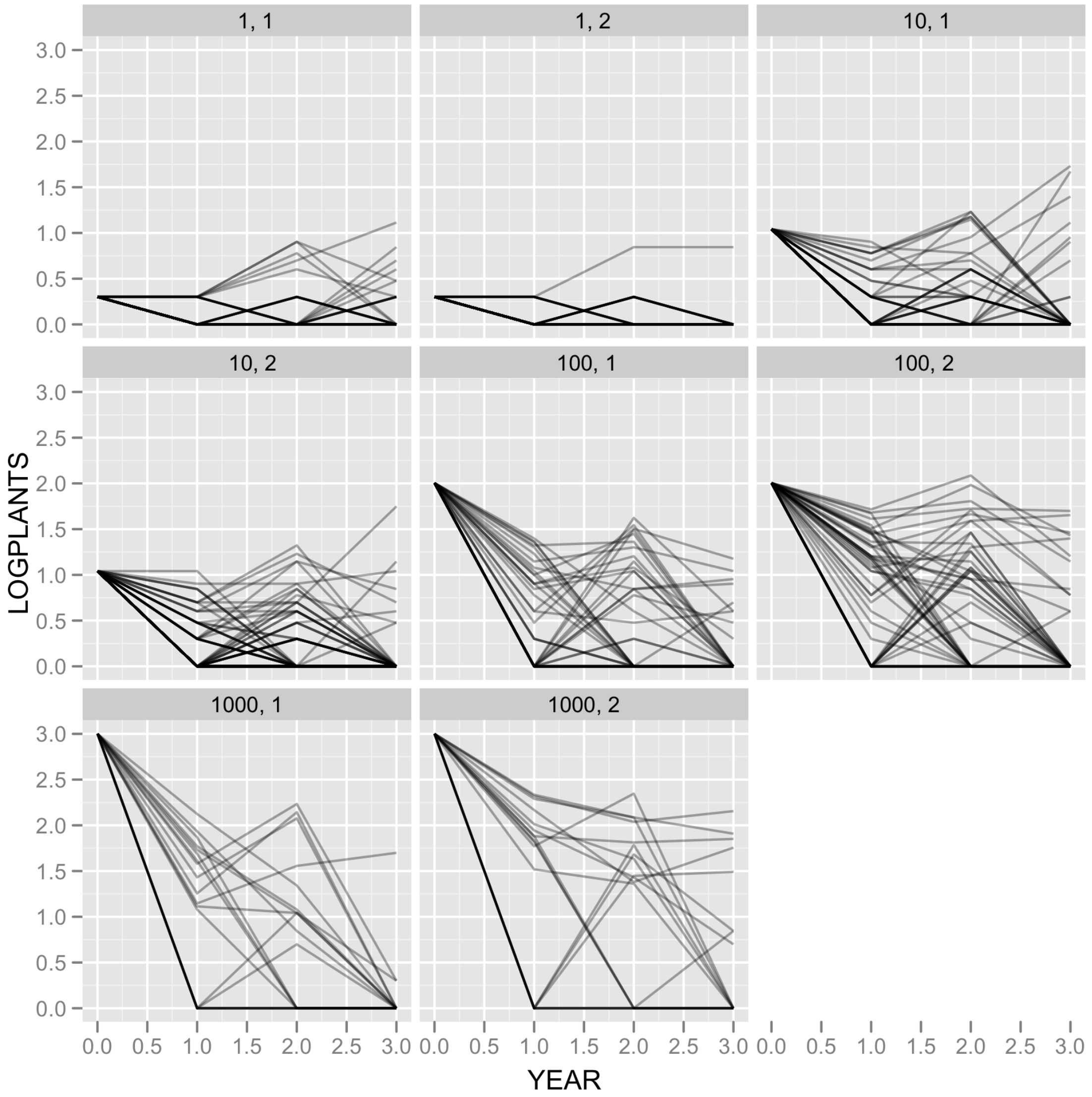

Figure 2.5b Time series plots of log (no. of plants emerging at a site +1) against year. Year zero indicates the number of seeds sown. Plots are separated according to year zero sowing density (1st digit in title), and by lineage (2nd digit in title; ‘1’ identifies monoecious-hexaploid sites, and ‘2’ dioecious-diploid sites

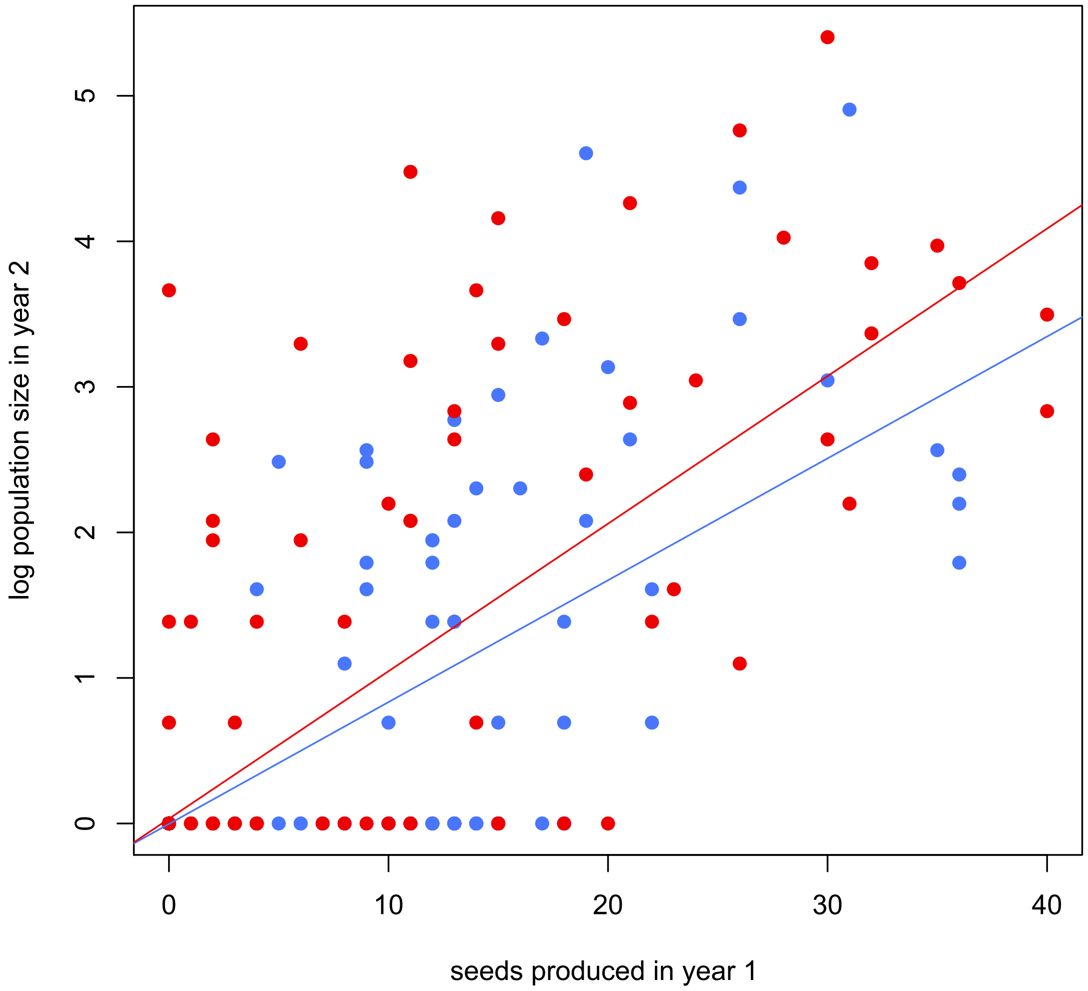

Figure 2.4 Log (1 + population size in 2009) plotted against seed production per sampled plants in the preceding year. Diploid line of best fit and points in red, hexaploids in blue; no significant difference was observed between lineages in terms of this relationship.

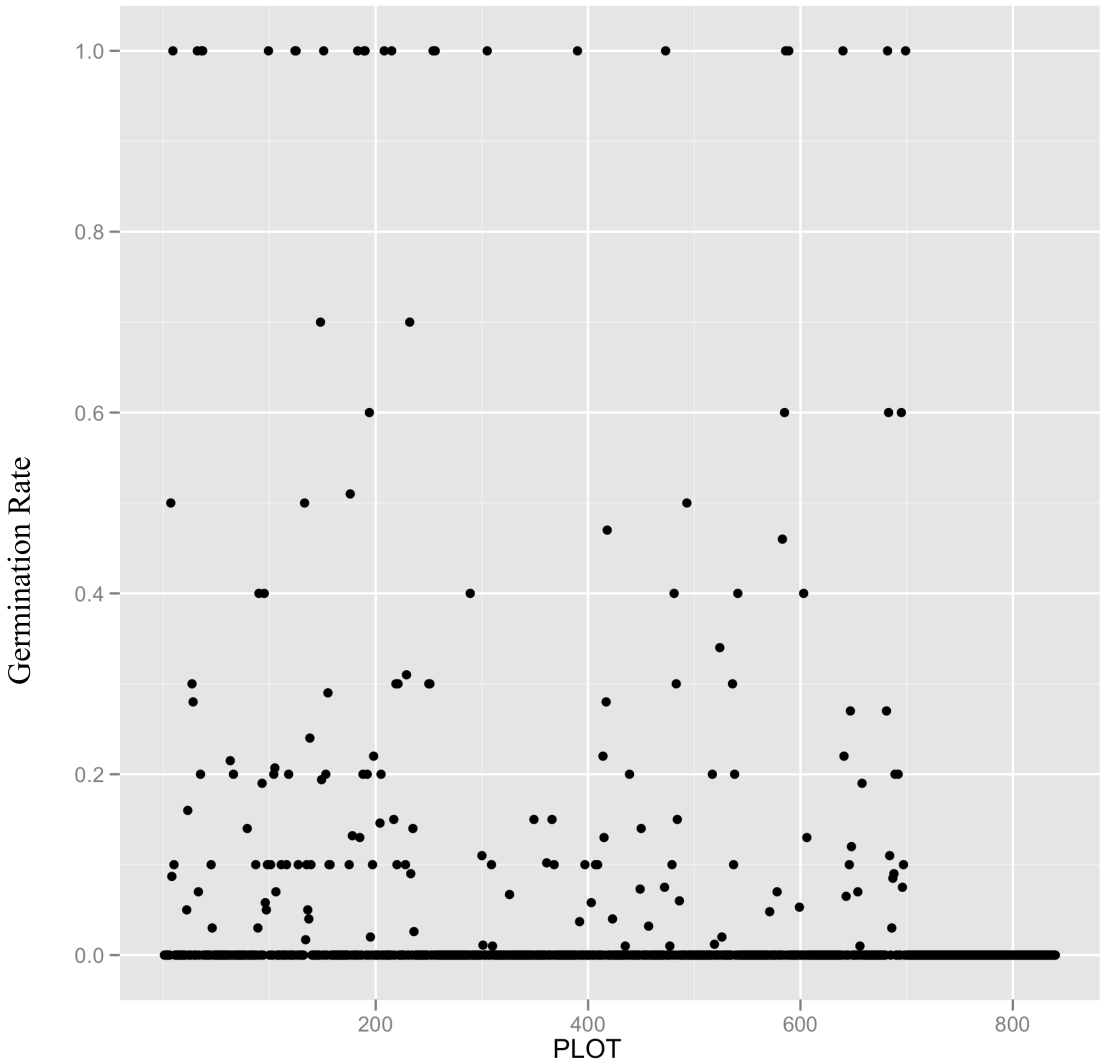

Figure 2.5 Germination rate plotted against sowing site showing no trend in site quality.

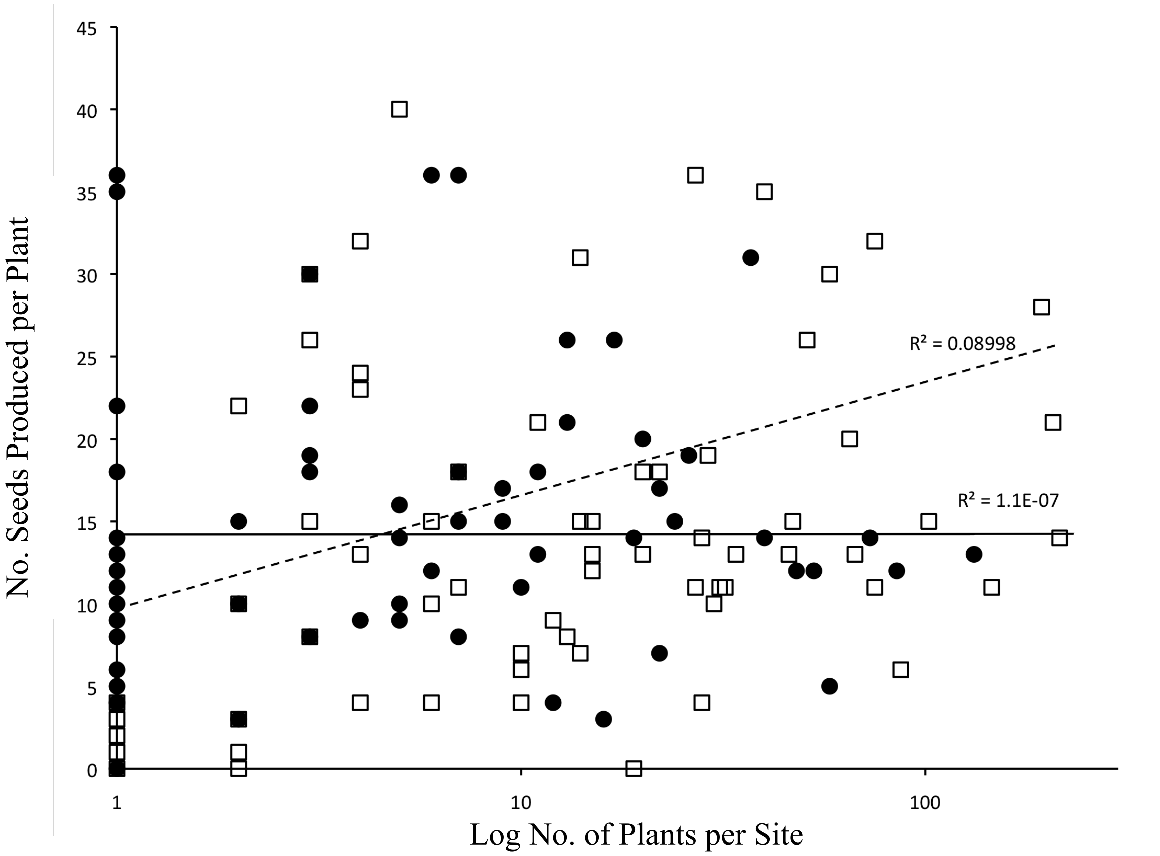

Figure 2.6 Plot of seed set against number of plants at a site (logarithmic scale population density). Filled circles and solid line represent hexaploid sites and line of best fit; open squares and dashed line refer to diploid sites.

CHAPTER 3. MINORITY CYTOTYPE EXCLUSION IN *MERCURIALIS ANNUA* CONTACT ZONE

3.1 INTRODUCTION

3.1.1 Origin and establishment of polyploids

Polyploidy is considered as one of the most significant mechanisms of evolution and speciation in plants (Levin 1983, Thompson and Lumaret 1992). The variation in and associations between sexual systems and ploidy documented in the genus *Mercurialis* (Thomas 1958, Durand 1963, Durand and Durand 1992) have provided a unique opportunity to understand the processes underpinning homoploid speciation, genome duplication and hybridisation. The genus comprises 7-10 European species and one Asian species, four of which have been recognised as annuals on the basis of morphology, ploidy and sexual system (Durand 1963): *M. huetii* Hanry, *M. annua* L., *M. ambigua* L.fil. and *M. monoica* (Moris) Durand. Recent long-range chloroplast capture is implicated in explaining these patterns by analyses of plastid ITS sequences from the woody perennial *M. huetii* (Obbard et al. 2006). Earlier work has attributed this variation solely to autopolyploidy (Durand and Durand 1985, 1992, Krahenbuhl et al. 2002). The success of polyploids in populations mixed with their diploid progenitors will be impacted by disassortative mating, and will be subjected to frequency-dependence (Levin 1975), with usually fewer potential mates, and more ineffectual matings. The ability of two ploidal levels to coexist has long been observed in numerous other species, with the consistent finding that the minority cytotype will decline due to the frequency dependence of effectual matings (e.g. diploid and tetraploid rye (Hageberg and Ellerstrom 1959); diploid and tetraploid maize (Cavanah and Alexander 1963)).

3.1.2 Secondary contact and minority cytotype exclusion

The identification of mixed ploidy populations raises questions about the processes that regulate the coexistence of alternate cytotypes, and whether they are subject to these frequency-dependent processes. In the event that the secondary contact of populations with different ploidy leads to hybridisation, and the production of offspring of fitness that is lower than that of the parental types, a process referred to as minority cytotype exclusion (MCE) has been shown to occur (Buggs and Pannell 2007). Because hybrids are of lower fitness, the establishment of one lineage in an area occupied by the other lineage depends on dispersal through seed. This reduced fitness may be a consequence of the high incidence of unpaired chromosomes at meiosis (Sybenga 1975).

In *M. annua*, pollen dispersal can facilitate this process of MCE, both by simultaneously increasing the likelihood that females will acquire compatible mates, and by reducing the seed fertility of hexaploids by swamping their ovules with pollen (Lexer and Van Loo 2006). The proportion of mating events for each cytotype that yield unfit hybrids will more frequently involve the least common cytotype, reducing its fitness relative to the common one, and eventually leading to its extinction (Rodriguez 1996, Husband 2000, Dorken and Pannell 2007). Hagberg and Ellerstrom (1959) for example monitored changes in cytotype frequencies over three years in diploid *vs*. tetraploid mixtures of rye and found that the minority cytotype declined.

The diploid and hexaploid lineages of *M. annua* occurred in the eastern and western ends of the Mediterranean basin respectively, during the Pleistocene glaciation, and have since come together in the Iberian Peninsula (Obbard et al. 2006). A comparison of population surveys in 1959 and 2003 revealed a remarkable shift in the position of the contact zone between lineages. The contact zones there between cytotypes have been moving at a rate of several kilometers per year however, and reciprocal transplant experiments of monoecious (hexaploid) and dioecious (diploid) populations have shown that diploids are fitter across all areas, and are preadapted to the area they are currently invading in Spain (Buggs and Pannell 2006).

Hybridisation is highly asymmetrical in favour of the diploids, mainly because they disperse substantially more pollen, as expected in a comparison between an obligate outcrosser and a facultative selfer (Buggs and Pannell 2006). Self-fertilisation of hexaploids is expected to reduce the proportion of sterile hybrids produced in mixed ploidy populations, and may allow hexaploids to avoid the effects of pollen swamping. Hexaploid *M. annua* exhibits little or no inbreeding depression, which is expected to be a concomitant result of its range expansion history, and as such this advantage is not expected to be offset by reduced viability of inbred offspring (Pujol et al. 2009). The experimental arrays of Buggs and Pannell (2007) found that regardless of population density however, the proportion of hybrid plants produced by diploid females was always near zero. Although experimental hybridisation rates of hexaploids were found to be high, they were lower than predicted by measures of relative pollen production (likely because of self-fertilisation). These findings led Buggs and Pannell (2006) to conclude that biased pollen dispersal between diploids and hexaploids is the primary factor responsible for the displacement of hexaploids. A similar experimental design with regular spacing of plants and no microhabitat variation was conducted on diploid and tetraploid *Chamerion angustifolium* (Husband 2000). However, clumping of the same cytotypes that occurs in natural populations due to patterns of localised dispersal can potentially affect the pollen pool composition around a focal plant. Experimental arrays that are randomised across cytotypes will not capture the role of this pollen pool composition in influencing hybridisation rates.

It is expected that unisexuals should be favoured under higher rates of site occupancy and abundance, i.e. lower rates of population turnover, and higher population densities (Dorken and Pannell 2007). Regional variation in the importance of metapopulation dynamics regulates the maintenance of unisexual individuals, with females, and especially males, occurring in areas where the rate of population turnover is not too high. In the experimental metapopulation study (Chapter 2) it was revealed that extinction rates are indeed relatively high in the area of study, as predicted by tension zone models wherein sites of contact will move towards low density-troughs, and especially at sites that are of low population density. Chapter 2, and earlier work in our laboratory has shown that diploid seeds from populations near the contact zone (indeed the populations that would be most likely to enter the contact zone) have a germination advantage over hexaploid seeds in that region (Buggs and Pannell 2007), suffering lower mortality, and attaining larger sizes and biomass. This confirms Durand's (1963) report that there are ecological differences between the cytotypes when in local sympatry, and should enable diploid populations to attain densities that approach hexaploid populations upon seed migration, given the germination advantage.

Reproductive assurance however, as may be identified through higher seedset in hermaphrodites relative to obligate outcrossing diploids at low density, should provide an advantage to cosexuals at low density that may lead to the exclusion of unisexuals from the monomorphic populations south of the contact zone. This may also provide a constancy of population density across years, preventing size fluctuations due to an Allee effect. Selfing in *M. annua* has been shown to occur below densities of 10 plants/m^2, with populations highly outcrossing at higher densities (Eppley and Pannell 2007). Females of more than a couple of meters from the nearest male become pollen limited at such densities (Hesse and Pannell 2011), suggesting that most pollination takes places within patches over small distances.

Competitive selfing at low densities should inhibit the spread of diploid populations by protecting hermaphrodite ovules from mating with diploid males. Diploids should therefore prevail under conditions of higher pollen dispersal, or high inbreeding depression, or high seed immigration. The yearly displacement of either hexaploids or diploids will identify the relative advantages of reproductive assurance of the hexaploids preventing hybridisation and promoting their spread at low density, and the asymmetric pollen production of male diploids in furthering their advance.

Assessing the causes and consequences of hybrid zone movement is critical if the extinction of one of the competing taxa is to be avoided (Ellstrand 1992, Rhymer and Simberloff 1996). In this study, I set out to examine the dynamics of mixed ploidy populations (both naturally and experimentally occurring) at the northeast contact zone of *M. annua*. Previous work examining MCE in *M. annua* was conducted under experimentally manipulated densities, and only a few naturally occurring mixed populations were identified and monitored at the northeast contact zone (Buggs 2004). I will quantify the relative advantage that diploids are expected to have over hexaploids in mixed populations at the study zone, given their sexual specialisation, differences in size, and their increased levels of pollen production that should lead to asymmetric mating. If minority cytotype exclusion were occurring, I would expect to observe increased rates of hybridisation in the minority cytotype, and decreased relative frequencies in the second year of study, as ovules are lost to hybridisation.

Here I derive a model that will assess the role of density, and relative frequency of cytotypes in mediating hybridisation rates. That is, given the other plants in the population, their sex, size, and distance from the focal plant, how can they be used to predict the hybridisation rate? Also, will the relationship between hybridisation rate and these population composition descriptors differ depending on whether the focal plant is a hexaploid hermaphrodite or a diploid female? This study uses naturally occurring mixed populations at the contact zone to test the extent to which pollen flow from males to focal hermaphrodites (as evidenced by the hybridisation rate), is greater than from hermaphrodites to females, or vice versa, given the same conditions (plant distances, sizes, relative frequencies). I expect that pollen flow should be disproportionately higher in the direction from males to hermaphrodites, than that from hermaphrodites to females, given the differences in the amounts of pollen produced by each, and also because males have specialised pollen-dispersing structures (Eppley and Pannell (2007). Hybridisation rates between the diploid and hexaploid lineages of *M. annua* have not previously been directly assessed in the field at this scale, and the process of MCE has only implicitly been observed under laboratory conditions. It is thus important to assess rates of hybridisation and MCE under field conditions, and to test assumptions in the field of the pollen pool models that predict these rates.

Under naturally occurring levels of seed and pollen dispersal, fluctuating population sizes and densities, it remains to be seen whether the presence of one cytotype in a population can preclude the establishment and persistence of the opposing cytotype in *M. annua*. Identifying whether MCE is currently occurring at the contact zone, and observing its process over a two year time course will enable an understanding of the fate of diploid populations as they continue their southward colonisation, and encounter monoecious populations of varying density. In this study, I follow a combination of experimental and naturally occurring mixed-cytotype populations in the diploid-hexaploid contact zone of the northeastern Iberian Peninsula over 2 years. I additionally perform a greenhouse-based hybridisation study to confirm field observations of hybrid morphology, and a greenhouse-based assessment of sex allocation in order to fit estimates of hybridisation rates and the factors influencing a bias, if present, in the direction of hybridisation.

3.2 MATERIALS AND METHODS

3.2.1 Source material and initial greenhouse assays

In March 2008, large amounts of seed were obtained from populations at opposite sides of a sexual system transition zone in Eastern Spain from Cambrils (dioecious) and Santa Carles de la Rapita (monoecious), approximately 40 km north and south of the contact zone, respectively. Greenhouse assessment of the proportion of male reproductive allocation was conducted on each of these populations so as to include this figure in the pollen pool model constructed from field data of hybridisation rates. Precisely 450 seeds per population were sown onto germination trays containing Emerald Green Pro-Mix soil-based compost (150 seeds evenly distributed per tray). Germination was monitored daily until a day at which at least 10 seedlings from each tray germinated at the same time. From the monoecious trays, 6 seedlings were individually transplanted into 10 cm pots after 4 days of growth in the germination trays, before passing the cotyledon stage. From the dioecious trays, 10 seedlings were transplanted, so as to ensure having at least 3 male and 3 female plants for reproductive allocation measurements. Pots were watered regularly and randomised once per week within the greenhouse.

I harvested the aboveground portions of the transplanted plants after 4 weeks of growth, when the relative production of seeds and pollen is expected to have reached a 'quasi-steady state', correlated with plant size (Pannell 1997), and thought to be representative of allocation patterns over the most productive period of growth. At this stage I recorded their height, and dissected them into their reproductive and vegetative components. After drying at 60ºC, the biomass for each component was recorded. For hermaphrodites, reproductive biomass was decomposed into fruits and pistillate and staminate flowers. Male reproductive allocation was estimated as the dry mass of staminate flowers, and also measured as a proportion of total vegetative biomass (cf. Samson and Werk 1986, Klinkhamer et al. 1992). Previous work in *M. annua* has shown that estimating pollen and fruit production in this way provides an effective measure of reproductive allocation (Pannell 1997, Buggs and Pannell 2006, Eppley and Pannell 2007, Dorken and Pannell 2008, Harris and Pannell 2008, Pujol and Pannell 2008).

A separate greenhouse assessment was conducted to verify the putative hybrid phenotypes that were observed in some of the mixed populations at the northeastern contact zone of this study. Using seed sourced from the aforementioned two populations, a pilot mating array between diploid and hexaploid plants was established at the John Krebs Field Station (Wytham, Oxon.). These were setup in two formats: [1] 3 focal hexaploid plants interspersed amongst 9 diploid male plants, and [2] 3 focal diploid female plants interspersed among 9 hexaploid plants. Each plant was contained in its own 10 cm diameter pot, and the 12 pots per setup were placed in a hexagonal array at maximum density (adjacent pots) so as to maximise the opportunity for pollen flow towards the focal maternal plant. Plants used in these arrays were initially grown in pure stands of identical cytotype under controlled greenhouse conditions for 4 weeks in the Department of Plant Sciences, University of Oxford, before being transported to the field station. The two mating arrays were grown in separate greenhouses that had not recently been used to grow *M. annua.* Focal plants were harvested after 3 weeks of growth in the mating array, subsequently dried, and sieved for seeds. Resultant seeds were germinated after a dormancy period of 14 weeks. Progeny with putative hybrid morphology were photographed and assessed for ploidy using flow cytometry.

3.2.2 Experimental mixed populations

At the Xerta-Calig canal in the diploid-hexaploid *M. annua* contact zone of northeastern Spain (N40.70059, E0.45547), experimental mixed populations were established and monitored over a subsequent two-year time course. Mixed hexaploid:diploid seed was sown in either a 100:100, or a 20:180 seed ratio. These ratios were chosen so as to provide a comparison between sites where each cytotype occurred at the same frequency, and sites in which the hexaploids would definitely be the minority cytotype, enabling a test of the minority cytotype exclusion principle. 10 sites of each ratio were sown in a straight 0.5 m long line and another 10 of each in a 5 m long line. Sowing lines were 10 cm deep and 2 cm across and occurred in freshly-tilled, bare and loose soil in a private farm site. Three plants randomly sampled from within each of these 40 experimentally established populations were measured for height, diameter at widest point, and seed production on second lowest branch.

3.2.3 Spatially explicit survey of naturally occurring mixed populations

In 2010, a further number of sites along the Xerta-Calig canal were identified in which hexaploid and diploid populations had naturally come into contact. 30 such sites were recorded with GPS coordinates, photographed, and described with respect to permanent landmarks so as to facilitate relocation. The plants at each of these sites were mapped out in a spatially explicit manner, with x-y coordinates measured in centimeters, as well as height and gender recorded for each plant. A number of plants were sampled (ranging from 3 to 16; mean ± s.e. = 4.88 ± 0.77) at representative intervals for each site, which varied depending on the size of the population. Sampled plants were individually placed into perforated polyethylene bags and were dried, then sieved for seeds.

Seeds from each of the sampled focal plants from these naturally occurring mixed populations were germinated three months after harvest in peat-based compost in a greenhouse in Oxford. Seed production of focal plants varied greatly, from 8 to 550 in hermaphrodites, and 4 to 1330 in females, as did germination rate (hermaphrodites: 0 to 96%, females: 0 to 78%). The viability of tetraploid seed resulting from diploid-hexaploid hybridisation is not well understood, not least because of the difficulty in assessing ploidy prior to germination, and as such hybridisation rate estimates must be viewed as an underestimate, with the assumption that a portion of hybrid seed was non-viable and thus not included in the flow cytometry analyses (see below).

Sites were revisited in 2011, and where present, plants were counted and categorised by a combination of gender, floral morphology, and female sterility (putative hybrids lacking seed set).

3.2.4 Laboratory-based F1 ploidy assessment

To determine which seeds of the field-sampled focal plants had been sired by males versus hermaphrodites, flow cytometry was used to measure the DNA content of progeny leaf cells and estimate the proportion of progeny that were tetraploids (diploid-hexaploid hybrids). Flow cytometry measures the DNA content (C-value) of cells, which is expected to increase linearly with ploidy (Leitch and Bennett 2004). Leaf samples were grouped according to their maternal plant and analysed using a Becton Dickinson FACScan flow cytometer in the Oxford Glycobiology Institute. For each sample, approximately 5 mg *Mercurialis annua* leaf material from up to 15 individuals was chopped in a petri dish on ice using a double-edged razor blade in 1 mL ice cold 'LB01' lysis buffer and staining solution. The buffer and staining solution, based on Dolezel et al. (1989), contained 15 mM Tris base, 2 mM Na2EDTA, 0.5 mM spermine tetrahydrochloride, 80 mM KCl, 20 mM NaCl, 0.1% (v/v) Triton X-100, 15mM ß-mercaptoethanol, 50 µg/ml propidium iodide and 50 µg/ml RNAse. The resulting suspension was filtered through 30 µm filter and run on a Becton Dickinson FACScan flow cytometer. For each sample, 10,000 events were recorded, and displayed as a histogram. Mean peak values were analysed using Becton Dickinson CellQuest software. When more than one peak was found in a sample, and thus the presence of tetraploid hybrids, the flow cytometer displayed the proportion of events attributed to each peak.

This enabled a rapid assessment of the proportion of hybrid progeny produced by a given maternal plant.

3.2.5 Data analyses

3.2.5.1 Hybridisation rates

The progeny of each field sampled maternal plant in this study represent a genetic array derived from ovules that either self-fertilise, or outcross to pollen of either the same or an alternate cytotype. The pollen pool model defined for the nonlinear regression (see below in section 3.2.5.2) was decomposed into its constituent parameters, each of which were estimated separately in a generalised linear model for their effect on hybridisation rate. A logit link function was used in order to translate the success (hybrid)/failure (non-hybrid) response into a continuous variable that may be linearly described. Two GLMs were constructed, one for focal hexaploids, and another for focal diploids. Measured parameters used as independent variables were: plant size (height and diameter), gender (frequency), logarithm of the distance from focal plant used to describe the pollen fallout curve, and population density. Population density was calculated as the number of plants contained in the area circumscribed by the outermost plants of a population, and was calculated using Spatial Point Pattern Analysis Code in S-Plus (Version 2 – Spatial and Space-Time analysis).

Each independent variable is defined as the sum of that parameter across all plants of the alternate cytotype as the focal plant for a population (i.e. all diploids for a hexaploid focal plant), divided by the sum of that parameter across all plants (of both cytotypes) in a population. For example, for a focal plant f, aside from self-pollen, there are two groups of plants contributing to the pollen pool: male plants and hermaphrodite plants that are denoted by M_f and H_f, respectively. The influence of the diameter (i.e., X_{Di}) of surrounding plants on the hybridisation rate of focal plants is calculated as in equation 3.1,

$$X_{Di} = \begin{cases} \dfrac{\sum_{i \in M_f} d_i}{\sum_{i \in M_f} d_i + \sum_{i \in H_f} d_i} & \textit{if } f \textit{ is hermaphrodite} \\ \dfrac{\sum_{i \in H_f} d_i}{\sum_{i \in M_f} d_i + \sum_{i \in H_f} d_i} & \textit{if } f \textit{ is female} \end{cases} \tag{3.1}$$

where d_i is diameter of the i^{th} plant in a population (either M_f or H_f). The relative influence of gender frequency, logarithmic distance from focal plant, and density were calculated in the above manner. The base model described above, excluding the inverse-logarithm of interplant distance parameter, can be considered as a form of mass-action model (Holsinger 1991), wherein it is assumed that the proportion of pollen of any type arriving on the stigma of a focal plant will be related to the relative frequency of that pollen type in the surrounding pollen cloud. This assumes an equal competitive ability of pollen types in siring ovules (although see Holsinger and Thomson 1994). I modified this model by accounting for the contribution of individual plants, as measured by their relative sizes and positions, to the population-level pollen pool (herein referred to as 'individual-based model'). The additional predictive power of this individual-based modification was assessed by incorporating the interplant distance parameter into the GLMs, and comparing models on the basis of AIC and χ^2 tests on differences in residual deviance.

In interpreting the coefficients from the GLM with logit link functions, the back-transformed (exp) of the coefficient is proportional to the increase in the odds of success in the response, assuming all other model independent variables are held constant. For instance, if the exponentiated coefficient is 2.5, then an increase in one step of the regressor corresponding to that coefficient will increase the odds of success by 2.5, i.e. the odds of an ovule being hybridised increase by 2.5 times.

3.2.5.2 Pollen pool modeling

Hybridisation rates were further explored using nonlinear regression with hybrid progeny classified as success, and non-hybrid as a failure. An individual-based assessment of the pollen pool surrounding each focal plant was developed as a regressor for hybridisation rate. The pool was calculated as the ratio of hybrid to total pollen available in the population, weighted by the distance of each pollen-contributing plant to the focal plant. To derive an expression for the hybridisation rate in an anemophilous plant population, consider a stigma on a particular cytotype: The pollen arriving at the stigma will be a mix of the same and the alternate cytotype, with the hybridisation rate expected to be proportionate to the fraction of each type available at a site. That is, the hybridisation rate of the average ovule on a focal plant of a particular cytotype will be the rate at which it is fertilised by foreign pollen of the alternate cytotype, which will depend on the density of that pollen relative to the density of pollen of the identical cytotype. In order to calculate the amount of pollen produced by plants of the same cytotype, and of the alternate cytotype that would lead to hybridisation, I calculated the summation across all plants for each cytotype (only males for diploids) of plant size (height • diameter) (as per the equation described above) divided by the logarithm of their distance from the focal plant:

$$\alpha = \beta \left(\frac{X_{height} \cdot X_{diameter}}{X_{dist}} \right) \tag{3.2}$$

wherein α represents the hybridisation rate of focal plants, and β is a coefficient indicating the relative effectiveness of each cytotype in effecting hybridisation, according to the relative to the composition of the pollen pool (hybrid to total). Distance (X_{dist}) of a pollen donor from the focal plant was calculated as the logarithm of the Euclidean distance using the x-y field coordinates to construct a matrix. This creates a context-dependency of the probability of a pollen grain from a particular plant and cytotype arriving at the stigma of the focal plant, accounting for both size ($X_{height} \bullet X_{diameter}$) (surrogate of pollen production), relative frequency of each cytotype, and density (through interplant distance). This provides a conservative estimate of the contribution of each of the parameters to the pollen pool as it does not account for the contribution of self-pollen to the ovules of the focal plant.

In comparison to previous mass-action models that assume a uniform (or random) distribution of males and hermaphrodites throughout a mixed population, where males and hermaphrodites are thought to contribute evenly to the pollen pool according to their relative frequencies in the population (see Eppley and Pannell 2007), the method used herein accounts for pollen pool compositional differences depending on the precise location from which a focal plant is sampled. Earlier experimental work has simply regressed the proportion of outcrossed offspring of the focal plant on total mass of all surrounding neighbours to assess the influence of plant size on outcrossing rate, not accounting for relative positions of neighbours (Eppley and Pannell 2007).

A nonlinear regression of the pollen pool was conducted against hybridisation rate separately for diploid and hexaploid focal plants of all populations, and the parameter estimates were then compared between cytotypes using a *t*-test. For example, in the case of a hermaphrodite focal plant in a population, the hybridisation rate is explained by the sum of the pollen contributed by each male plant in the same population, divided by the sum of the pollen contributed by all males and hermaphrodites in that same population. The amount of pollen contributed by an individual plant is the inverse logarithm of its distance from the focal plant, multiplied by its size (height × diameter). Hybrids were again characterised as 'successes' and non-hybrids as 'failures'. The β coefficients for models with female focal plants were compared to those with hermaphrodite focal plants using *t*-tests. The comparison of this parameter estimate between hexaploids and diploids allows inference as to the relative advantage males might have over hermaphrodites (or vice versa) in effecting hybridisation of the ovules of the alternate cytotype; i.e. to determine whether for a given population structure (in terms of plant sizes, relative positions, relative frequencies), the rate of pollen flow from males to focal hermaphrodites (as evidenced by the hybridisation rate) is greater than from hermaphrodites to females, or vice versa. These parameter estimates may be further broken down according to the relative dispersal advantage enjoyed by males (a_m) versus hermaphrodites (a_h) ($\alpha = a_m/a_h$ as per Eppley and Pannell 2007) and relative differences in pollen production of males (p_m) versus hermaphrodites (p_h) ($\lambda = p_m/p_h$) using estimates from greenhouse grown plants.

3.3 RESULTS

3.3.1 Greenhouse estimates

Pollen production was more than twice as high in diploid males (mean ± s.e.: 0.128 ± 0.013 g) as in hexaploid hermaphrodite plants (mean ± s.e.: 0.058 ± 0.005 g) as determined by Welch's two-sample *t*-test ($t_{13} = 4.812$, $p = 0.0003$). Calculating reproductive allocation to male function as a proportion of total biomass, there was no significant difference between males (10.29 ± 1.082 %) and hermaphrodites (7.52 ± 1.67%) ($t_{29} = 1.387$, $p = 0.176$). When grown in the greenhouse, plants of both cytotypes did not differ significantly in terms of height (monoecious: 20.80 ± 1.15 cm, dioecious: 22.26 ± 0.57 g, $t_{29} = 1.133$, $p = 0.266$), although diploid plants had significantly greater total dry biomass (1.362 ± 0.0580 g) than hexaploids (0.941 ± 0.063 g) ($t_{39} = 4.911$, $p =$ 1.666e-5).

Germination rates of the progeny from the six focal plants from the greenhouse hybridisation pilot study were higher in maternal hexaploids (mean ± s.e.: 40.4 ± 0.14 %) than maternal diploids (16.8 ± 9.6 %). The phenotype that was used to characterise naturally occurring hybrids in the field-sampled populations included sterile female flowers (often borne on peduncles), and male flowers bearing little or no pollen or no male flowers at all (Fig. 4.1 and 4.2). When these phenotypes were observed in the greenhouse grown progeny of the mating arrays flow cytometry revealed that they were only associated with tetraploid hybrids.

3.3.2 Experimental setup in northeastern contact zone

3.3.2.1 Change in hexaploid:diploid ratio

In 2009 when the experimental mixed populations in the contact zone were first re-visited, the proportion of hexaploid:diploid plants that had established was 0.078 for the 20 hexaploid:180 diploid seed sowings, and 0.765 for the 100 hexaploid:100 diploid sowings. In the following year, these proportions had become 0.008 (20:180 sowing) and

0.446 (100:100 sowing) respectively, although these changes were not found to be statistically significant ($t_{75} = 0.625$, $p = 0.534$). This slight change in the ratio of hexaploid:diploid plants in the experimental mixed populations was not influenced by the ratio in which seeds were initially sown ($t_{34} = 0.581$, $p = 0.564$), nor the density at which they were sown ($t_{34} = 1.256$, $p = 0.218$). The minimum adequate linear model on the change in the ratio of hexaploid:diploid plants at a site between years ($F_{3,36} = 19.23$, $p = 1.297\text{e-}7$, adjusted $R^2 = 0.584$) identified a significant but minimal negative influence of the relative sizes of hexaploid:diploid plants (coefficient: -0.394, $t_{34} = 5.741$, $p = 1.54\text{e-}6$) and a small positive influence of their relative seed production in 2009 (coefficient: 0.161, $t_{34} = 3.236$, $p = 0.003$).

3.3.2.2 Population growth rate

Numbers of hexaploid plants per experimental site decreased on average between 2009 and 2010 from 6.80 ± 0.95 to 3.68 ± 0.69 (mean ± s.e.) ($t_{71} = 2.66$, $p = 0.00963$). The minimum adequate linear model (summarised in Table 3.6) identified a weak relationship between the change in population size and the sowing density, with sites sown over a larger area experiencing an increase in population size. The number of diploid plants present at a site in 2009 had a negative influence on overall growth of the mixed populations into 2010.

Numbers of diploid plants per experimental site increased on average between 2009 and 2010 from 22.75 ± 1.82 to 27.125 ± 2.48 (mean ± s.e.) although this change was not statistically significant ($t_{71} = 1.422$, $p = 0.1595$). Diploid population growth was positively related to sowing area (coefficient = 0.9001 ± 0.4709, $t_{36} = 1.912$, $p = 0.0639$), with population size increasing most at low density as determined by the minimum adequate linear model ($F_{3,36} = 4.405$, $p = 0.0097$, adjusted $R^2 = 0.208$). Diploid population growth was not significantly influenced by the number of hexaploid plants appearing at a site in the preceding year (coefficient = -0.168 ± 0.265, $t_{36} = 0.634$, $p = 0.5299$). The production of sterile hybrids in 2010 was negatively related to sowing area (highest at high density) (coefficient = -0.7889 ± 0.3056, $t_{36} = 2.581$, $p = 0.0139$), and positively influenced by the ratio of hexaploid:diploid seeds initially sown (coefficient = 4.9209 ± 1.5557, $t_{36} = 3.163$, $p = 0.00311$), as determined by the minimum adequate linear model ($F_{2,37} = 8.34$, $p = 0.001$, adjusted $R^2 = 0.273$).

3.3.3 Spatially explicit survey of naturally occurring mixed populations

3.3.3.1 Plant sizes and hybrid production

Considering all plants in the naturally occurring mixed populations, diploid plants were significantly taller (diploid: 26.19 ± 0.69 cm, hexaploid: 23.06 ± 0.32 cm, $t_{474} = 4.135$, $p = 4.192e\text{-}5$), and wider (diploid: 11.87 ± 0.64 cm, hexaploid: 11.50 ± 0.20 cm, $t_{392} = 4.99$, $p = 9.094e\text{-}7$), than hexaploids. Considering all focal plants, hexaploids produced a significantly higher proportion of hybrids (acting as maternal parent) (45.34 ± 4.17 %) than did diploid females (28.6 ± 0.029 %) across all sites ($t_{89} = 4.99$, $p = 1.447e\text{-}3$).

3.3.3.2 Hybrid production

All terms in the general linear model of pollen production had a significant impact on the hybridisation rate on both hexaploid hermaphrodite (Table 3.1) and diploid female (Table 3.2) ovules separately. Coefficients of each of the parameters differed significantly between the hexaploid and diploid pollen pool models as determined by *t*-tests (Table 3.3). Plant sizes in a population had a disproportionately higher effect on hybridisation of hexaploid focal plants than of diploids ($t_{76} = 56.61$, $p = 6.75e\text{-}64$). The relative frequency of plants of the opposite cytotype in a population also had a much higher effect on the hybridisation rate of hexaploids than diploids ($t_{76} = 71.53$, $p = 1.78e\text{-}71$), meaning that for an equal proportion of males to hermaphrodites (in the case of focal hermaphrodite plants) and hermaphrodites to females (in the case of focal female plants) the hybridisation rate would be significantly higher in hermaphrodite focal plants, assuming all other independent variables are held constant. Similarly, this pattern wherein significantly higher hybridisation rates were experienced by focal hermaphrodites was also produced for a given set of interplant distances ($t_{76} = 50.06$, $p = 6.06e\text{-}60$) and population densities ($t_{76} = 63.05$, $p = 2.21\text{e-}67$). Interplant distances calculated in the individual-based version of the mass-action model as described above added significant predictive power to the mass-action model for both hexaploid and diploid focal plants as determined by a comparison of AIC values between models and by comparing differences in residual deviance by in a χ^2 test (see Table 3.4).

3.3.3.3 Pollen pool parameterisation

The nonlinear regression of the pollen pool model using the Gauss-Newton iterative method (starting value of 2) produced an estimate for β_h in hexaploid populations of 3.833 ± 1.058 (mean ± s.e.) ($t_{90} = 3.623$, $p = 6.01\text{e-}4$). This indicates in a hypothetical pollen pool, a focal hexaploid plant, surrounded by an equivalent amount of diploid and hexaploid pollen, will have 3.833 ovules hybridised, for each ovule not hybridised. This was much higher than for the β_d estimate for focal diploid females of 0.627 ± 0.0936 ($t_{90} = 6.776$, $p = 1.63\text{e-}7$), indicating that in an equivalent hypothetical pollen pool to that described above, only 0.627 ovules of a female plant would be hybridised for each ovule not hybridised. A *t*-test comparison of these parameter estimates revealed that they were significantly different between cytotypes ($t_{90} = 3.022$, $p = 3.27\text{e-}3$).

3.3.3.4 Population size changes

All naturally occurring mixed survey sites were revisited the following year. 18 of the 30 sites were apparently extinct when revisited, and of the 12 remaining, 4 had fewer than 6 plants (counts of hexaploid and diploid plants from each remaining population shown on Table 3.5). Sterile plants were not observed at the sites over the two-year time course. Only one population was of mixed-ploidy in the second year, starting from almost equal proportions of each cytotype in the first year, leading to a 23.1% : 76.0% dioecious:monoecious ratio the following year, with a reduction in population size from 75 to 26 individuals. Of the remaining 11 populations, 7 settled at a stable state of complete diploidy, and 4 at complete hexaploidy. 8 out of 12 populations suffered a reduction in population size.

3.4 DISCUSSION

3.4.1 Hybrid Production

Considering the parameters that were measured in this study, it was found that hexaploid ovules were significantly more likely to be hybridised than those of diploids. Diploids were indeed significantly larger than hexaploids and produced more pollen than hexaploids, which is in contrast to earlier work that recorded males attaining smaller aboveground vegetative sizes than hermaphrodites (Hesse and Pannell 2011). In the current study, biomass was actually higher in diploids, although this is not to be taken to reflect differences in soil nutrient availability or site quality, which have been shown previously to be independent of plant size (Hesse and Pannell 2011). This supports the idea that diploids are pre-adapted to the region they are currently invading in northeastern Spain, and their relatively larger plant size should facilitate pollen production and accelerate their advance past the generally female-biased hexaploid populations, as absolute allocation to male function increases with size (Hesse and Pannell 2011). The diploids have been shown to harbour greater levels of genetic diversity, which might make them more capable of dealing with harsh or novel environments; a pattern that has been observed in the diploid *vs*. tetraploid *Rorippa amphibia* (Luttikhuizen et al. 2007).

Considering the recorded differences in male fertility between the lineages ($p_m = 0.128$g, $p_h = 0.058$g), the relative difference in male fertility ($\lambda = 2.21$) can be accounted for in the estimates of β_d (3.833) and β_h (0.627) to obtain the siring success ($\alpha = (\beta_d / \beta_h) \cdot \lambda^{-1}$) of pollen grains from males relative to that of hermaphrodites. This simple calculation illustrates an $\alpha = 6.11/2.21 = 2.77$-times advantage of diploid males over hexaploid hermaphrodites in effecting hybridisation in the competing lineage at the contact zone. That is to say that pollen dispersed from male inflorescences are 2.77-times more likely to sire hybrid progeny than hexaploids are to sire hybrids in the same population. This is likely due to the specialised pollen dispersal structures of males. The field-based estimate of β in this study is substantially lower than the $\beta = 35.2$ previously estimated in experimental arrays (Eppley and Pannell 2007). The relative difference in male fertility λ

is also lower than that estimated by Buggs and Pannell (2006) ($\lambda = 21$), however the estimate of α in this study is higher (cf. $\alpha = 1.67$; Buggs and Pannell 2006). The discrepancy in values can be largely attributed to the context-dependency of the pollen pool model used in this study; earlier work has assumed that males and hermaphrodites are distributed uniformly (or randomly) throughout the population, so that at any distance from a focal plant, males and hermaphrodites will contribute evenly (in proportion to their relative frequency) to the pollen pool surrounding the stigmas of the focal plant (Eppley and Pannell 2007). Clumping and uneven densities across a natural population render this assumption implausible in the field. High density patches within a larger population matrix can alter the amount of stationary air surrounding a focal plant (Burd and Allen 1988), and act as a further barrier to gene flow by diverting pollen higher into the velocity gradient where air is more rapid (Vogel 1994). Earlier work in *M. annua* did not identify any significant effect of plant spacing or the hexaploid:diploid ratio on the rate of hybridisation in diploids, however there was a significant effect in hexaploids (Buggs 2004). The range of densities, relative frequencies, and interplant distance combinations assessed under natural field conditions in this study did however identify a significant effect of plant spacing on both diploids and hexaploids, albeit the relationships were much more pronounced in hexaploid focal plants. The specialised pollen dispersing peduncles of diploid males might enable them to better disperse pollen under high-density conditions in comparison to hexaploid hermaphrodites that bear male flowers basally at leaf axils. The mass-action model used in the earlier studies described above, and that was compared to the individual-based form of the mass-action model developed herein, ignores the possible role of spatial variation in the density of individuals. Explicit analytical results for nonuniform spatial distributions have proved difficult to obtain, both in this study and by other authors (Holsinger 1991), and are of particular importance at lower densities to that where uniform outcrossing occurs. The studies of Buggs (2004) were performed at highest densities that could be obtained using hexagonal arrays, under which mass-action models may possibly be reliable in describing pollen pool dynamics. Beyond such densities however, as were observed in naturally occurring mixed cytotype field populations, significant additional predictive power on hybridisation rates was gained through the use of an individual-based assessment of pollen pool composition. It may be noted however, in populations with high spatial variance a disproportionate proportion of the offspring will be produced in sections of the population that contain the most individuals. Focal plants from the naturally occurring populations of this study were sampled at regular intervals so as to capture the spatial variation of the entire population, thus necessitating individual-based pollen pool modeling.

Previous work has noted that the mass-action model is reliable providing that self- and outcross pollen grains (or equally diploid *vs*. hexaploid in this case) compete equally for access to ovules (Holsinger and Thomson 1994). There has been little work in this species to specifically identify the type of self-fertilisation that occurs in hexaploids, be it prior self-fertilisation, delayed, or competing (Lloyd 1979). The mass-action model would only hold if competing self-fertilisation were occurring (Holsinger 1991), and additionally if diploid and hexaploid pollen are equally able to fertilise ovules of the alternate cytotype. In the context of this contact zone, hexaploid variants that are able to effect substantial prior self-fertilisation, possibly through a reduction in the degree of protogyny that has been observed to occur (pers. obs.), could be at a distinct advantage in high density mixed populations through the avoidance of hybridisation. Further work is needed to assess the relative competitive abilities of diploid and hexaploid pollen in effecting fertilization of the alternate cytotype.

The results also support the possibility of pre-zygotic isolating mechanisms that prevent the fertilisation of diploid ovules by pollen of alternate cytotypes (Petit et al. 1999). The low seed-set of females in mixed populations with few males, and the lower hybrid production relative to hermaphrodites indicate that the strength of such isolation would be stronger than in the hexaploids. In the greenhouse hybrid phenotype assessments, it was found that hexaploids produced more viable seeds than diploids; this can be accounted for by the amount of conspecific hexaploid pollen produced by the two hermaphrodites in the array, where even under conditions of high density with high male frequency, the amount of hermaphrodite pollen led to fertilisation. Previous work has recorded that beyond interplant distances of 30 cm, outcrossing rates become negligible (Eppley and Pannell 2007), and indeed the isolated females occurring in low density monoecious patches did not produce any hybrid progeny.

Asymmetrical crossing has been implicated in contact zone movement in other species as well. In a hybrid zone between chickadees *Poecile atricapillus* and *Poecile carolinensis*, dominance of *P. carolinensis* males, or their preference by *P. atricapilis* females are implicated in the movement of their contact zone in favour of *P. carolinensis* (Bronson et al. 2003, Woodcock et al. 2005, Reudink et al. 2006). Movement biases may also arise where a preference exists for males of a certain type, as with females of the katydid *Orchelimum nigripes* that prefer conspecific mates, compared to *O. pulchellum* females that exhibit no preference; movement is thus biased in favour of *O. nigripes* (Shapiro 2001).

The production of sterile hybrids represents a loss of both viable ovules, and also the resources required to produce hybrid seeds. This study and earlier work has shown that the viability of hybrid seed is generally very low (Buggs 2004). The phenotypic assessment of hybrids confirmed that apparent male and female sterility are highly associated with tetraploids, with all of the plants that exhibited these traits having being confirmed as hybrids through the use of flow cytometry. This knowledge is a very helpful tool for unequivocally identifying hybrids in the field, with non-viable or no male flowers, and infertile female flowers, but should be taken as an underestimate of actual hybrid production. An earlier glasshouse assessment found that, of hybrids with hexaploid mothers, only 1 of 48 plants set more than one seed, and 11 of 52 plants producing any pollen at all (Buggs 2004). Across the *M. annua* species complex, tetraploid and hexaploid cytotypes are the least morphologically distinct, between which more than 10% have previously been misclassified in each direction (Obbard 2004). The morphological assessment in this study provides a reliable phenotypic indicator that can be easily used to identify tetraploid hybrids in the field.

The loss of resources due to competition with sterile hybrid offspring within a mixed population should not be a significant detriment to the fitness of either cytotype, given their apparent rarity in the field. Under conditions of increased population density in mixed populations, and thus increased production of hybrids that attain a vegetative state, the effect of competition from hybrids on non-hybrids remains to be observed. However, the expectation is that competition should occur against both parental cytotypes, thereby accelerating the process of MCE because the proportion of minority seed contributed to the seed pool would be reduced regardless of its minority frequency. Differences in competitive ability between cytotypes at a contact zone has previously been observed in *Senecio carniolicus* in the Eastern Alps (Hulber et al. 2009), with hexaploids found in more species-rich and denser communities relative to diploids. This environmentally mediated distribution of cytotypes in *S. carniolicus* provides a basis for reproductive isolation and thus prevents the occurrence of MCE in the natural environment, leading to less than 1% of plants across populations being of secondary hybrid cytotypes. Narrow stable contact zones have also been observed in *Knautia arvensis* (Kolar et al. 2009), *Melampodium* (Stuessy et al. 2004) and *Ranunculus adoneus* (Baack 2004).

3.4.2 Tension zone dynamics and demographic effects

In a tension zone, the area of contact is expected to stabilise due to selection against hybrids, thereby moving the zone towards areas of minimal dispersal between lineages. Such areas can be characterised by relatively low density and population establishment rates (as has been identified in Chapters 2). The selection against hybrids leads to positive frequency-dependent selection in outcrossing species (Levin 1975), wherein the proportion of mating events for each cytotype that yield unfit hybrids will more frequently involve the minority cytotype, reducing its frequency in the population relative to the alternate cytotype, and further reinforcing tension zone dynamics. These patterns were indeed identified in the field, in both naturally occurring, and experimentally established populations, with the ratio of hexaploid:diploid plants significantly decreasing into year 2. The starting densities and relative frequencies of each cytotype were highly variable even among neighbouring populations. Such variability has also characterised populations of mixed ploidy in *Solidago altissima* (Halverson et al. 2008). This provided ample opportunity to assess the role of density and relative ploidal frequency in mediating fluctuations in population size for each cytotype. The reduction in relative hexaploid frequency in the *M. annua* populations was inversely related to sowing density; at higher densities, the reduction in hexaploid frequency was further exaggerated. Hybridisation rates of diploid ovules were uniformly low, and there was no significant positive relationship between their hybridisation and the frequency of hexaploids in a population, thereby verifying in the field earlier laboratory based experiments (Buggs 2004).

Despite the relative advantages of the diploids over the hexaploids in effecting hybridisation of the alternate cytotype, most populations went extinct into the second year of study, and those that did not rapidly reached an equilibrium of only one cytotype per population. With 11 populations settling in pure diploidy, and only 4 in pure hexaploidy, this observation is a testament to the facility with which diploids may be able to expand their range into the predominantly monoecious region south of the current diploid-hexaploid interface. The reproductive handicap in a given generation leads to a greater handicap in the next, with the minority cytotype being rapidly excluded from the population. Self-pollination is expected to reduce the minority disadvantage and the tempo of the exclusion process (Levin 1975). However, the observed population dynamics did not support the view that reproductive assurance at low densities of hermaphrodite hexaploids should mitigate the effects of asymmetric pollen swamping by the diploids. The diploid advantage may additionally be supported by more effective post-zygotic isolating mechanisms that act to abort seed sired by hexaploids, relative to any similar mechanisms in the hexaploid counterparts. There are few explicit studies of population differentiation in polyploids, let alone the mechanisms governing such isolation. A study by Werth and Windham (1991) proposed that the silencing of the same gene in different populations in polyploids composed of differentiated chromosome sets (i.e. allopolyploids, putatively like that of hexaploid *M. annua*) would lead to 25% of F1 hybrids in which neither copy of the gene is functioning. In this manner it is thought that post-zygotic isolation may accrue rapidly. Further studies of population differentiation in polyploids as compared to their diploid progenitors are needed to reveal evolutionary patterns associated with polyploidy.

A study of interpopulation hybrids in *Campanula americana* that have been shown to differ in genome size did not identify any relationship between parental genome size and offspring fitness (Galloway and Etterson 2005). Although DNA content was shown to vary among populations of *C. americana*, differences were not associated with the loss or gain of genes that influence cross-compatibility. A review of experiments on 14 genera of angiosperms by Tiffin et al. (2001) found that the strength of postzygotic isolation may differ depending on which species is used as a pollen parent and which is used as a seed parent. Asymmetries in post-zygotic isolation are likely to be caused by nuclear-cytoplasmic interactions (Grun 1976, Levin 1978). Hybrids from crosses involving parents with different chromosome structure may have low fitness because of abnormal segregation during meiosis, which may in turn result in gametes with genetic duplications and deficiencies that reduce hybrid fertility (Rieseberg et al. 1999)

Isolating mechanisms between cytotypes that have been found to be more effective in other species include the example of diploid and polyploid populations of *Dactylis glomerata* (Lumaret et al. 1987, Lumaret and Barrientos 1990) and *Claytonia virginica* (Lewis 1976) wherein flowering phenology prevents pollen pool overlap. Flowering phenology has also enabled stable sympatry in the different cytotypes of *Heuchera grossulariifolia* (Segraves and Thompson 1999). In *M. annua* however, hybridisation indeed occurred across all densities in favour of the diploids, which also experience greater population growth into the second year of study in the low-density experimental sites. Barton and Hewitt (1985) argued that tension zones will be stable in troughs of low population density and these would be frequent and deep enough to resist movement due to fitness differences or asymmetry of hybridisation. The performance of diploid *M. annua* observed at low-densities in this study however should enable the diploid advance to rapidly sweep through the current low-density trough in which the contact zone currently resides. Thus the low-density area is not expected to act as a hybrid sink *per se* (Hall 1973, Barton 1980) where more organisms are expected to migrate into the region than emigrate out.

Movement of the tension zone is expected to occur when there are biases in dispersal, caused, for example, by differences in fertility between lineages. The bias in pollen production of diploids over hexaploids observed in this study has clearly contributed to their relative advantage in effecting hybridisation, and can be attributed to the advantage of sexual specialisation and concomitant morphological adaptations. These adaptations may be largely held responsible for the bias in movement of the contact zone in favour of the diploids. Bias in pollen flow from tetraploids to diploids in *Chamerion angustifolium* (Husband and Schemske 2000, Husband et al. 2002) provides a relevant comparison to the *M. annua* study system. It is expected that the directional biases experienced in *C. angustifolium* pollen flow should similarly allow tetraploids to expand their range at the expense of diploids that produce triploid hybrid progeny of reduced fitness (Husband and Sabara 2004).

3.4.3 Concluding remarks

The rapid rate over which mixed-ploidy populations became homoploid in this study offers further explanation to the relative rarity of mixed-ploidy populations in Catalonia and Galicia. Additionally, the narrow, unsuitable coastal strip where the contact zone resides and is currently moving further into supports the hypothesis of tension zone dynamics in mediating the relative frequency and positioning of diploid and hexaploid *M. annua* in this region. Despite the advantage of self-fertilisation experienced by hexaploid hermaphrodites, diploids were still capable of effectively hybridising the ovules of hexaploids under a range of densities and relative frequencies due to asymmetric pollen production. This advantage could also be supported by differences in post-zygotic isolating mechanisms between lineages, wherein diploids may be more effective at aborting seed sired by hexaploids than *vice versa*. Asymmetrical reproductive isolation in plant taxa will have important implications for the dynamics of hybrid zones, the direction of genetic introgression and the probability of enforcement. This should serve to tighten the area of contact, with mixed-ploidy populations becoming localised to only the most isolated and lowest density habitat available in the northeastern contact zone, with the demise of hexaploid populations being otherwise inevitable. Even under conditions of low population density and frequent turnover in a natural environment, the significantly greater pollen fertility of the diploids will allow them to overcome the minimal effects MCE upon migration into monomorphic populations, and accelerate the geographic displacement of the hexaploids.

3.5 TABLES AND FIGURES

Figure 3.1 Diploid (paternal) / hexaploid (maternal) hybrid bearing male and female flowers on a peduncle. Male flowers did not produce pollen and female flowers did not set fruit.

Figure 3.2 Diploid female (left) with full seedset, and hexaploid (paternal) / diploid (maternal) tetraploid hybrid bearing sterile female flowers.

Table 3.1 Back-transformed parameter estimates from GLM of pollen pool parameters on hybridisation rate of ovules of focal hexaploid hermaphrodite plants in naturally occurring mixed populations.

Parameter	Estimate	s.e.	*z*-value	*P*
Intercept	0.33	1.01	-128.88	<2e-16
log Distance	47.08	1.40	50.66	<2e-16
Size	2.79e+08	1.49	69.84	<2e-16
Ploidy	8.02+e08	1.48	13.86	<2e-16
Density	8.57e+29	2.67	7.07	<2e-16

Table 3.2 Back-transformed parameter estimates from GLM of pollen pool parameters on hybridisation rate in ovules of focal diploid female plants in naturally occurring mixed populations.

Parameter	Estimate	s.e.	*z*-value	*P*
Intercept	0.12	1.07	-32.65	<2e-16
log Distance	4.31+18	2.03	60.9	<2e-16
Size	3.39e-08	1.67	-33.59	<2e-16
Ploidy	2.00e-16	1.99	-52.6	<2e-16
Density	3.09e+02	1.21	29.9	<2e-16

Table 3.3 Results from *t*-tests on coefficients of hermaphrodite *vs*. female GLMs of pollen pool on hybridisation rate. This tests for the disproportionate influence of any of the model parameters on hybridisation rates on hexaploids and diploids, with the difference between estimates and test result displayed.

Parameter	Δ t_{76} estimate	*P*
Height	9.30	3.49e-14
Diameter	47.19	4.73e-58
Distance	50.06	6.06e-60
Size	56.61	6.75e-64
Ploidy	71.53	1.78e-71
Density	63.05	2.21e-67

Table 3.4 Comparison of mass-action and 'individual-based' pollen pool GLMs for focal hexaploid and diploid plants.

Model Type	Focal Plant Ploidy	AIC	Resid. Dev.	χ^2
Mass-action	Diploid	12978	12706	$P < 2.2e-16$
Individual-based	Diploid	8974	8700	
Mass-action	Hexaploid	80708	80095	$P < 2.2e-16$
Individual-based	Hexaploid	80578	80227	

Table 3.5 Percentage of dioecious and monoecious *Mercurialis annua* in the 12 naturally occurring mixed-ploidy populations that persisted from 2010 into 2011.

Population (synonym)	**Plant type**	**2010**	**2011**
A (1)	Dioecious	5.0%	100%
	Monoecious	95.0%	0%
	n.	180	4
B (5)	Dioecious	72.7%	100%
	Monoecious	27.3%	0%
	n.	77	49
C (6)	Dioecious	15.0%	0%
	Monoecious	85.0%	100%
	n.	20	12
D (7)	Dioecious	61.1%	100%
	Monoecious	38.9%	0%
	n.	72	142
E (9)	Dioecious	22.2%	0%
	Monoecious	77.8%	100%
	n.	30	55
F (10)	Dioecious	10.0%	100%
	Monoecious	90.0%	0%
	n.	9	10
G (13)	Dioecious	50.7%	23.1%
	Monoecious	49.3%	76.9%
	n.	75	26
H (18)	Dioecious	24.1%	100%
	Monoecious	75.9%	0%
	n.	83	23
I (19)	Dioecious	6.3%	0%
	Monoecious	73.7%	100%
	n.	16	1
J (24)	Dioecious	47.4%	100%
	Monoecious	52.6%	0%
	n.	19	24
K (26)	Dioecious	12.3%	100%
	Monoecious	87.7%	0%
	n.	65	6
L (28)	Dioecious	16.7%	0%
	Monoecious	83.3%	100%
	n.	18	3

Table 3.6 Minimum adequate linear model on the change in the ratio of hexaploid:diploid plants from 2009 to 2010 across experimental sites. Adjusted $R^2 = 0.33$, $F_{2,37} = 7.42$, $p = 5.41e-4$.

Parameter	Estimate	s.e.	*z*-value	*P*
Intercept	4.95	2.45	2.03	0.05
Sowing area	0.42	0.23	1.82	0.08
# Diploids in year 1	-0.24	0.07	-3.69	7.35e-4

CHAPTER 4. GENERAL DISCUSSION

Studies of hybrid zones offer ‘windows on the evolutionary process’ (Harrison 1990) and have provided fruitful research avenues for a wide variety of studies, many of which have focused on trying to understand how patterns of gene flow and introgression between taxa influence the processes important in creating and maintaining biological diversity (e.g. Szymura and Barton 1986, 1991, Porter et al. 1997, Rieseberg et al. 1999, Payseur et al. 2004, Brumfield 2005, Carling and Brumfield 2008, Nolte et al. 2009, Teeter et al. 2010). The primary aim of this study was to create a detailed picture of the behaviour of the northeastern diploid-hexaploid tension hybrid zone of *M. annua*, with a window to the role of density and lineage interaction in governing its long-term persistence, or demise. This was done through a series of both multi-year field experiments in the zone itself, and studies conducted under controlled laboratory conditions.

Although the causes of hybrid zone movement are seen to be varied (Buggs 2007), two general models of hybrid zone structure have emerged: 1-the tension zone model (Key 1968), and 2- the environmental selection gradient, or ecotone model (May et al. 1975), which together provide a framework for understanding the factors underpinning hybrid zone movement. Under the tension zone model, the hybrid zone is necessarily maintained by a continual influx of parental types into the contact zone from allopatric populations as endogenous selection against unfit hybrids causes their disappearance (Barton and Hewitt 1989). Given that selection is endogenous in the case of a tension zone, such zones are expected to move according to gradients in population density as the interface between the lineages tightens, and dispersal occurs so as to avoid contact. Tension zones are thereby predicted to settle in areas of low population density or low population suitability. By contrast, exogenous selection, as that which occurs in an ecotonal hybrid zone, leads to fitness differences along environmental gradients, and can give rise to population shifts along such gradients (Carling and Zuckerberg 2011). It follows then that if one of the lineages in a contact zone is expanding its range, hybrid zone movement may then ensue, as has indeed been documented in *Mercuralis annua* (Buggs 2007), and populations of red and sika deer (Goodman et al. 1999), among other examples.

To disentangle the various factors influencing the structuring and maintenance of this hybrid zone, I first considered the colonisation and extinction dynamics of the competing lineages *in situ* (Chapter 2). This provides a picture of how diploid and hexaploid populations can be expected to persist in isolation of each other, assessing whether the advantages of self-fertilisation and reproductive assurance at low densities provide a quantifiable benefit to the hexaploid lineage, over the diploids, which may be pollen limited under such conditions. In the second study (Chapter 3), I consider migration into habitat occupied by the alternate cytotype through a setup of both experimental and naturally occurring mixed populations. The naturally occurring populations were mapped in a spatially explicit manner that enabled calculation of the precise distance to and frequency of neighbours of each cytotype in a population. I was then able to quantify the rate at which the minority cytotype of a population would produce hybrid progeny, and the precise nature of the advantage that diploids have over hexaploids in mixed populations.

The large-scale experimental metapopulation setup of Chapter 2 enabled the first explicit assessment of the role of seed bank dynamics in buffering populations of *M. annua* against environmental uncertainty leading to extinction. The production of dormant seed was equally likely in both lineages, and thus should not provide either cytotype with asymmetric benefits. The direct 3-year observation of 840 experimental introductions of varying density across the northeastern diploid-hexaploid contact zone has permitted precise estimates of the likelihood establishment and extinction in this vicinity. Both emergence and population size declined throughout years, and germination rates were marginally higher in the diploid stands, showing that this lineage is better adapted to the zone than the hexaploids that currently reside there. Seed production however was significantly density-dependent in the diploids, which produced lower amounts of seed than the hexaploids under conditions of low density, likely given their inability to self-fertilize. Establishment was generally very low throughout the 20-km long study area, with virtually all sites experiencing a notable reduction in population size into the third year of study, and less than 10% of sites remaining by the end of the study period.

Given previous surveys that have identified a low frequency of occurrence of *M. annua* populations surrounding the study region (Eppley and Pannell 2007), it becomes apparent the amount of seed rain possibly arriving in the contact zone will not be of a sufficiently high volume to sustain the long-term persistence of either lineage in this area, or to deliver populations of a sufficiently high density to avoid an Allee effect through pollen limitation in the diploids. The area can thus be characterised as a low-density trough that should cause the contact zone to tighten in its spread across the area, narrowing the north-south diploid-hexaploid cline. The advantage of reproductive assurance in the hexaploids will only serve as a temporary benefit against a backdrop of widespread population decline. It is not expected that populations will attain densities sufficiently high to produce a notable frequency of mixed populations in this contact zone (indeed fewer than 40 were identified through this work over 4 years of detailed survey across a 40 km^2 area), which should further enhance the spread, where suitable sites do exist, of the ecophysiologically superior diploid lineage. The putatively stable habitats (in the sense of long-run metapopulation viability) surrounding this area of contact is expected to produce an excess of immigrants (source type populations), whereas the low-suitability area under study should be more 'permeable to migration' (cf. Dias 1996). It must be noted however that this study did identify the formation of a viable seed bank that persisted for at least two years. In many of the high-density sowing treatments, it was observed that the majority of the population was contained below ground in the form of a dormant seed bank than above ground in the vegetative phase. I have discussed the importance of a seed bank in buffering populations against short-term patterns of population extinction, allowing re-emergence (colonisation in time rather than space) when habitat becomes suitable, however an additional view that needs to be addressed is that the majority of the life-cycle of *M. annua* is actually spent in the seed phase. Re-emergence from the seed bank was not however observed to mitigate the overarching pattern of population size and frequency decline in the four years of study. This reiterates the view that the presence of *M. annua* in the study region is microsite-limited, and not only seed-limited. Furthermore, reductions in the contribution to the seed bank, through the aboveground decline in population size as might be experienced in a low-density trough, can be expected to lead to extinction both apparent and non-apparent. In this way, the seed bank has been said to act as a memory that "stores" the cumulative effects of recurrent extinction through time. In the simulations of Maron and Gardner (2000), even infrequent pulses of seed-limited recruitment enabled accumulated seed loss from herbivory to be expressed in terms of reduced seedling recruitment and adult abundance. Significant reductions in population size were detected even when averaged across many years where recruitment was safe-site-limited and therefore uncorrelated with seed bank abundance

Similar patterns of hybrid zone movement towards low-density troughs have recently been documented in the house mouse hybrid zone (Nunes et al. 2009), in which the interactions between neighbouring lineages are restricted. Moreover, given that population sizes obtained within this *M. annua* contact zone have been observed to be low relative to the surrounding regions (Eppley and Pannell 2008), dispersal out of this density trough is expected to be rare, thereby enhancing geographical isolation of the two lineages. The contact between diploid and hexaploid *M. annua* may have settled into this region without actually being maintained by climate-associated selection, given the observed importance of endogenous selection and demographic parameters in mediating patterns of population colonisation and extinction across the region. Such environmentally independent movement leading to settlement in a density trough has also been characterised recently in the *Plethodon metcalfi*/*P. jordani* hybrid zone, and attributes asymmetric population density to the primary cause of movement (Chatfield et al. 2010).

The demes under study within the experimental metapopulation framework of Chapter 2 largely exhibited negative long-run growth rates for the duration of the study. The observation of population size declines at even the highest starting sizes and densities confirms seminal works in this field of Ludwig (1976) and Brockwell (1985), wherein the persistence of populations already undergoing long-run negative growth will be independent of populations founding size. The units under study have been shown to be particularly susceptible to demographic stochasticity, with extinction influenced by an Allee effect at low densities in the case of the diploid lineage, and an inability to establish generally in the hexaploids. The susceptibility of the study populations to demographic stochasticity can be traced to their low maximum size, and follows intuitively from the fact that the importance of demographic stochasticity in causing fluctuations in population growth rate, is inversely proportional to population size (Lande 1992). The concentration of hybrid zones across areas low suitability for population growth relative to adjacent refugia is a recurring observation in studies secondary contact (Barton and Hewitt 1989), and has been observed even more broadly at the scale of assemblages of taxa occurring in tropical sutures (Moritz et al. 2009).

Over the course of four years of observations at the northeastern diploid-hexaploid contact zone, I was able to identify a number of naturally occurring mixed populations, in addition to experimentally mixed populations that I had established in the first year of study. Spatially explicit mapping of individual populations has allowed me to quantify the precise nature of the advantage that diploids maintain over hexaploids in effecting hybridisation of the ovules of the alternate competing cytotype. It is expected that the proportion of mating events for each cytotype that yield unfit hybrids will more frequently involve the least common cytotype, reducing its fitness relative to the common one, and eventually leading to its extinction (Rodriguez 1996, Husband 2000, Dorken and Pannell 2007). To my knowledge this is the first study to explicitly document diploid-hexaploid hybridization rates and the occurrence of minority cytotype exclusion in this moving *M. annua* contact zone. Diploids were found to be almost 3-times as likely to induce hybridisation of ovules in the alternate cytotype that were hexaploids, for a given set of measured parameters (density of each cytotype, plant sizes, cytotype frequencies, interplant distances), beyond accounting for relative differences in pollen production between lineages. It is expected that this disparity reflects the advantage afforded by specialised pollen dispersing structures and floral architecture, but the possibility of prezygotic selection against mismatched pollen cannot be excluded. Most of the mixed populations either went extinct into the final year of study, or settled into a pure population of only one cytoype (11 into diploidy *vs.* 4 polyploidy). It is clear that although the zone represents a trough of low density, the possibility of establishment is not altogether excluded given the availability, albeit in low frequency, of suitable microsites across the study region. Persistence of populations across multiple years is however low in the area, but should be sufficient to enable diploid populations to sweep through into more regionally abundant hexaploid populations further south. This would of course depend on the intensity of seed rain arriving in the region, and although difficult to assess, is an important aspect in need of experimental quantification.

The studies within this thesis began almost half-a-century ago with the hypothesis of Durand (1963) that differential environmental adaptation is the driving force in the positioning of the diploid-hexaploid *M. annua* contact zone. Through a combination of glasshouse-based studies, *in situ* field experiments and multi-year observations I have conducted a detailed assessment of the *M. annua* tension hybrid zone, and the forces governing its structure. The contact zone does not appear to be maintained by differential habitat preferences between cytotypes and their local adaptation, and as such its movement should not be impeded by habitat characteristics that are better suited to the hexaploids that are currently being displaced. Ecological niche modelling approaches have been used to identify environmental factors underpinning the geographic location of hybrid zones, such as that which occurs in the *Passerina amoena*/*P. cyanea* hybrid zone (Swenson 2006), positing that *P. amona* is better adapated to the western xeric environment and *P. cyanea* to the wetter mesic environment of the east, and that of Bullock's Oriole *Icterus bullockii* and the Baltimore Oriole *I. galbula* (Rising 1969). Indeed adaptation to a xeric environment was initially proposed by Durand (1963) to explain the distribution of hexaploid *M. annua* because of their ability to self-fertilise under low population density, as compared to obligate outcrossing diploid *M. annua* which were thought to be restricted to the more mesic northerly environment. This study in contrast finds that diploids are better suited throughout the contact zone under study, and thus environmental shifts would do little to engender changes in the structure of the hybrid zone.

I have identified strong evidence of endogenous selection acting against hybrids in the northeastern *M. annua* contact zone, and evidence of exogenous selection acting through differential adaptation of the lineages, with diploids outperforming hexaploids throughout the study area. The low suitability of the area generally represents a low-density trough that should act to slow the current rapid movement of this northeastern contact zone. This should serve to tighten the area of contact, with mixed-ploidy populations becoming localised to only the most isolated and lowest density habitat available in the northeastern contact zone, with the demise of hexaploid populations being otherwise inevitable. Similar patterns of narrowing have been observed in the *Passerina* bunting hybrid zone (Carling and Zuckerberg 2011), where there was a nearly threefold reduction in cline width between two temporal samples, suggesting a movement rate of approximately 2.5 km per year.

Even under conditions of low population density and frequent turnover in a natural environment, the significantly greater pollen fertility of the diploids will allow them to overcome the minimal effects MCE upon migration into monomorphic populations, and accelerate the geographic displacement of the hexaploids. Revisiting this study site in future years will prove valuable in supporting or refuting this interpretation of the dynamics currently observed.

Hybrid zones, particularly those in which movement has definitively been documented, provide excellent opportunities to view evolution in action. The secondary contact of lineages that had remained isolated in disjunct refugia during the glacial periods that dominated much of the past two million years now provide an opportunity to compare levels and patterns of reproductive isolation, in relation to divergence history and phenotypic evolution (Remington 1968, Hewitt 1988). The continued use of detailed field studies at contact zones (Graham et al. 1995), coupled with novel distributional modelling approaches currently being developed (Swenson 2008, Moritz et al. 2009), will ensure that hybrid zones continue to provide unique windows for the observation evolutionary process.

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