PLASTIC AND GENETIC RESPONSES TO ENVIRONMENTAL CHANGES

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By
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\[ R^2 = 77.4\%, \; H: y = \frac{2.445}{1 + e^{(-4.923 - 2.187x)}} \]
\[ R^2 = 50.4\%, \; S_g: y = 0.036x + 0.512, \; R^2 = 38.8\% \]

Free-to-vary genome size:

\[ G: y = \frac{0.869}{1 + e^{(-2.317/h - 0.967)}} \]
\[ R^2 = 79.5\%, \; H: y = \frac{2.064}{1 + e^{(-4.05 - x/1.837)}} \]
\[ R^2 = 40.8\%, \; S_g: y = 0.028x + 0.513, \; R^2 = 20.9\% \] Each point represents a single replicate of 100 total replicates at each fluctuation rate. All curves are significant at P<0.00001. .......................... 94

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Abstract

Human activity is causing climates to change more rapidly than at any time in the last 10,000 years. If populations of organisms are unable to effectively respond to changing environments, they will be at risk of extinction. In plants, two of the most important mechanisms of response to environmental change are phenotypic plasticity, where the same genotype expresses different phenotypes in different environments, and adaptation, which requires changes in allele frequency in populations as exposed individuals show variable survival and reproduction. Although most researchers accept the importance of both of these mechanisms, they are most commonly considered in isolation in models of response and persistence to climate change. Here, I use the model species *Arabidopsis thaliana* to investigate the interaction of plasticity and selection in fitness and phenology response to simulated climate warming, the effect of artificial selection on variation for plastic response and cross-generational effects of environmentally induced variation in flowering time. I also study the effects of varying rates of environmental fluctuation on evolvability on populations of self-replicating computer programs using the artificial life platform *Avida*. I find that a small increase in ambient temperature, in line with predictions for the next few decades, is able to elicit significant plastic responses and that these responses have the potential to alter population genetic structure and affect future evolution. I also find that selection on flowering time can reduce variation for plastic response and that non-genetic effects on flowering time can significantly alter germination in the next generation. Lastly, I find that rapidly changing environments in the long term can select for more evolvable populations and genotypes. These results highlight the importance of considering plasticity and evolution together if we are going to make accurate predictions of climate change response.
Declaration

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This thesis is presented in the “alternative format” according to the University of Manchester procedures and guidelines, which allows the inclusion of work presented in a format suitable for publication in a peer-reviewed journal. All chapters in this thesis are in one consistent format, but each chapter has its own abstract, introduction and discussion in preparation for publication as an original contribution of research.

All chapters are the sole work and authorship of myself under the supervision of Paula Kover, with the following exceptions: In chapter 4, the experimental design and execution of the experiment itself was by Nora Scarcelli, Jennifer Rowntree and Paula Kover. I performed the analysis and wrote the paper. Chapter 6 was a collaboration between myself, Robert Platt and Daniel Rozen. In this case, myself, Daniel Rozen and Robert Platt designed the experiment; myself and Robert Platt performed the experimental runs and collected data; I performed the analysis and wrote the paper.
Chapter 1

Introduction

1.1 Response to changing environments

“Nothing endures but change” - Heraclitus (535 BCE - 475 BCE), from Diogenes Laertius, Lives of Eminent Philosophers

“There is almost no species for which we know enough relevant ecology, physiology and genetics to predict its evolutionary response to climate change” (Holt, 1990)

All environments are heterogeneous to some extent, whether it be through space, for example as an organism forages or disperses from its natal territory, or time, due to daily variation, seasonal fluctuations or longer term climate change. The ability of organisms and populations to respond effectively to changes in their environment is critical for their persistence and survival. The structure and composition of organisms’ environments are partly determined by abiotic factors, but organisms also do not exist in isolation and are influenced by interactions with other organisms. The response of plants and animals to a changing environment will be dependent on the responses of these other organisms as well as to the changing physical and chemical properties of that environment. Whereas in the past, environmental variation in organism traits was seen as noise to be eliminated in order to get to the true response, studying genotype-by-environment interactions between organisms and their environment is now one of the most active fields of research in ecology and evolutionary biology. Climate scientists have identified five major types of human-induced environmental change: habitat loss and fragmentation, the spread of exotic and invasive species, harvesting by humans, environmental pollution and, of course, climate change (including global warming and an increase in occurrence of extreme weather conditions) (Young et al., 1996; Rohr et al., 2006; Lockwood and Frohlich, 2007; IPCC, 2007; Salo et al., 2007). As the effects of human activity on global climates have become generally acknowledged in the scientific literature, the study of organismal and genetic response to changing environments is now of critical importance to conservation biology and agronomics: Predicting accurately how changes in climates through, for example, warming or invasive species will affect populations should directly influence policy, and there are huge economic and social benefits if plant breeders are able to produce crops that can grow beyond current climatic tolerances.
1.1. RESPONSE TO CHANGING ENVIRONMENTS

Living systems have evolved a broad range of mechanisms to cope with environmental change that occurs over different scales of time and space. Some organisms may be able to move in response to change, either seasonally, as in a migratory bird, or over a number of generations as seen in shifting ranges of boreal forest trees in response to polar warming. Several animal species are also known to have shifted range in response to climate change in the 20th century (Walther et al., 2002). Evolutionary ecologists have traditionally recognised four strategies that organisms employ for physiologically coping with environmental change in situ: Specialism (a single, invariant phenotype is produced that is optimal in one environment, even though it might find itself in other environments), Generalism (an intermediate phenotype is produced that is at least moderately successful in different environments), Bet Hedging (where an organism produces different phenotypes probabilistically, for example through having many diverse offspring) and phenotypic plasticity, where different phenotypes are triggered by environmental conditions. Although processes like range shifts and plasticity may be the most immediate forms of response to a changing environment, directional selection will ultimately result in changes in allele frequency in populations as individuals show variable survival and reproduction, given certain genetic and demographic constraints. There is increasing evidence that the ability to adapt to new and heterogeneous environments (evolvability) is itself able to evolve in response to selection.

Many climate models indicate alarming consequences for biodiversity, with the worst-case scenarios leading to extinction rates that would qualify as the sixth mass extinction in the history of the Earth. Despite this, different models often give wildly differing predictions as to the response of organisms, communities and ecosystems, depending on the predicted factors and assumptions (Bellard et al., 2012). Until fairly recently, most climate studies have made the implicit assumption that there is no evolutionary component to the response to climate change (e.g. Thomas et al. (2004)). This is based on the common perception of evolution as a slow and gradual process. However, many recent studies have reported rapid or “contemporary” evolution on a timescale of a few generations (Hendry and Kinnison, 1999; Reznick and Ghalmor, 2001; Stockwell et al., 2003; Carroll et al., 2007). These observed cases of evolution on ecological timescales were often in response to anthropogenic environmental change, for example air pollution and heavy metal tolerance, herbicide and insecticide resistance, introduced species and hybridization between wild and domestic species (Reznick et al., 1997; Stockwell et al., 2003). Studies such as these resulted in a call to explicitly include evolutionary biology in climate models (O’Connor et al., 2012; Skelly and Freidenburg, 2010), since evolution is a key, and inevitable, response of organisms to changes in their environment. Explicitly including adaptation and plasticity in models has a significant effect on predicted species ranges (Garzon et al., 2011), with less dramatic estimates of species loss than simple climate envelope models. Recent studies also suggest that interactions between phenotypic plasticity and evolution should be incorporated into climate models, since plasticity is capable of having cross-generational effects (Reed et al., 2010).

In this introduction, I will discuss these different mechanisms of coping with environmental change, with particular focus on phenotypic plasticity, evolutionary response and evolvability, since these are the main themes of the experimental chapters in this thesis.
1.1. RESPONSE TO CHANGING ENVIRONMENTS

1.1.1 Range shift

One of the major predicted consequences of climate change is that species will move to higher latitudes and elevations as they are displaced from the climates to which they are adapted (Jump and Penuelas, 2005). There is already considerable evidence that species distributions are altered in response to climate change (Grabbherr et al., 1994; Davis and Shaw, 2001; Walther et al., 2002; Jump and Penuelas, 2005). For example, Lenoir et al. (2008) compared the recorded distribution of 171 western European forest plant species for the periods 1905-1985 and 1986-2005 (This threshold was chosen because analysis of mean surface temperature anomalies showed a shift in 1986, staying above the average baseline level since then). They found that climate warming had resulted in a significant upward average altitudinal shift of 29 meters per decade. The shift was greater for species restricted to mountain habitats. Similar patterns of significant elevational rises have also been observed in Alaska (Lloyd and Fastie, 2003), (Leif, 2002), the Alps (Grabbherr et al., 1994) and the Mediterranean region (Penuelas and Boada, 2003). A global meta-analysis of species distribution (Parmesan and Yohe, 2003) documented significant range shifts averaging 6.1km towards the poles, and Sturm et al. (2005) show how shrubs have advanced latitudinally into the Arctic tundra.

Range shift will often be constrained by geographical factors. The migration of alpine plants experiencing an upward shift in optimum elevation will be constrained by the height of the mountain and by competition from other populations better adapted to the environment it is being forced into. Habitat fragmentation is likely to further impede migration, and migration is expected to be slower than during the last recession of the glaciers as it relies on seedling establishment in occupied habitats (Etterson and Shaw, 2001). We may therefore expect to see greater pressures on organisms to adjust phenotypically or genetically in future than in past climate change events as range shift is more constrained.

Rates of natural climate change in the past have allowed for a balance between adaptation and migration, but predicted rapid, human mediated, climate change, in conjunction with reduced gene flow due to habitat fragmentation and land use changes, has the potential to disrupt this balance. This means that populations are forced into environments to which they are poorly adapted. Davis and Shaw (2001) note that current climate predictions for the 21st century necessitate range shifts for tree species of 300-500km per century, in contrast to recent past migration rates of 20-40km per century. Fossil evidence from exceptionally rapid migration during the Quaternary record maximum migration rates of 100-150km per century. This suggests that predicted rates of climate change would require unrealistically fast rates of migration, resulting in many species being unable to cope and facing extinction.

1.1.2 Physiological tolerance

The following four strategies can be grouped into those which adopt a single phenotype (specialism and generalism) and those that adopt variable phenotypes (bet-hedging and phenotypic plasticity). Note that there is no necessary exclusivity between these strategies and it is perfectly possible (and even likely) for integrated strategies combining two or more of the pure strategies to evolve. Models have shown that, depending on the granularity of the environment and other factors
1.1. RESPONSE TO CHANGING ENVIRONMENTS

(such as costs of plasticity, see below), these integrated solutions can confer significant fitness benefits over pure strategies (DeWitt and Langerhans, 2004)

1.1.2.1 Specialism and Generalism

A specialist produces a single phenotype that is optimal in a single environment, but sub-optimal in others, while a generalist will maintain a single phenotype that is intermediate between the environments it experiences, so that it is not penalised too heavily in any one environment. There is clearly an evolutionary trade-off between these two strategies, (Wilson and Yoshimura, 1994) identified four factors influencing the relative fitness of generalists and specialists:

1. The fitness set (Figure 1.1 on the following page). To what degree do the fitness functions in different environments overlap? The curve of the fitness sets must be concave in order for specialism to evolve.

2. Activity/habitat selection. For example diapause, hibernation, seed dormancy. If organisms are able to select one habitat and avoid the other, specialism can be favored even with a convex fitness set, since the cost of specializing (lower fitness in the less favoured environment) is seldom experienced. It can be argued that habitat selection is often due to Phenotypic plasticity (for example in germination timing (Donohue et al., 2005b)), so this in fact is an example of an integrated strategy (DeWitt and Langerhans, 2004).

3. Negative density dependence. Fitness associated with a particular environment depends not only on the abilities of the organisms to exploit the resources available in that environment but also on the number of other organisms exploiting the same resources.

4. Temporally varying environments. Seasonal fluctuations and stochastic variation force organisms to perform a range of different activities, so increasing the cost of specialism.

1.1.2.2 Bet-hedging

Bet-hedging strategies achieve a decrease in temporal fitness variation at the expense of a reduction in average fitness. (Childs et al., 2010). Bet-hedging is expected to evolve under conditions of unpredictable environmental variance and where environmental cues and the evolution of phenotypic plasticity (See 1.1.2.3 on page 21) are partially impeded. It is a response to environmental variation itself, rather than to any particular environmental cues.

Bet-hedging strategies aim to increase geometric mean fitness (the geometric mean of $n$ numbers is the product of those $n$ numbers raised to the power of $1/n$) over the long run, even if arithmetic mean fitness may be consequently lower. This is because fitness, like population growth, is an inherently multiplicative process that is very sensitive to occasional low values (Childs et al., 2010). So, if a genotype experiences even rare occurrences of zero fitness, it has an expected geometric mean of zero and will ultimately become extinct, even if high fitness at other times results in a high arithmetic mean fitness.

Philippi and Seger (1989) identified two distinct classes of bet-hedging strategy:
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Figure 1.1: Fitness functions (top) and fitness sets (bottom) for two environments. The lines in the fitness sets represent the set of genotypes that can potentially exist in the population. (a) Fitness functions for the two environments ($w_1(x) = \text{black}$, $w_2(x) = \text{red}$) are closely overlapping, resulting in a convex fitness set conferring a higher cost of specialism. (b) The two fitness functions are only slightly overlapping, resulting in a concave fitness set conferring a higher cost of generalism. Adapted from DeWitt and Langerhans (2004).

- **Conservative bet-hedging** entails individual risk avoidance, where an individual sacrifices expected fitness in order to reduce temporal variance in fitness. Examples include semelparous perennial plants initiating flowering early in life to avoid an occasional high mortality year (Rees et al., 2006) and iteroparous plant species storing limited resources (e.g. water in arid habitat) in order to mitigate future scarcity, instead of using it immediately for reproduction and growth. These strategies can only be classed as bet-hedging strategies if they involve a trade-off between mean and variance of fitness, making this form of bet-hedging difficult to identify (Childs et al., 2010).

- **Diversified bet-hedging** is when the same genotype produces a range of different phenotypes probabilistically, spreading the risk to ensure non-zero survival overall. The classic example of diversified bet-hedging is seed dormancy in annual plants, for example in the formation of a “rosette bank”, whereby individuals of one genotype initiate flowering at a range of different ages so that their seeds are dispersed in time (Rees et al., 2006). Even though individuals are subject to increased mortality as a consequence of the delay, dormancy (or delayed flowering) is selected because it reduces fitness variation at the genotype level.

Despite much recent interest and active research in bet hedging, conceptual and practical difficulties (compounded by confusion over what constitutes evidence for its existence) make its study challenging (Simons and Johnston, 1997). Because bet-hedging strategies maximize geometric-mean fitness across generations but do not maximize the expected fitness within a generation, adaptive bet-hedging can often only be detected in the long run and often may
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actually increase the variance in fitness among individuals within a generation, such that individual-level analyses can be misleading. Appraisals of optimality are rare even over a single generation, let alone over longer time scales, and the absence of a framework for evaluating the strength of evidence where it does exist means that we still do not know how common bet-hedging is.

1.1.2.3 Phenotypic plasticity

“Why is there not just one species, infinitely plastic, with individuals capable of producing the appropriate phenotype in the ecological context they find themselves?” (Whitlock, 1996)

Phenotypic plasticity is the property of a given genotype to produce different phenotypes in response to distinct environmental conditions (Pigliucci, 2001).

Historically, plasticity has been seen as non-heritable phenotypic variation producing no evolutionary response and so has been dismissed as unimportant in adaptive evolution (Wright, 1931). It was Bradshaw (1965) who first proposed that phenotypic plasticity can be an adaptive trait for coping with environmental heterogeneity, particularly in plants and other sessile organisms that must endure changing environments without the option of behavioural flexibility.

Perfect plasticity, in the absence of constraints, must always be superior to specialization, generalism and bet-hedging in variable environments (DeWitt and Langerhans, 2004). An organism that is able to maximize its fitness over a wide range of environmental conditions is expected to be more successful than one that is constrained to a narrow niche. In reality, there are many millions of species and the majority occupy narrow niches and appear to be obligate specialists constrained to particular, very specific, environments (Futuyma and Moreno, 1988; Whitlock, 1996). The fact that plasticity is not ubiquitous, (in that it is not evident for all traits in all species) and also that there are actually very few definitive examples of adaptive plasticity in plants and that most cases of plasticity are passive responses to, for example nutrient conditions suggests that adaptive plasticity will often be highly constrained by various costs and limits (van Kleunen and Fischer, 2005). Costs to plasticity include negative pleiotropic effects in different environments constraining the evolution of characters in phenotype space (The “Jack of all trades is master of none”), costs of expressing phenotypes through plastic rather than fixed development, costs of sensing the environment to respond to and developmental instability resulting from maintenance of plasticity. Limits to the long term potential of plasticity include the time lag between environmental change and plastic response and the likelihood that plastic responses are typically unable to produce as extreme phenotypes as are micro-evolutionary responses (van Kleunen and Fischer, 2005; DeWitt et al., 1998). This becomes particularly important as plastic responses are pushed to their extremes and they are no longer able to reduce the impact of selection. In addition, plastic responses are only effective at coping with changing environments if the relationship between the existing reaction norm and fitness remains unchanged (Gienapp, 2008). This would be important in, for example, migratory birds, whose life cycles cross different seasons in different regions. If the temperatures in the seasons and regions change inconsistently, the existing reaction norm is unlikely to remain adaptive. This was demonstrated by Visser et al. (1998) in great tits (parus major), where spring warming
induced earlier breeding and consequently a mistiming of hatching chicks and the peak of food abundance.

There has been remarkably little empirical evidence of cross-environment genetic correlations or other costs of plasticity, despite determined searching (e.g. DeWitt et al. (1998); Scheiner and Berrigan (1998); van Kleunen and Fischer (2005); Relyea (2002); Weinig et al. (2006) and see reviews in Van Buskirk and Steiner (2009); Auld et al. (2010)). This makes the fact that most species do exhibit a range of fixed phenotypes rather than ubiquitous phenotypic plasticity still more paradoxical. The high potential fitness benefit of being able to modify one’s phenotype to adjust to a particular environment, particularly in sessile organisms such as plants, would surely lead us to expect that adaptive plasticity is common, and some models suggest that the absence of plasticity is due to the nature of the evolutionary process itself (Whitlock, 1996) - even if negative genetic constraints are not present, more plastic species may have a slower rate of evolutionary response, due to the buffering effects of plasticity, effectively shielding the genotype from selection pressure.

In this work, I concentrate on adaptive plasticity, which is explained by evolutionary theory and emphasises the interplay of selection and gene flow in favouring or disfavouring plasticity in heterogeneous environments (Scheiner and Holt, 2012), rather than non-adaptive trait plasticity which depends instead upon organismal physiology, morphology, and development. Heterogeneous environments are a necessary (but not sufficient) prerequisite for the evolution of phenotypic plasticity, since environmental change ensures that no single phenotype is optimal. If the environment is constant and selection is stabilizing and frequency independent, one phenotype should outperform all others, so there is no reason for plasticity to evolve. Other prerequisites for the evolution of plasticity include reliable environmental cues, benefits that outweigh the costs of plasticity, and a genetic basis (i.e. heritable genetic variation) for plasticity (Berrigan and Scheiner, 2004).

Scheiner and Lyman (1991) identified three models for the genetic basis of phenotypic plasticity:

- The Overdominance model (Gillespie and Turelli, 1989) states that plasticity is a negative function of heterozygosity and that as populations become more genetically diverse their fitness increases and they become more buffered to environmental change.

- The Pleiotropy model (Via and Lande, 1985) states that plasticity is a function of the differential expression of the same genes in different environments. In this model plasticity for a trait is under control of the same genes as those for the trait itself.

- The Epistasis model (Scheiner and Lyman, 1989). Here, plasticity is due to genes that determine the magnitude of response to environmental effects which interact with genes that determine the average expression of a character, i.e. plasticity is under control of different genes from the trait itself.

(Scheiner and Lyman, 1991) tested the predictions of these models by selecting for phenotypic plasticity of thorax size in response to temperature in Drosophila melanogaster. In this experiment they found most support for the epistasis model, although they accepted that the models are mutually exclusive. Since that time, the overdominance model has found very little empirical
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support and is now largely ignored as a serious explanation of the genetics of phenotypic plasticity (Pigliucci, 2001). The slightly confusing nomenclature of “epistasis” and “pleiotropy” models is now generally discussed in terms of either environmentally sensitive loci or regulatory genes specifically associated with a phenotype. It should also be noted that these two models are not necessarily conflicting and organisms may well possess alleles with differing response to different environments alongside separate regulatory genes (see e.g. Tetard-Jones et al. (2011)).

There is still no general consensus on the role of phenotypic plasticity in evolution, the conditions under which it arises or its genetic control (Via et al., 1995; Pigliucci et al., 2006; Crispo, 2007, 2008). There is no standardised measure of plasticity (Valladares et al., 2006) and still debate even over whether plasticity is more meaningful when discussed in terms of slopes of reaction norms across environments or genetic variance for genotype-by-environment interactions (Pigliucci, 2005). It is now generally accepted that phenotypic plasticity is a property of genotypes that can evolve in response to selection, but the form of that evolution is still contentious (Scheiner and DeWitt, 2004): Is plasticity a direct target of selection like any other phenotypic trait, or does it evolve as a correlated response to selection on other traits? We still do not know just how important plasticity is to the process of evolution, from local adaptation to speciation.

Part of this gap in our knowledge is due to the difficulty in conducting experiments on phenotypic plasticity. Since plasticity is a property of a genotype and not a single individual, one needs to raise individuals of the same genotype in different environments in order to detect it. This drastically increases the size of experiments (particularly as more environments are tested). In addition, it limits the organisms that can be tested to those that are amenable to large-scale quantitative genetics experiments (i.e. those that can be easily bred or cloned, and that do not have prohibitively long life spans), particularly if the investigation is into the genetics of plasticity (although see Gianoli and Valladares (2012) for a discussion of a broader approach to detecting plasticity in an ecological setting). The approach I take in this thesis, using the model organism Arabidopsis thaliana allows me to tackle some of the important questions surrounding plasticity despite these difficulties.

1.1.3 Adaptive evolution

Although range shifts, bet-hedging and plasticity may be the most immediate forms of response to changing environments, directional selection will ultimately result in changes in allele frequency in populations as exposed individuals show variable survival and reproduction. It has been suggested that adaptation is relatively unimportant for response to climate change, based on the similarity of species relationships with climate in their past and present distributions (Bradshaw and McNelley, 1991; Huntley, 1991). Adaptation has been portrayed as a gradual process that is too slow to track rapid environmental fluctuations. While it may be true that species cope with short term environmental variability largely through plasticity (Jump and Penuelas, 2005), local adaptation has frequently been observed (Joshi et al., 2001; Etterson, 2004a; Turner et al., 2010) suggesting that strong selective pressures are exerted on natural populations by climate. This may well mean that the role of adaptation in response to climate change has been underestimated (Davis and Shaw, 2001). Several studies have also shown that evolution
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is quite capable of occurring on a contemporary or “ecological” timescale, particularly during invasions and colonisations and in response to anthropogenic habitat modification (Reznick and Ghalambor, 2001). The fact that contemporary evolution is accepted as a real and observable phenomena, possibly even representing the norm rather than the exception, in combination with predictions of rapid near-future climate change, mean that studying response to evolution in the short-term is of great importance both to species conservation (Stockwell et al., 2003) and to ecological studies: two disciplines that have historically underestimated the importance of evolutionary processes (Carroll et al., 2007). Adaptation is likely to play an increasingly important role because human mediated habitat destruction and fragmentation will limit species’ ability to cope with change by shifting range.

Populations are expected to gradually adapt to evolutionary change. However, strong natural selection will result in an average reduction in fitness (Lynch, 1991). If the rate of environmental change is sufficiently fast, adaptation will not be able to keep up with this reduction in fitness and the population will be at risk from extinction, particularly if the magnitude of change forces organisms to the limits of their ability to cope by range-shifting, bet-hedging or plasticity. Theoretical and experimental studies of adaptation to rapid climate warming demonstrate that strong selection pressures may result in high levels of inbreeding, steep declines in fitness and erosion of genetic diversity, particularly at loci linked to climate response (Billington and Pelham, 1991; Lynch and Lande, 1993; Etterson and Shaw, 2001). There are various factors that can potentially limit adaptive evolutionary response which I broadly delineate into population and genetic/developmental factors:

1.1.3.1 Population and demographic constraints on adaptation

The amount of standing genetic variation in a population is the main limit on its ability to respond to change in the short term. Mutation is a slow, stochastic process and populations of plants and animals generally cannot rely on de novo adaptive genetic change to respond to strong directional selection. Evidence for this is that some species show large initial responses to environmental change, followed by a decay in evolutionary rate over time (Kinnison and Hendry, 2001). Low standing genetic variation leads to a lack of response to selection because there is little variation for selection to act on.

Many populations of conservation concern have low effective population sizes \((N_e)\), and such populations often have low genetic variation (Skelly et al., 2007). Natural selection imposes demographic costs on a population as small populations are more influenced by genetic drift, which erodes variation and limits natural selection (Burger and Lynch, 1995). Smaller populations also have a higher incidence of inbreeding, which leads to further loss of genetic diversity. This problem can be exacerbated by bottlenecks or founder effects, which may leave a population genetically impoverished, even after subsequent expansion.

The genetic variation at neutral markers is a commonly used measure of genetic diversity, and populations with high neutral marker variation are thought to be able to respond best to selection and so proliferate. However, variation at neutral markers does not always accurately represent a population’s ability to adapt and even though genetic variation across a genome may be high, lack of segregating variation for ecologically important traits can result in a lack
of response to potential climatic stress (e.g. Hoffmann et al. (2003)).

There is debate over whether population structure can actually assist populations to adaptation to environmental change. Under an extension of Wright’s shifting balance theory (Wright, 1931), selection and random genetic drift in a small population (e.g. after a bottleneck event) can convert epistatic genetic variation into additive variation that can then be a target for selection. To illustrate this, if a hypothetical trait is under the control of two epistatically interacting genes and, due to drift or selection, the homozygote of one of these genes becomes fixed in the population, the variation at the other locus then effectively becomes additive as there is no variation at the first locus to interact with. This process has the potential to release cryptic variation into the population (Wade and Goodnight, 1998). Others, while accepting that there is some evidence for the increase of additive variation following a population bottleneck and that this could facilitate divergence by increasing selection response or by generating extreme phenotypes, argue that many putative cases of conversion simply represent an increase in the frequency of rare alleles (Coyne et al., 2000). This is more an example of the conversion of dominance variation to additive variation and as these rare recessive alleles are likely to be deleterious, they will be quickly removed by purifying selection, leaving little target for adaptive evolution.

1.1.3.2 Genetic and developmental constraints to adaptive evolution

Even if populations have adequate genetic variation to respond to natural selection, they may not be able to respond effectively due to genetic or developmental constraints (Maynard Smith et al., 1985). Genetic constraints are trade-offs among components of fitness (Mitchell-Olds, 1996). The main genetic constraints on evolution are pleiotropy (where two or more traits are under control of the same gene) and epistasis (where several unlinked genes have control over a single trait).

Evolution can be constrained by antagonistic pleiotropy, where selection favours high values of two traits, but they are negatively correlated. For example, plants would experience a trade-off when under selection for early flowering and also for increased leaf number before reproduction (Pigliucci, 2007), resulting in a population that is only able to produce intermediate forms. If between-trait correlations are opposed to the direction of selection, the evolutionary response can be retarded and consequently the rate of adaptive evolution will be slowed (Etterson and Shaw, 2001).

Many groups of organisms targeted for conservation protection are slow growing and have long generation times (e.g. large mammals, forest vegetation: (Skelly et al., 2007). This can lead to a lag between change and response, and there is evidence that some species take hundreds or thousands of years to respond to changing climates (Davis and Shaw, 2001). Theoretical work (Burger and Lynch, 1995; Gomulkiewicz and Holt, 1995) suggests that the magnitude of this lag determines a population’s vulnerability to extinction.

1.1.4 Evolvability

Evolutionary biologists have long discussed the “evolutionary potential” of a population or species (e.g. Milkman (1961)). More recently, researchers have attempted to quantify evolutionary
Potential as a measure of “evolvability”, which depends upon a population’s standing genetic variance, life history, demography and genetic architecture. Unfortunately there is no generally agreed definition of evolvability and the term has been used to describe a range of subtly different phenomena (Pigliucci, 2008) operating at different levels of organisation. For example, evolvability is, variously:

1. “The ability to maintain potentially adaptive genetic variants” (Hansen, 2006). Fisher’s Fundamental Theorem of Natural Selection states that “The rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time.” (Fisher, 1958). From this, it follows that a population’s standing heritable genetic variance will determine how it copes with environmental change (Houle, 1992).

2. “The capacity to evolve at the phenotypic level, irrespective of the action of natural selection” (Dichtel-Danjoy and Felix, 2004). This is the opposite to mutational robustness which is defined as the ability of a genome to maintain constant phenotypes in the face of mutation (Wagner, 2005b). The relationship between mutational robustness and evolvability is critical to the understanding of how organisms can withstand generally deleterious mutations while still maintaining enough variation to allow for evolutionary adaptation and to cope with heterogeneous environments. High evolvability (in this sense) means a population would be better able to adapt to unpredictable environmental change, but may inhibit a populations ability to maintain optimum fitness in any given environment. Conversely a very robust population could maintain fitness in a stable environment, but would be less able to respond to environmental change. We should therefore expect there to be a balance between robustness and evolvability that is dependent on environmental heterogeneity (Draghi et al., 2010). It is this definition of evolvability that is kept in mind when discussing the evolution of evolvability (see below).

3. “The ability of a population to undergo adaptive evolution in the direction of selection” (Hansen and Houle, 2008; Flatt, 2005). This measure has the advantage over (1) of including both selection and genetic architecture, but is also much simpler to estimate than (2), given appropriate experimental designs and using statistical methods based on the multivariate breeders equation (Hansen and Houle, 2008).

The concept of the “evolution of evolvability” was coined by Dawkins (1987) who suggests that the success of many groups of organisms depends upon their capacity to be “champion evolvers”. One example Dawkins gives is the arthropod segmented body plan, which has facilitated phyla-level radiation. If successful species are those that are better able to throw up new lines for future evolution, this capacity would be most beneficial when environmental change exerts new selection pressures. Recent theoretical work suggests that evolvability can be viewed as a trait like any other that can be targeted by selection (Wilke et al., 2001; Earl and Deem, 2004; Draghi and Wagner, 2008; Pigliucci, 2008) and that one way populations can respond to environmental change is by tuning their evolvability. If a population evolves in a heterogeneous environment, there will be selection for evolvability as it has to respond quickly to environmental change, in a similar way that a heterogeneous environment may also select for phenotypic plasticity.
In homogeneous environments with populations under stabilizing selection, genomes will be selected to become more robust so that deviation from the mean optimum trait value is reduced. The issue of different populations having different evolvabilities is of practical conservation concern. If we can identify populations with low evolvability, then we can predict those most at risk from rapid climate change. For example, the impact of global warming in the tropics has been predicted to be less intense in than in temperate regions because the rate of climate warming is predicted to be lower. However, tropical organisms have evolved at constant, stable, near-stressful temperatures with strong stabilizing selection and so are likely to have canalized genomes with poor evolvability and therefore slow responses to climate change (Tewksbury et al., 2008). Higher latitude species will have experienced more environmental heterogeneity in their recent evolutionary history and so may be more evolvable.

### 1.1.5 The relationship between adaptation and plasticity

Although there is clear evidence of phenotypic response to anthropogenic effects such as over-harvesting and herbicide resistance, and ecological systems are showing clear evidence of response to climate warming (Visser et al., 1998; Davis and Shaw, 2001; Walther et al., 2002; Ackerly, 2003; Lloyd and Fastie, 2003; Parmesan and Yohe, 2003; Root et al., 2003; Parmesan, 2006; Colwell et al., 2008; Lenoir et al., 2008), information about the genetic and evolutionary basis of these responses remain scant, and the studies conducted are overwhelmingly based on phenotypic or ecological data, and often do not feature any quantitative genetic data, which makes it impossible to differentiate between plastic and adaptive responses. Gienapp (2008) compared the number of studies using phenotypic and genetic data in two meta-analyses of climate mediated responses (Parmesan and Yohe, 2003; Root et al., 2003) and three recent general reviews of micro-evolutionary responses (Hendry and Kinnison, 1999; Kinnison and Hendry, 2001; Reznick and Ghalambor, 2001). Out of a total of 219 studies across a wide range of taxa, only 70 (32%) made use of genetic evidence.

Many studies only consider one aspect of response to environmental change and assume that this is the most important factor governing response. For example, some stress the importance of range shifting (Bradshaw and McNeilliy, 1991; Huntley, 1991), some plasticity (Price et al., 2003) and some adaptation (Davis and Shaw, 2001). However, experimental and theoretical work suggests that plasticity can affect adaptation in both positive and negative ways. It is important to disentangle adaptive from plastic responses to environmental change if we are going to understand and predict organismal response (Gienapp, 2008).

The relationship between plasticity and adaptation is still very much a topic of debate. Some suggest that plasticity is able to reduce the cost of directional selection, effectively flattening the fitness landscape, allowing easier movement between adaptive peaks and enabling populations to become established enough to generate the genetic variation by mutation and recombination to produce a range of heritable phenotypes to respond to local selection pressures (Ghalambor et al., 2007). However, others contend that the buffering effect of phenotypic plasticity will slow down adaptive responses in the long run, resulting in a less evolvable population (Ancel, 2000).
1.2 Investigating plastic and genetic responses to environmental changes

Humans are impacting on natural systems in a wide variety of ways, including climate warming and associated effects resulting from burning of fossil fuels and the release of other greenhouse gases into the atmosphere, widespread habitat fragmentation, land use change and introduction of invasive non-native species (Gurevitch and Padilla, 2004; IPCC, 2007). Due to the complexity of ecological communities, the inability of a few critical species to cope with the long-term environmental change may have cascading effects through the ecosystem on other interdependent species (Holt, 1990), possibly resulting in major changes in community structure (Lynch and Lande, 1993). In addition, changing environments are likely to select for colonising and invasive species, since these are the ones that are most able to cope with environmental change. It is therefore important that we further develop our understanding of the limits, costs and constraints on individual, population and species response to changing environments. We also need to know more about how plastic and evolutionary responses to such change interact.

Previous studies into the genetic and plastic response to changing environments have been hampered by a number of shortcomings. Natural population experiments with climate manipulations have shown clear environmental responses (e.g. Grime et al. (2000); Ineson et al. (1998)), but because genotypes cannot be replicated, it is impossible to determine if these responses are due to plasticity or adaptation, or tell how these forces interact to give the observed phenotypic response. Attempts have been made to measure plasticity in natural populations (Gianoli and Valladares, 2012). These are useful for determining the ecological significance of plasticity, but again are not able to ascribe observed phenotypic variation in the field to phenotypic plasticity rather than to genetic variation. Others have used reciprocal transplant experiments along latitudinal or altitudinal gradients to simulate climate change (e.g. Etterson and Shaw (2001)). These studies suffer from the confounding effects of different cues, making it difficult to isolate the particular environmental effects (e.g. temperature). Other studies have used recombinant inbred lines in model species such as Arabidopsis thaliana to determine the direct effect of increased temperature in growth chambers or greenhouses (Balasubramanian et al., 2006b). These are able to strictly control experimental conditions but suffer because genetic responses in the lab are often a poor match to responses in the field.

In this thesis, I investigate a range of questions related to individual and population response to changing environments, plasticity and evolvability. To do this I use the model plant species Arabidopsis thaliana and the Avida artificial life platform.

A. thaliana has been used as a model plant species in physiology and genetics for over 50 years. It was the first plant to have its full genome sequenced (Dangl et al., 2000) and is now the de facto model for understanding complex traits such as flowering time (Simpson and Dean, 2002). More recently it has been used as a model in evolutionary ecology (Pigliucci, 2002; Mitchell-Olds, 2001) and has been used to approach a range of problems including phenotypic plasticity (Pigliucci and Hayden, 2001), local adaptation (Mitchell-Olds and Schmitt, 2006), experimental evolution (Scarcelli and Kover, 2009) and maternal effects (Donohue et al., 2012). Because it is relatively easy to manipulate A. thaliana in the lab to create inbred lines, it has
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been extensively used in quantitative trait loci mapping experiments (e.g. Kover et al. (2009b); Koornneef et al. (2004)). I am particularly interested in this system because it is a weedy, colonising annual plant which, although it is rarely locally abundant, has a wide geographical and ecological range stretching from Scandinavia, North Africa and the Middle East. This, in combination with *A. thaliana*’s large amount of natural variation in quantitative traits, makes it an ideal subject for studies investigating phenotypic plasticity and the response to changing environments.

*Avida* is a platform for studying the evolution in silico of self-replicating computer programs (Adami, 1998; Ofria and Wilke, 2004). “Avidians” compete for resources and space by optimising their reproductive rate and performing logical tasks in order to be rewarded with more CPU time which allows them to to execute their genomes faster relative to their neighbours. Because the digital organisms in *Avida* undergo “true” open-ended Darwinian selection, rather than having a target set by the programmer, the system represents a considerable advantage over genetic algorithms and other simulations of evolution and are able to express levels of complexity far beyond what would be possible in an analytic simulation (Adami, 2006). The short generation times in *Avida* combined with complete control over environmental variables, mutation rates and a perfect fossil record have meant that the system has been used to address some of the fundamental questions in evolutionary biology that are difficult or impossible to address in the “wet” lab, such as the role of robustness in evolution (Wilke et al., 2001), resource competition in adaptive radiation (Chow et al., 2004) and the evolution of complex features (Lenski et al., 2003).

My first two experimental chapters deal with plant response to simulated climate warming. The 20th century experienced the strongest climate warming trend of the last 1000 years, with a mean temperature rise of 0.6°C Jones et al. (2001). Thermometer records show that, over the past 30 years, global average surface temperatures increased by 0.28°C per decade (Hansen et al., 2006). This rate of warming is expected to increase with predicted rises of 0.1°C - 0.4°C per decade across Europe (IPCC, 2007). In Chapter 2, I investigate the effect of a climate warming manipulation under natural conditions on an experimental population of *Arabidopsis thaliana*. I find that a small, ecologically relevant increase in temperature is enough to cause significant changes in flowering time, vegetative size and fitness. I also find that elevated temperatures cause a change in the fitness rank order of genotypes, making predictions of response to future climate change problematic and having the potential to have negative effects on community structure. In chapter 3, I further investigate the implications of elevated temperature on *A. thaliana* by looking at how patterns of selection and plasticity change with temperature. By separating out the effects of plasticity and evolution, I find that, although plasticity seems to assist in immediate response to climate warming, it can have consequences over the longer term for population evolvability.

Phenotypic plasticity is an important strategy for coping with changing environments, but little is known about how strong directional selection due to environmental change affects plasticity. If the same genes affect both a trait and its plasticity, selection on that trait should also affect plasticity. In chapter 4, I use an artificial selection experiment to investigate the effect of selection for early flowering on flowering time plasticity in *A. thaliana*. I find that the
indirect effects of selection on plasticity are environment-dependent and show that, in some cases, the magnitude of plasticity can be increased while genotype-by-environment variation is reduced. I discuss the consequences of this for plasticity theory and for plant persistence under future climate change.

The coordination of seed germination timing with favourable environmental conditions is critical for the fitness of many plants, and plastic germination responses to the environment are effectively mechanisms of habitat selection. In chapter 5, I present a study into the genetic basis of germination timing in *A. thaliana* under natural conditions. Here I show how environmental variation in maternal flowering time can result in an unpredictable plastic response on germination traits in the next generation. I also demonstrate that the genes underlying germination response in the field are different from previous studies in controlled environments.

Classic evolutionary theory shows that environmental change selects for ecological generalists. More recent work suggests that changing environments should also select for increased evolvability. In chapter 6, I use the Avida artificial life platform to investigate how different rates of environmental change affect ecological generalism and measures corresponding to three main definitions of evolvability in the literature: diversity, phenotypic sensitivity to mutation and the ability to evolve in the direction of selection. I find that high rates of environmental change select for high levels of ecological generalism and of all three measures of evolvability. This ties together classical ecological theory with recent theoretical and computational evolutionary biology.

In the final chapter I review and synthesis my findings and present them in the light of recent work in similar areas.
Chapter 2

Response in fitness and flowering time to simulated climate warming

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Abstract

Mean global temperatures are expected to increase by 2-3°C by the end of the century and effects on plant phenology are already being observed. Even though higher temperatures may initially increase fitness and crop yield, there are concerns that warming may also affect population genetic structure, resulting in poorly adapted populations. Here we investigate the phenotypic response to predicted climate warming by exposing an experimental population of Arabidopsis thaliana to an increase in ambient temperature under field conditions. Although warming increases mean fitness (fruit production) by ~ 25%, there is a significant genotype-by-environment interaction, which means that rank-fitness is only weakly correlated between plants grown in ambient and elevated temperature conditions. In addition, different QTL for fitness are detected across treatments. We find that plants exposed to elevated temperatures are larger and flower earlier in a predictable way, in line with thermal models. Fitness response is not mediated by flowering time, but is correlated with vegetative size, meaning that warming probably causes an acceleration in vegetative development. Changes in fitness rank due to warming are likely to cause changes in population genetic structure and indicate that imminent climate change is likely to have detrimental effects on current population mean fitness and cause populations to become maladapted in their new environment.

2.1 Introduction

Global climates are changing at an unprecedented rate, largely due to human activities. Mean global temperatures have risen by around 0.8°C in the last hundred years and increases of
Plant phenology is expected to produce the strongest and most immediate biotic response to climate warming (Walther, 2003). Although the initiation of flowering is typically mediated by changes in photoperiod and is therefore thought to be independent of temperature, the time required for flowers to develop to maturity is strongly dependent upon temperature (Minorsky, 2002). Changes in phenology due to increased temperatures have already been observed in many species despite an unchanged photoperiod (Sparks et al., 2000; Menzel et al., 2006; Abu-Asab et al., 2001). For example, the average first flowering date of 385 British plant species has advanced by 4.5 days during the past decade compared with the previous four (Fitter and Fitter, 2002).

Flowering time is a complex trait considered to be of great importance to plant fitness (Korves et al., 2007; Johanson et al., 2000; Kover et al., 2009a; Simpson and Dean, 2002). The transition to flowering depends on a range of environmental and physiological factors such as photoperiod, ambient temperature, vernalisation and plant growth (Brachi et al., 2010; Brock et al., 2009). The time to flower in turn affects the timing of later developmental transitions, can be critical for pollination and ultimately can affect fruit set and dispersal. Flowering time is therefore thought to be under strong selection (Le Corre et al., 2002) and has been found to have direct correlated effects on fitness (e.g. Korves et al. (2007); Kover et al. (2009a)). However, the precise effect of flowering time on fitness varies with different environments and genetic backgrounds (Korves et al., 2007). Selection is expected to favour both early flowering and increased vegetative size (Scarcelli et al., 2007). This means that there could be a fitness trade-off for early flowering: if earlier flowering is achieved by earlier onset of reproductive development, we would expect to observe a negative relationship between early flowering and both size and fitness (Blazquez et al., 2003; Korves et al., 2007). On the other hand, if early flowering is achieved by a general increase in the rate of vegetative development, we would expect to see early flowering plants that are larger and have higher fitness.

Although most studies have emphasised the importance of photoperiod and vernalisation, ambient temperature is also well known to have significant and complex effects on flowering time (Blazquez et al., 2003; Long and Woodward, 1988; Balasubramanian et al., 2006b). In general, plants may be expected to benefit from slightly elevated temperatures, but genotypes might not all benefit equally from these conditions. Accelerated phenologies may therefore alter patterns of resource allocation, interactions with pollinators and the size and diversity of the soil seed bank. (Minorsky, 2002; Fitter and Fitter, 2002). Variable response to warming could also cause cascading effects on community structure due to the breakage of co-evolved relationships caused by mismatched responses or to invasive species that are better able to respond to the new conditions (Petchey et al., 1999; Walther et al., 2002; Hegland et al., 2009; Post and Pedersen, 2008). These problems can be exacerbated because phenological changes can have secondary ecological consequences on animals which depend on pollen, nectar and fruits as a resource (Visser and Holleman, 2001). There has also been growing concern for food security due to the effects of climate warming on crop yields, since increased temperatures have been found to have a negative impact on global yields of maize, wheat, rice and barley (Lobell and Field, 2007; Lobell et al., 2011; Peng et al., 2004).

Studies of the effect of temperature on fitness, growth and phenology have typically been
conducted in controlled growth environments - either in glasshouses or laboratory growth chambers. Much of this work has been done in the model plant Arabidopsis thaliana (e.g., Balasubramanian et al. (2006b); Lempe et al. (2005); Blazquez et al. (2003)) because this species is easily experimentally manipulated, amenable to the construction of inbred lines and is genetically well characterised. However, plants often respond very differently under field conditions because they experience a much wider range of environmental cues including variations in light quality, temperature and photoperiod (Bradshaw et al., 2004). Studies have shown that, as a result of these more complex environmental cues, plants under field conditions often behave quite differently from those grown in the laboratory and many of the Quantitative trait loci (QTL) found in lab-based studies are different to those found in the field (Brachi et al., 2010; Weinig et al., 2002).

An alternative approach to determining the effect of elevated temperatures is to perform climate manipulations in field conditions: Grime et al. (2000; 2008) used surface-level heating cables to determine the effect of simulated climate change on natural limestone grassland communities. Ineson et al. (1998) used a similar setup to investigate nutrient release in upland soils, while Totland (1999) used open top chambers to investigate the effects of temperature on performance and phenotypic selection on plant traits in the alpine perennial herb Ranunculus acris. This approach is particularly powerful for studying the effect of elevated temperature in flowering time and its consequence on fitness because it allows plants to be exposed to small elevated temperatures (in line with predictions for the next few decades), without losing information about daily variation and other environmental cues that should be present but equal across treatments.

Here, we combine a climate manipulation approach championed by Grime et al. (2000) and Ineson et al. (1998), with a set of A. thaliana inbred lines from heterogeneous stocks that allow us to explore the underlying genetic basis of response in fitness and phenology to elevated temperature.

Specifically, we investigate the effect of elevated temperature on fitness and whether this effect is mediated by flowering time. By also investigating the effect of temperature on vegetative size, we can determine whether effects of temperature on flowering time have detrimental effects on plant fitness as predicted by life-history trade-offs. Lastly, we investigate if elevated temperature differentially affects fitness in different genotypes, resulting in a change in the relative fitness ranking of genotypes in the population. This will allow us to explore whether elevated temperatures are likely to cause detrimental changes in population genetic structure and whether fitness under current temperatures can accurately predict responses under elevated temperatures.

2.2 Materials and methods

We used a set of 320 Multiparental Advanced Intercross (MAGIC) A. thaliana lines (Kover et al., 2009b). These isogenic lines are derived from an outbred population composed of 19 natural accessions of A. thaliana that have been SNP genotyped at 1500 loci. Thus, they can be used for QTL mapping as well as for side-by-side comparisons of plastic response. Five seeds
2.2. MATERIALS AND METHODS

from each line were placed in a 0.2% agar solution and cold-stratified for 7 days prior to being planted outdoors. Seeds were then directly planted in the soil in April 2009 into 10 plots at the Botanical research station at the University of Manchester, UK, with 10 cm spacing between individual plantings. The position of each line within a plot was randomly assigned. Warming cables (600W, 48m soil warming cables, Thermoforce ltd, UK) were connected to differential thermostats which maintained the surface temperature a constant 2-3°C above ambient. Thus, the elevated temperature plots experienced the same variation in temperature, day length, light quality and humidity the other plots experience, the only difference is that the temperature was constantly elevated by 2-3°C. Data loggers (Hobo U2 Temperature data loggers) were set up to record temperatures in both treatments. The plots were treated with Roundup herbicide three weeks prior to planting then tilled and smoothed one week later. A fruit cage was constructed over the plots to protect from herbivores. Two weeks after planting, all plots were treated with slug pellets. Plants were inspected daily and flowering time was recorded as the first day an open flower was visible. At flowering time, we also estimated the plant’s vegetative size by measuring rosette diameter at a 45° and 135° angles relative to the plant label. After senescence, all plants were collected and the number of fruits on each individual was counted in the lab to give an estimate of fitness.

Because we were interested in the effect of temperature on phenology in a complex environment with multiple environmental cues, we analysed flowering time both in its raw form (as days from planting) and converted into photothermal units (PTUs) using a phenology model that includes both photoperiod length and temperature (Brachi et al., 2010):

\[ PTT = \sum_{i=p}^{ft} \lambda_i(\mu_i - \mu_b) \]

where \( PTT \) stands for “photothermal time” in photothermal units (PTU, °C . daylight hours); \( p \) is the planting date; \( ft \) the flowering date; \( i \) spans the time from planting date to the flowering date; \( \mu_b \) is the optimal base temperature for the developmental rate of the natural accession Col-0 (3°C, Granier et al. (2002)); \( \mu_i \) is the mean daily temperature during daylight and \( \lambda_i \) is the daily photoperiod as a proportion of 24 h. Temperature data was collected by dataloggers. Photoperiod data was extracted from http://www.timeanddate.com. The use of a transformation to PTT has been advocated because it allows for the comparison of flowering time different environments (Brachi et al., 2010; Chew et al., 2012; Wilczek et al., 2009). A photothermal model represents the threshold temperature and photoperiod needed for a genotype to flower. Because this threshold is determined genetically it should be the same independent of the environment. Here, since the only difference between the two treatments should be in ambient temperature, we would expect no difference in PTT across treatments if time to flower is a linear function of temperature (Chew et al., 2012).

To determine the effect of elevated temperatures on flowering time, fitness and size, as well as genetic variation for these traits and to identify any genotype-by-environment interaction, we fitted the following mixed effects model using the R package lme4 (Bates and Maechler, 2009):

\[ \text{Trait} = \text{Treatment} + \text{MAGIC line} + \text{Treatment} \times \text{MAGIC line} + \text{density} + \text{Edge} + \text{Plot} + \text{error} \]
2.2. MATERIALS AND METHODS

In the model, treatment was set as a fixed effect while genotype was set as a random effect. Plot was set as a random effect nested within treatment. Each planting was scored as being on the edge of a plot or not and edge effects were controlled for as a fixed effect. Five seeds of the same genotype were planted at each individual planting and it was impossible to thin this number down to a single successful seedling in all cases. Because of this, multiple plants were observed in some plantings. We controlled for density effects of multiple plants at individual plantings in all models by setting density (number of plants in a planting) as a fixed effect. MCMC p-values (10000 MCMC samples) were calculated for the fixed effects using \textit{pvals.fnc} from the \textit{R} package \textit{languageR} (Baayen, 2011). Significance of random effect variance components were determined by likelihood-ratio tests. To provide the final unbiased variance components, the model was then re-fitted using restricted maximum likelihood (REML). Genotype-by-environment (gxe) interactions can arise from either changes in genetic variance across environments or a change in the rank order of genotypes across environments (crossing of reaction norms). Only the second case is likely to result in changes in population genetic structure because they represent a change in the fitness rank order of genotypes. We used the methods outlined in Cockerham (1963, p.88) and Johnson (2007) to test for the percentage of significant gxe interactions that can be attributed to crossing of reaction norms.

We estimated heritabilities for all traits using the REML variance components for genetic and total phenotypic variance from the above model. Heritability was calculated as $h^2 = \frac{V_g}{V_p}$.

To estimate the level to which phenology, fitness and size responses in ambient temperatures could predict responses in elevated temperatures, we calculated cross-environment genetic correlations as the Pearson correlation coefficients of the best linear unbiased predictors (BLUPs) for the MAGIC line random effects of each trait (Johnson, 2007). We produced standard errors for heritabilities and genetic correlations by jackknife.

To ensure that density effects due to multiple individual plants in a planting were not confounding our results, we ran a series of models for the effect of number of plants on flowering time, rosette diameter and number of fruits. We also calculated summary statistics on a subset of the data containing only plantings with a density of 1 (i.e. containing a single individual).

All statistical analysis was performed in \textit{R} version 2.13.0 (R Development Core Team, 2011)

2.2.1 QTL mapping

We used a quantitative trait loci approach to determine if the genetic architecture underlying phenology, flowering time and fitness were affected by elevated temperatures. We used the BLUPs for the MAGIC lines from a REML mixed effect model to give estimates of the line effects for each trait. The use of BLUPs from mixed effect models has been criticised for being anti-conservative (Hadfield, 2010), but using this method we were able to control for experimental noise as much as possible before attempting to map QTL, rather than just using the line means, as has been done previously (Kover et al., 2009b). The specification of REML models also reduces the bias associated with these techniques. The model used to generate the BLUPs was:

\[ \text{Trait} = \text{MAGIC line} + \text{plot} + \text{Edge} + \text{density}, \]

with MAGIC line set as a random effect, and the others as fixed effects. The models were
2.3. RESULTS

Table 2.1: Mean trait values for four traits in *Arabidopsis thaliana* MAGIC lines grown in ambient and elevated temperature treatments. MS, F and P are for the difference between control and elevated treatments. P values calculated by MCMC resampling.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Control mean (se)</th>
<th>Elevated mean (se)</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosette diameter</td>
<td>36.69 (2.54)</td>
<td>49.92 (3.41)</td>
<td>1</td>
<td>4219.83</td>
<td>13.17</td>
<td>0.0036</td>
</tr>
<tr>
<td>Flowering time</td>
<td>53.15 (0.77)</td>
<td>49.02 (1.04)</td>
<td>1</td>
<td>263.63</td>
<td>14.96</td>
<td>0.0036</td>
</tr>
<tr>
<td>PTT</td>
<td>540.51 (9.45)</td>
<td>522.91 (12.68)</td>
<td>1</td>
<td>4015.5</td>
<td>1.4633</td>
<td>0.2170</td>
</tr>
<tr>
<td>Number of fruits</td>
<td>786.63 (52.78)</td>
<td>989.93 (68.75)</td>
<td>1</td>
<td>3061850</td>
<td>9.78</td>
<td>0.0192</td>
</tr>
</tbody>
</table>

run for all traits in both ambient and elevated temperature treatments. To determine if there were QTL affecting the plastic response of traits independent of QTL that directly affect the trait, we also mapped QTLs for the magnitude of plasticity (measured as the difference between the mean trait values for each line in the two treatments).

We mapped QTLs using a hierarchical Bayesian random effects model implemented in the R package happy.hbrem (http://spud.well.ox.ac.uk/arabidopsis/) (See Kover et al. (2009b) for full details). To identify a QTL, first a probabilistic reconstruction of the haplotype mosaic of each MAGIC line was calculated, taking into account information from multiple markers and the genetic map. Then, the genome was scanned for evidence of a QTL in each SNP interval using a fixed effects model ignoring the effects of other QTLs. Finally, the evidence in favour of a QTL was evaluated by re-sampling the data 500 times and fitting multiple QTL models. Each re-sampling produces a different set of QTL, and the fraction of models containing a given QTL is the measure of support for that QTL.

If different QTL were identified in the ambient and elevated temperature treatments, the full genome scans were compared. If the region of the QTL in the other treatment was flat, we could infer that there is no QTL in the other environment. If this were not the case, there may have been evidence for a QTL at that position, but not enough power to fully resolve it.

2.3 Results

2.3.1 Effect of elevated temperature on phenology, size and fitness

All lines that had >= 3 replicates that had survived to the end of the experiment with full data for all phenotypes in each treatment were retained for analysis, leaving 278 lines of the 320 initially planted.

Elevated temperatures had a significant effect on mean flowering time, rosette diameter and number of fruits. Plants under elevated temperature, on average, flowered ~4 days earlier; had rosettes ~12mm larger and produced ~200 more fruits (Table 2.1). However, there was no significant effect on flowering time in photothermal units (PTT), suggesting that temperature affects flowering time in a predictable manner. Significant heritable genetic variance was observed for all traits, as indicated by the between MAGIC line tests ($V_g$) and the significant heritability ($h^2$) for all traits (Table 2.2). Heritabilities were significantly lower than typically observed in growth chambers, ranging from 35% for flowering time to 8% for number of fruits. No significant
interaction between line and treatment was observed for rosette diameter (P_{gxe} = 0.254), also showing that elevated temperature increases vegetative size in a predictable manner. However, gxe for flowering time was approaching significance (untransformed flowering time P_{gxe} = 0.071; PTT P_{gxe} = 0.072), suggesting that although most lines tend to accelerate flowering in response to elevated temperature, this was not the case for all lines. More importantly, a significant gxe interaction was observed for number of fruits (P_{gxe} = 0.019) and 75% of this interaction was due to crossing of reaction norms, meaning that the relative ranking of genotype fitness has been significantly altered under elevated temperatures. Only a weak relationship between relative fitness rank in ambient and elevated temperatures (r = 0.26, t = 4.5, df = 275, P < 0.0001), and a relatively low cross-environment genetic correlation (r_g = 23%) for number of fruits compared to flowering time (r_g = 64%) and rosette diameter (r_g = 45%) were observed. Together, these results imply that fitness in ambient temperatures is a poor predictor of fitness under small increases in temperature.

Pairwise genetic correlations (based on genotype mean trait values) among traits for ambient and elevated temperature treatments are shown in figure 2.2 (PTT is not shown because it is perfectly correlated with flowering time within treatments). Rosette diameter is significantly correlated with number of fruits in both treatments, meaning that larger plants also have more fruits. Flowering time is also positively correlated with rosette diameter, so large plants also tend to be later flowering. However, flowering time is uncorrelated with number of fruits in either treatment, showing that fruit production is more correlated with rosette size than flowering time.

Tests for the confounding effects of plant density at individual plantings revealed that the effect of density on trait means was small: The R^2 values from linear models of the effects of number of plants on the different traits were: 3.6% (Flowering time), 3.8% (PTT), 1.8% (Rosette diameter) and 1.7% (number of fruits). Treatment means and variances in a subset of the data with only a single plant in each planting (Appendix A, tables A.1 and A.2) are similar to that of the full dataset suggesting that we are justified in using our full dataset with density included in the BLUPs in the analysis.

### 2.3.2 QTL mapping

Full genome scans for traits in ambient and elevated treatments, as well as for plasticity are shown in Appendix A. A total of 18 QTLs were detected among the 278 lines across treatments
Figure 2.1: Mean reaction norms between ambient and elevated temperature treatments for four traits in *A. thaliana* MAGIC lines.

Figure 2.2: Correlations between traits in ambient and elevated temperature treatments. Histograms and kernel density plots of the univariate distributions are shown on the diagonal. Pairwise Pearson correlations with starred significance levels are shown right of the diagonal. Scatter plots of the correlations with LOESS smoothers are shown left of the diagonal.

(a) Ambient treatment

(b) Elevated temperature treatment
2.3. RESULTS

Table 2.3: List of QTL identified in each treatment and their locations, as well as variance explained by the SNP.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phenotype</th>
<th>chr</th>
<th>SNP</th>
<th>position</th>
<th>logP</th>
<th>p-value</th>
<th>% Var</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient</td>
<td>Flowering time</td>
<td>chr1</td>
<td>MN1_24322296</td>
<td>24318694</td>
<td>6.85</td>
<td>&lt;0.001</td>
<td>20.2</td>
</tr>
<tr>
<td>Ambient</td>
<td>Flowering time</td>
<td>chr4</td>
<td>LD_5094</td>
<td>1123896</td>
<td>3.83</td>
<td>0.023</td>
<td>19.9</td>
</tr>
<tr>
<td>Ambient</td>
<td>Flowering time</td>
<td>chr5</td>
<td>MN5_25963543</td>
<td>25946317</td>
<td>6.61</td>
<td>&lt;0.001</td>
<td>16.2</td>
</tr>
<tr>
<td>Elevated</td>
<td>Flowering time</td>
<td>chr1</td>
<td>RGL1_1171</td>
<td>24748272</td>
<td>3.78</td>
<td>0.031</td>
<td>14.3</td>
</tr>
<tr>
<td>Elevated</td>
<td>Flowering time</td>
<td>chr1</td>
<td>PERL0235052</td>
<td>25849927</td>
<td>4.45</td>
<td>0.005</td>
<td>11.9</td>
</tr>
<tr>
<td>Elevated</td>
<td>Flowering time</td>
<td>chr4</td>
<td>MN4_541323</td>
<td>541323</td>
<td>3.63</td>
<td>0.046</td>
<td>14.9</td>
</tr>
<tr>
<td>Elevated</td>
<td>Flowering time</td>
<td>chr5</td>
<td>MN5_26029439</td>
<td>26012213</td>
<td>3.85</td>
<td>0.028</td>
<td>12.2</td>
</tr>
<tr>
<td>Ambient</td>
<td>PTT</td>
<td>chr1</td>
<td>MN1_24322296</td>
<td>24318694</td>
<td>6.82</td>
<td>&lt;0.001</td>
<td>20.5</td>
</tr>
<tr>
<td>Ambient</td>
<td>PTT</td>
<td>chr4</td>
<td>LD_5094</td>
<td>1123896</td>
<td>3.78</td>
<td>0.03</td>
<td>20.2</td>
</tr>
<tr>
<td>Ambient</td>
<td>PTT</td>
<td>chr5</td>
<td>MN5_25963543</td>
<td>25946317</td>
<td>6.74</td>
<td>&lt;0.001</td>
<td>16</td>
</tr>
<tr>
<td>Elevated</td>
<td>PTT</td>
<td>chr1</td>
<td>PERL0235052</td>
<td>25849927</td>
<td>4.3</td>
<td>0.012</td>
<td>11.3</td>
</tr>
<tr>
<td>Elevated</td>
<td>PTT</td>
<td>chr4</td>
<td>MASC04651</td>
<td>903543</td>
<td>3.75</td>
<td>0.044</td>
<td>23.4</td>
</tr>
<tr>
<td>Elevated</td>
<td>PTT</td>
<td>chr5</td>
<td>MN5_26029439</td>
<td>26012213</td>
<td>3.72</td>
<td>0.045</td>
<td>11.8</td>
</tr>
<tr>
<td>Elevated</td>
<td>Rosette diameter</td>
<td>chr1</td>
<td>MN1_11553514</td>
<td>11553534</td>
<td>4.26</td>
<td>0.013</td>
<td>13.9</td>
</tr>
<tr>
<td>Elevated</td>
<td>Rosette diameter</td>
<td>chr5</td>
<td>MN5_3227635</td>
<td>3227634</td>
<td>5.19</td>
<td>0.001</td>
<td>23</td>
</tr>
<tr>
<td>Plasticity</td>
<td>Rosette diameter</td>
<td>chr1</td>
<td>PERL0147872</td>
<td>17474215</td>
<td>4.2</td>
<td>0.016</td>
<td>9</td>
</tr>
<tr>
<td>Plasticity</td>
<td>Rosette diameter</td>
<td>chr5</td>
<td>MN5_625679</td>
<td>625681</td>
<td>4.75</td>
<td>0.003</td>
<td>14.9</td>
</tr>
<tr>
<td>Ambient</td>
<td>Fitness</td>
<td>chr1</td>
<td>MN1_395107</td>
<td>395107</td>
<td>3.66</td>
<td>0.047</td>
<td>14.8</td>
</tr>
</tbody>
</table>

for all 12 traits mapped (4 traits in each treatment, and their plasticity). Three QTL for flowering time were detected in ambient temperatures in chromosomes 1, 4 and 5. Together they explain 56.3% of $V_p$. Under elevated temperatures, Four QTLs were observed in overlapping position with ambient temperatures, which together explain 53.3% of $V_p$ (Table 2.3). The flowering time QTL detected in the elevated treatment but not in the ambient treatment was on chromosome 1, but this did overlap with the ambient QTL (Figure 2.3). No QTL for plasticity in flowering time was detected, in agreement with previous result that most genotypes respond similarly in flowering time. Three QTL for PTT were found in both treatments, one on each of chromosomes 1, 4 and 5. These explained 47.5% of $V_p$ in the ambient treatment and 46.5% of $V_p$ in the elevated temperature treatment (Table 2.3). All QTLs for PTT overlapped with those for flowering time. Two QTL for rosette size in the elevated temperature treatment were detected on chromosomes 1 and 5 explaining 36.9% of $V_p$. No QTLs for rosette size were detected in ambient temperatures, but two QTLs for rosette size plasticity on chromosomes 1 and 5, explaining 23.9% of $V_p$. The plasticity QTL on chromosome 1 did not co-locate with QTL for rosette diameter itself, suggesting that there is some separate genetic control over plastic response in vegetative size. One QTL was found for number of fruits on chromosome 1 in the ambient treatment explaining 14.8% of $V_p$. There was no evidence for a QTL for fruit number in elevated temperatures corresponding to the QTL in ambient temperatures (Appendix A figure A.2). This QTL could in part explain the gxe variance for fruit number observed above.
Table 2.4: Estimated QTL effects in ambient and elevated temperature treatments. Figures are the estimated variance explained by that founder ecotype (Kover et al., 2009b). Positive values are in black while negative values are in red. Maximum and minimum values for each QTL are bordered.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Treatment</th>
<th>QTL</th>
<th>Bur</th>
<th>Can</th>
<th>Col</th>
<th>Ct</th>
<th>Edi</th>
<th>Hi</th>
<th>Kn</th>
<th>Ler</th>
<th>Mt</th>
<th>No</th>
<th>Oy</th>
<th>Po</th>
<th>Rich</th>
<th>Sf</th>
<th>Tsu</th>
<th>Wil</th>
<th>Wu</th>
<th>Zu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient</td>
<td>Flowering time</td>
<td>MN1_24322296</td>
<td>-0.52</td>
<td>-0.36</td>
<td>-0.86</td>
<td>1.65</td>
<td>2.74</td>
<td>-1.11</td>
<td>-1.33</td>
<td>0.13</td>
<td>-1.11</td>
<td>0.28</td>
<td>0.37</td>
<td>0.13</td>
<td>-0.77</td>
<td>-0.56</td>
<td>2.92</td>
<td>-0.55</td>
<td>0.84</td>
<td>-0.63</td>
</tr>
<tr>
<td>Ambient</td>
<td>Flowering time</td>
<td>LD_5094</td>
<td>0.9</td>
<td>3.42</td>
<td>1.07</td>
<td>0.06</td>
<td>1.41</td>
<td>-0.61</td>
<td>0.97</td>
<td>-0.69</td>
<td>0.89</td>
<td>0.16</td>
<td>-0.58</td>
<td>0.49</td>
<td>-0.35</td>
<td>2.52</td>
<td>0.92</td>
<td>-1.31</td>
<td>-0.61</td>
<td>0.78</td>
</tr>
<tr>
<td>Ambient</td>
<td>Flowering time</td>
<td>MN5_25963543</td>
<td>0.03</td>
<td>-1.49</td>
<td>-0.6</td>
<td>-1.09</td>
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<td>-0.94</td>
<td>0.17</td>
<td>0.79</td>
<td>-0.31</td>
<td>-1.61</td>
<td>1.54</td>
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Figure 2.3: QTL for traits in ambient and elevated temperature treatments, and for phenotypic plasticity. The x axis is in Mb pairs along the chromosome.
2.4 Discussion

Previous studies have demonstrated or predicted an advance in phenology due to climate warming (Sparks et al., 2000; Menzel et al., 2006; Abu-Asab et al., 2001; Fitter and Fitter, 2002). It has been suggested that earlier flowering is likely to cause reductions in fitness as flowering is triggered early, cutting off vegetative development and leading to smaller rosettes and lower seed production in spring conditions (Korves et al., 2007). Here, we confirm that small increases in temperature can lead to earlier flowering. However, we also observed an increase in vegetative size, leading to an increase in fitness despite the earlier flowering. This suggests that development is actually being accelerated, rather than cut short. Another possible explanation would be that elevated temperatures release the correlation between flowering time and vegetative size, but this seems unlikely because there is little noticeable treatment effect on correlation structure between traits.

Some studies have suggested that flowering time has important direct effects on fitness, and in particular that earlier flowering plants tend to have higher fitness (e.g. Kover et al. (2009a)) in spring conditions. However, others have noted that the very responsiveness of flowering time to small environmental changes implies that flowering time is not under strong selection at all (Ollerton and Lack, 1992; Stenoien et al., 2002) and so when selection is observed on flowering time (particularly in annual, weedy and selfing species such as *A. thaliana*) this is actually just a correlated effect to selection for accelerated development. Our results support this hypothesis: contrary to previous work (Kover et al., 2009a) we find that flowering time is not well correlated with fitness in either ambient or elevated temperatures. However, rosette diameter is correlated with both early flowering and number of fruits, so the increase in fruit production (and therefore, fitness) due to elevated temperature is not mediated by flowering time and appears to be due to an acceleration of vegetative development.

The response in flowering time to elevated temperature is consistent across lines and accordingly the same QTL underlie flowering time in both ambient and elevated temperatures. The small gxe interaction associated with flowering time is therefore probably due to differential allelic sensitivity rather than different alleles being expressed in the two environments. The allelic effects of the QTLs in the two environments (Table 2.4) support this: QTLs for flowering time in elevated temperatures that overlap with QTLs for flowering time in ambient temperatures show some degree of different patterns of allelic effects. The effect of temperature on flowering time is in agreement with thermal models, in that temperature affects flowering time predictably and independently of photoperiod (Balasubramanian et al., 2006b; Wilczek et al., 2009; Chew et al., 2012). The lack of a significant effect of elevated temperature on PTT shows that temperature affects flowering in a linear and predictable way. Overlapping QTLs are detected for flowering time and PTT, implying that PTT is accurately modeling flowering time.

The temperature response is also consistent for rosette diameter, with no evidence of gxe and little crossing of reaction norms (figure 2.1). However, there is evidence of different genes controlling the response to elevated temperatures. It is possible that the QTLs found in the elevated temperatures are involved in control over accelerated development, and so are not observed in the ambient treatment.
2.4. DISCUSSION

We found two QTLs for plasticity in rosette diameter, one of which (17.4 Mb along Chromosome 1) was distinct from QTLs detected for rosette diameter. This underlines the importance of specificity when discussing the meaning of phenotypic plasticity. Plasticity, or more specifically, magnitude of phenotypic plasticity is measured as the difference in mean trait value between the two treatments, and indicates the potential plastic response to the different environments. By contrast, gxe measures the variation in the population in plastic responses among genotypes. In chapter 4 we show that it is possible for selection to increase plasticity even whilst reducing gxe, and it is the reduction in gxe due to directional selection which is potentially more problematic for maintaining the evolutionary potential of populations subjected to environmental change.

For rosette diameter, we observe QTLs that appear to underlie the difference in response to high and low temperatures, but little variation in the slope of reaction norms is observed. If the fitness response to elevated temperatures was also consistent across genotypes, we might deduce that climate warming has a positive effect on populations, in that crops and plants in natural populations should be more vigorous and productive. However, we observe significant gxe for fruit production, most of which is due to changes in rank order of genotypes, and only a weak correlation between fitness rank across treatments. We also observe QTL for fitness in ambient temperatures, but no QTL at this position in elevated temperatures, which may well contribute to this observed gxe interaction. Previous work has also demonstrated gxe for fitness (or fitness related traits) and QTL-by-environment effects on fitness in *A. thaliana* in response to environmental changes (Weinig et al., 2003). Although fitness is moderately well correlated with rosette diameter, the response of rosette diameter to elevated temperature is consistent across lines (and so with thermal models), so the difference in rank fitness between treatments appears to be independent of vegetative size. This means that the current distribution of fitness is a poor predictor of future responses to climate warming. Therefore, under further climate warming, current populations are likely to be immediately maladapted because the increased temperature means that the most common individuals under previous conditions are likely to be less fit under the new conditions. Thus, increases in temperature will likely result in significant changes in population genetic structure.

The same lines we used in this study were previously used in greenhouse and growth chamber experiments (Kover et al., 2009b). In the previous study, flowering time was recorded under glass and in short and long day conditions in the growth chamber. We compared the confidence intervals of QTLs for flowering time and PTT found in this study with flowering time QTL in the previous studies. Non-overlapping confidence intervals were taken to be indicative of different QTL. Most of the QTLs for flowering time identified here agreed with those identified by Kover et al. (2009b). The exception is the QTL on Chromosome 4 (at around 286kb) for which there is no support for the presence of a QTL in this study (i.e. the region near the QTL is fairly flat, see figure A.1 in appendix A). This strongly indicates that the loci under this QTL found at this position by Kover et al. is not likely to affect flowering time under the field conditions of this experiment. Looking at the full genome scans, the QTL identified by Kover et al. near the start of chromosome 5 would probably have been picked up if we had a larger set of lines. There is a small peak here between the 50% and 90% confidence intervals in all of the measures, indicating that there may well be a true QTL here, but we are lacking the power to fully resolve
this because we use significantly fewer lines (278 vs. 459 lines). Heritability in flowering time is far lower than has been previously observed (Kover et al., 2009a; Brachi et al., 2010). This is probably due to the wider and more complex range of environmental cues the plants in this experiment are exposed to, underlining the importance of performing experiments in natural conditions in order to obtain accurate predictions of response to environmental change.

Plasticity has played a prominent role in phenotypic response to climate change (Anderson et al., 2012). The novel combination of temperature manipulation in a natural environment and MAGIC lines in this experiment has allowed us to analyse significant plastic changes in phenology, plant size and fitness in response to a small change in ambient temperature. This study is a useful step forward from previous climate manipulation studies (Grime et al., 2000; Ineson et al., 1998) because the inbred lines we use allow us to grow the same genotypes in ambient and elevated temperatures to identify plastic responses, genotype-by-environment interactions and map QTLs to investigate the genetic basis of these responses. The in situ climate manipulation approach also represents a considerable advantage over previous transplant experiments (Etterson and Shaw, 2001) because we can explore the response of temperature in isolation, without it being confounded by different cues in different locations. Here, we have demonstrated that small changes in ambient temperatures in line with climate change predictions can cause not only predictable, linear changes in phenology and size, but significant changes in fitness rank order that are not mediated by flowering time, as has been suggested in the past. This is concerning because it means we cannot use current estimates of fitness to predict response to future climate change, and because imminent climate change is likely to have detrimental effects on current population mean fitness, and is likely to cause populations to become maladapted in their new environment.
Chapter 3

The effect of plasticity and evolvability on response to simulated climate warming

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Abstract

Climate change models predict that there will be an increase in mean global temperatures over the next 50 years. Previous work has shown that temperature affects many aspects of plant development and life-history, and there is concern whether plants can adapt physiologically or genetically to the new conditions. To determine the possible impact of elevated temperature on population persistence it is important to determine whether temperature affects both the pattern of selection and the ability of populations to respond to selection. Furthermore, it is important to separately evaluate the plastic and evolutionary response to changing environments. Here we grew a set of recombinant inbred lines of Arabidopsis thaliana in both ambient and elevated temperature conditions in the field to estimate patterns of selection, evolutionary response and evolvability in five ecologically relevant traits. We found that a small increase in ambient temperature was enough to significantly change the direction of selection and response. Plants did respond plastically to the increase in temperature, and this response was generally in the same direction as selection. However, comparison of the G matrices in ambient and elevated temperatures revealed that plasticity to elevated temperatures causes reduced evolvability in comparison to what would be expected had there been no plasticity. These results suggest that although plastic response assists in coping with environmental change within a generation, plastic response may well be detrimental in the longer term to future change.
3.1 Introduction

Global climates are undergoing rapid change and climate warming in particular is occurring ten times faster than at any period in the last 10,000 years (IPCC, 2007). Although populations have encountered environmental change throughout evolutionary history, the current unprecedented rate of change is of concern because it may exceed the capacity of the genetic, physiological and demographic mechanisms that organisms have evolved to cope with environmental change (Root et al., 2003; Chevin et al., 2010). Organisms have three main mechanisms for coping with changing climates: range shifting (tracking their preferred environment in space), phenotypic plasticity (expressing different phenotypes depending on the environment) and genetic change. If habitat fragmentation disrupts demographic response, the persistence of some populations will depend on plasticity and adaptation. While phenotypic plasticity has the potential to assist organisms in coping with changing environments, plastic responses are limited and less able to produce extreme phenotypes than genetic adaptation (Gienapp, 2008). Thus, in the long term, adaptive evolutionary response is critical if populations are to persist in rapidly changing environments.

Historically, models of species responses to climate change have implicitly made the assumption that the evolutionary process is not important to the ability of populations to cope with environmental change (Malcolm et al., 2006; Thomas et al., 2004; Huntley, 1991; Thuiller et al., 2005). However, several recent studies have shown that adaptive evolutionary change can happen on contemporary, or “ecological”, timescales (Hendry and Kinnison, 1999; Reznick and Ghalambor, 2001; Stockwell et al., 2003; Carroll et al., 2007). While a meta-analysis has shown a distinctive "fingerprint" of phenotypic response to climate change in many natural populations (Root et al., 2003), most studies cannot determine if these phenotypic changes are the result of evolutionary change or plastic responses. There is therefore still debate as to the relative importance of plastic and adaptive responses to climate change. Some have assumed micro-evolutionary adaptation to be ubiquitous (Hendry and Kinnison, 1999). Others maintain that climate change will still often outpace adaptation, particularly in fragmented environments (Jump and Penuelas, 2005) and that most response to changing environments will be plastic (Gienapp, 2008). There is also some concern that plastic responses can actually slow evolutionary response by reducing the strength of selection (Ancel, 2000). The direction of plasticity is also important when determining response to climate change. This is because the direction of plastic response in relation to changes in selection patterns will determine whether or not plasticity will be adaptive. Different environments are expected to select in different directions (Chevin et al., 2010) and if these changes in patterns of selection result in plastic response being in the opposite direction to selection, environmental change will result in organisms that are maladapted (Visser, 2008), particularly if climate change is rapid and organisms are exposed to environments they have not experienced in their recent evolutionary history. On the other hand, if plastic responses are in an adaptive direction, they may be able to assist in response to changing environments by reducing the strength of selection, allowing populations to maintain larger population sizes while adapting to the new conditions (Crispo, 2008).

Here, we expose a set of recombinant inbred lines to simulated climate warming and attempt to disentangle plastic from adaptive evolutionary responses. Specifically, we determine if patterns
3.1. INTRODUCTION

of selection are changed with increased warming, whether plastic responses are adaptive in this new environment and if a diverse population is able to produce an observable adaptive response to an ecologically plausible environmental change.

Reciprocal transplant experiments across latitudinal gradients (e.g. Etterson and Shaw (2001); Etterson (2004b,a)) have previously been used to investigate response to climate change. These studies have shown clear contemporary responses to climate change, but were not able to isolate the effects of temperature from the range of other cues (such as light quality and precipitation). Many of these cues are likely to change in unpredictable ways along a latitudinal gradient and so the effects of these cues will be confounded. This makes it impossible to determine the causal effect of any single cue, for example ambient temperature. Several other studies have investigated the effect of simulated climate change on plant populations using in situ temperature manipulations (e.g. Grime et al. (2000, 2008); Ineson et al. (1998); Jump et al. (2008)) and precipitation (Grime et al., 2000). These studies used natural populations to estimate effects of simulated climate change at the community level. However, because these studies did not use inbred lines they could not address the relative roles of plasticity and genetic adaptation. While these studies are useful for determining how populations respond to climate change, we still lack empirical data under realistic environmental conditions of the relative contributions of plasticity and adaptation to this. To address this void, we used a combination of these two approaches. We installed above-ground warming cables to provide an in situ temperature manipulation that enabled us to maintain temperature at a consistent 2-3°C above ambient (whilst leaving other environmental conditions unchanged), with a diverse set of recombinant inbred lines that allowed us to plant the exact same genotypes in ambient and experimentally elevated temperatures, so they also experienced otherwise the same environmental conditions. This experimental design allowed us to separate selection from evolutionary response and to quantify the contribution of plasticity to this response. It also allowed us to estimate evolvability of the experimental population and determine how temperature and plasticity affect this.

We used a set of Arabidopsis thaliana Multiparental Advanced Genetic InterCross (MAGIC) lines, which are inbred lines derived from an intercross of 19 natural accessions as described in (Kover et al., 2009b). A. thaliana is a good system to investigate the effect of climate change because it has been previously shown to respond to environmental cues such as elevated temperature (Balasubramanian et al., 2006b). In addition, as a model organism for genetics and physiology, A. thaliana has a wide range of genetic tools available. In terms of evolutionary ecology, it is a fast growing annual plant with a wide distribution that is capable of coping with a wide range of environmental conditions. The experimental lines used have had historical linkage between loci broken up by five generations of random inter-mating, before being inbred. We have previously shown (Chapter 2) that even a relatively small increase in ambient temperature can have significant effects on a range of plant traits, including flowering time, vegetative size and fitness, and that genotype x environment interactions on fitness can potentially lead to changes in population structure. Here, we use a range of quantitative genetics tools to analyse the effect of simulated climate warming in accordance with climate model predictions on the multivariate evolution and evolvability of a set of ecologically relevant traits. Specifically, this experimental design allows us to ask whether plastic responses to elevated temperature are in
the same direction as selection and if small changes in mean temperature, under field condition where there are many cues and wide variation in temperature, can significantly affect patterns of selection or predicted response to selection.

3.2 Materials and Methods

3.2.1 Experimental design

Seeds from each of the 320 MAGIC lines were placed in a 0.2% agar solution and cold-stratified for 7 days prior to being planted outdoors. Seeds were directly planted into the soil in April 2009 into 10 plots at the Botanical research station at the University of Manchester (Manchester, UK). Each 3.3m x 1.1m plot contained a single replicate of each line, with 10 cm spacing between seeds. The position of each line within a plot was randomly assigned and half of the plots were assigned to an elevated temperature treatment. Elevated Temperature plots had warming cables (600W, 48m soil warming cables, Thermoforce ltd, UK) laid in between each row of seedlings. The cables were connected to differential thermostats which maintained the surface temperature in these plots a constant 2-3°C above ambient temperature. Thus, the elevated temperature plots experienced the same diurnal and seasonal variation in temperature, as well as day length, light quality and humidity that the ambient plots experienced, the only difference being in the temperature. Data loggers were set up to record temperatures in both treatments, and ensure treatment worked as expected. Two weeks after planting, all plots were treated with slug pellets. Plants were inspected daily and flowering time was recorded as the first day an open flower was visible. At flowering time, we also estimated the plant’s size by measuring rosette diameter at a 45° and 135° angles relative to the plant label. After senescence, all plants were collected and taken to the lab for further phenotyping. For each individual we measured the height of the inflorescence stalk and counted the number of branches on the main inflorescence stem and the total number of branches, as well as the number of fruits (to give an estimate of fitness).

3.2.2 Data analysis

To test for treatment effects and to estimate variance components for total, genetic and genotype by environment variance we fitted the following mixed effect model, using the R package lme4 (Bates et al 2011):

Trait = Treatment + Line + Treatment x Line + Plot + Density

Treatment is the temperature treatment and was a fixed effect. Line and Plot were set as random effects. Density was the number of plants in a planting and was also fixed. MCMC p-values (10000 MCMC samples) were calculated for the fixed effects using `pvals.fnc` from the R package `languageR` (Baayen, 2011). Significance of random effects variance components were determined by likelihood-ratio tests. To provide the final unbiased variance components, the model was re-fit using restricted maximum likelihood (REML).

To test whether the patterns of selection imposed by ambient and elevated temperatures differ, we estimated selection gradients for each trait using a linear regression approach (Lande and Arnold, 1983). First, we mean standardised all traits by dividing each observation by the
Mean of that trait. Although selection gradients are generally calculated using z-standardised or variance standardised scales for traits it makes more sense when comparing traits to use proportional, rather than absolute, changes in traits (Hansen and Houle, 2008). With mean standardised selection gradients, if an element has a value of 1, this means a 1% increase in a trait yields a 1% increase in fitness (Hansen et al., 2011; Walsh and Lynch, 2011). Conversely, a value of 0 means the trait is completely uncorrelated with fitness. This gives a natural measure for comparing the relative strength of selection representing the increase in relative fitness for a proportional change in a trait and therefore is better suited for the comparison of qualitatively different traits (Hansen et al., 2011). Mean standardised vectors of selection gradients, $\beta_{\mu}$, for all traits in both treatments were calculated using multiple regression (Lande and Arnold 1983). The model was first fit with quadratic and linear terms for all traits. The quadratic terms were subsequently dropped from the model because they showed clear evidence of over-fitting. The mean standardised vector of selection gradients, $\beta_{\mu}$, is the vector of partial regression coefficients for each mean standardised trait on relative fitness. Residual bootstrapping was used to generate 95% confidence intervals for the partial regression coefficients. We tested for a difference in both length and direction of vectors of selection gradients in ambient ($\beta_{\mu}^{ambient}$) and elevated temperatures ($\beta_{\mu}^{elevated}$), by comparing the angle between and the difference in vector norms of 10000 bootstrap replicates of the two vectors (Walsh and Lynch, 2011). Since the same genotypes were grown in the two environments (ambient and elevated temperatures), the differences in phenotype observed among the two treatments represent the plastic response to the environment. To test if this response is adaptive, we conducted a fitness analysis of plasticity (Lande and Arnold, 1983; Weinig et al., 2006), where selection gradients were calculated for magnitude of plasticity (calculated as the difference in line mean trait value across treatments for all traits) in the same way as for the first order traits above.

We were able to determine the contribution of phenotypic plasticity to evolutionary response by calculating the additive genetic variance-covariance matrix ($G$) for the populations in the ambient and elevated temperatures. Because we have the same genotypes in both treatments, the differences in $G$ represent plasticity. We estimated the $G$ for the populations separately for each treatment using the R package MCMCglmm (Hadfield, 2010), which implements Markov chain Monte Carlo routines for fitting generalised linear mixed models. With Bayesian inference via MCMC methods, one can avoid many of the problems of parameter estimation and hypothesis testing in restricted maximum likelihood (REML) mixed effects models (Bolker et al., 2009) by examining the posterior distribution of a parameter of interest and testing if the 95% credible interval overlaps zero. The MCMC sampling process is computationally intensive however and does require the specification of prior distributions for unknown parameters, although in larger datasets, these priors should have little impact on the final parameter estimates. We followed the method for specifying animal models outlined in Wilson et al. (2010). Because we used inbred lines and were able to have replicates within and across treatments of the same genotype, we did not need to specify a pedigree. The priors matrix to seed the chain was specified by the phenotypic variances and the degree of belief in these was set to the number of traits to be investigated. Genetic and residual matrices were set as unstructured Gaussian matrices, the most general available for this type of data. Fixed effects in the covariance model were
3.3. RESULTS

the covarying traits (minus the intercept), plants and edge (both nested in trait). Random
effects were genotype and plot (both nested in trait). The model was run for 500000 MCMC
iterations with a 50000 iteration burn-in time. Results were collected every 100 iterations.
Genetic variances and covariances were estimated by the posterior modes of the sets of MCMC
estimates of genetic variance and covariance for each trait. Heritabilities were estimated by
calculating the posterior modes of the ratios of genetic variances to the sums of variances for all
random effects (Wilson et al., 2010). G matrices were standardised by the trait means so all
of the traits were on a common scale for comparison. The mean standardised G matrix, $G_\mu$,
was yielded by element-wise division by the products of the trait means, $\bar{z}$ (Hansen and Houle,
2008).

The mean standardised vector of evolutionary response, $\Delta \bar{z}_\mu$, was estimated by substituting
the mean standardised G matrix and selection gradients into the multivariate breeders equation:
$\Delta \bar{z}_\mu = G \beta_\mu$ (Lande, 1979; Lande and Arnold, 1983). Because we are interested on the effect of
plasticity on the response to selection we calculated the response to selection in the elevated
plots using the G matrix from both the elevated and control plots. The former is the true
estimate of the response to selection and the latter gives an estimate of what the response to
selection would have been had there been no plasticity. To determine whether the population
would have been able to respond better without or without the plastic response, we estimated
the angle between the selection gradient vector and response vectors with and without plasticity.
A shallower angle indicates that the population is better able to respond in the selected direction.

We also estimated the evolvability of the two populations, $e(\beta_\mu)$, as the ability to respond in the direction of selection, given the genetic constraints imposed by G (Hansen and Houle,
2008). This is the projection of the mean standardised response to selection, $\Delta \bar{z}_\mu$, on the
mean standardised selection gradient, $\beta_\mu$, and gives the predicted proportional change in the
mean-standardised trait vector when selection is as strong as that on fitness:

$$e(\beta_\mu) = \frac{\beta_\mu^T G \mu \beta_\mu}{|\beta_\mu|^2}$$ (3.1)

This measure of evolvability corresponds to definition of evolvability given by Flatt (2005),
which is the ability to respond phenotypically in the direction of natural selection. Further
discussion of the different meanings of evolvability can be found in chapter 6. The validity of
this index relies on the assumptions of no linkage and no epistasis (Hansen and Houle, 2008).
The assortative mating of parental lines in this set of RILs means that these effects have been
largely broken up, so it is reasonable to ignore these effects for the purposes of this study.

3.3 Results

Plants grown in the elevated temperature plots were on average larger, flowered earlier and
produced more branches and fruits (table 3.1). Although plants were slightly taller and produced
more branches on the main stem, these differences were not statistically significant. Significant
3.3. RESULTS

Table 3.1: Treatment effects, variance components, and cross-environment genetic correlations for six traits in Arabidopsis thaliana MAGIC lines grown in ambient and elevated temperature treatments. The results are in part from Chapter 2.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Treatment mean (se)</th>
<th>MS F (P)</th>
<th>V_g (P)</th>
<th>V_{gxe} (P)</th>
<th>r_g (se)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering time (days)</td>
<td>53.15 (0.77)</td>
<td>49.02 (1.03)</td>
<td>428.88</td>
<td>11.224 (&lt;0.001)</td>
<td>0.677 (0.08)</td>
</tr>
<tr>
<td>Rosette diameter (mm)</td>
<td>36.64 (2.54)</td>
<td>49.9 (3.41)</td>
<td>4266.27</td>
<td>13.28 (&lt;0.005)</td>
<td>1.956 (0.42)</td>
</tr>
<tr>
<td>Inflorescence height (cm)</td>
<td>48.35 (1.59)</td>
<td>50.4 (2.11)</td>
<td>78.91</td>
<td>47.293 (&lt;0.001)</td>
<td>5.073 (0.05)</td>
</tr>
<tr>
<td>Branches on main stem</td>
<td>9.83 (0.6)</td>
<td>11.19 (0.73)</td>
<td>88.9</td>
<td>10.322 (&lt;0.001)</td>
<td>2.448 (0.03)</td>
</tr>
<tr>
<td>Total branches</td>
<td>27.79 (1.66)</td>
<td>34.52 (2.08)</td>
<td>3726.32</td>
<td>12.47 (&lt;0.016)</td>
<td>41.551 (0.001)</td>
</tr>
<tr>
<td>Number of fruits</td>
<td>777.91 (51.16)</td>
<td>989.35 (66.4)</td>
<td>3670939</td>
<td>32008.0 (&lt;0.001)</td>
<td>163051 (0.03)</td>
</tr>
</tbody>
</table>

Table 3.2: Estimates of genetic parameters for five traits in A. thaliana MAGIC lines grown in ambient and elevated temperature treatments. Trait heritabilities (with MCMC standard errors) are shown in the first column. In the remaining columns, additive genetic variances and covariances are shown on the diagonal and above the diagonal. Genetic correlations are shown in bold below the diagonal. All traits were mean standardised as described in the methods and in Hansen and Houle (2008).

(a) Ambient treatment

<table>
<thead>
<tr>
<th></th>
<th>h^2</th>
<th>Flower</th>
<th>Rosette</th>
<th>height</th>
<th>main stem</th>
<th>total branches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering time</td>
<td>0.30 (0.09)</td>
<td>0.04</td>
<td>0.008</td>
<td>0.004</td>
<td>0.011</td>
<td>0.004</td>
</tr>
<tr>
<td>Rosette diameter</td>
<td>0.12 (0.04)</td>
<td><strong>0.66</strong></td>
<td><strong>0.038</strong></td>
<td>0.014</td>
<td>0.032</td>
<td>0.019</td>
</tr>
<tr>
<td>Inflorescence height</td>
<td>0.23 (0.07)</td>
<td><strong>0.45</strong></td>
<td>0.5</td>
<td>0.021</td>
<td>0.011</td>
<td>0.005</td>
</tr>
<tr>
<td>Main stem branches</td>
<td>0.17 (0.06)</td>
<td><strong>0.56</strong></td>
<td><strong>0.53</strong></td>
<td>0.25</td>
<td>0.095</td>
<td>0.055</td>
</tr>
<tr>
<td>Total branches</td>
<td>0.11 (0.04)</td>
<td><strong>0.24</strong></td>
<td><strong>0.38</strong></td>
<td><strong>0.12</strong></td>
<td>0.69</td>
<td>0.067</td>
</tr>
</tbody>
</table>

(b) Elevated treatment

<table>
<thead>
<tr>
<th></th>
<th>h^2</th>
<th>Flower</th>
<th>Rosette</th>
<th>height</th>
<th>main stem</th>
<th>total branches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering time</td>
<td>0.26 (0.08)</td>
<td>0.006</td>
<td>0.011</td>
<td>0.004</td>
<td>0.007</td>
<td>0.001</td>
</tr>
<tr>
<td>Rosette diameter</td>
<td>0.20 (0.06)</td>
<td><strong>0.63</strong></td>
<td>0.052</td>
<td>0.017</td>
<td>0.037</td>
<td>0.022</td>
</tr>
<tr>
<td>Inflorescence height</td>
<td>0.27 (0.07)</td>
<td><strong>0.39</strong></td>
<td><strong>0.52</strong></td>
<td>0.019</td>
<td>0.016</td>
<td>0.007</td>
</tr>
<tr>
<td>Main stem branches</td>
<td>0.16 (0.06)</td>
<td><strong>0.34</strong></td>
<td><strong>0.55</strong></td>
<td><strong>0.38</strong></td>
<td>0.09</td>
<td>0.048</td>
</tr>
<tr>
<td>Total branches</td>
<td>0.10 (0.04)</td>
<td><strong>0.05</strong></td>
<td><strong>0.39</strong></td>
<td><strong>0.19</strong></td>
<td><strong>0.66</strong></td>
<td>0.062</td>
</tr>
</tbody>
</table>

genetic variance was observed for all traits, indicating that genetic differences contribute to the phenotypic variance, but lines tended to respond differently to the elevated temperature as indicated by the significant genotype x treatment variance for inflorescence height, number of branches on the main stem and number of fruits.

All traits for plants in both ambient and elevated temperature plots exhibited significant heritability (Table 3.2). Flowering time was the trait with highest heritability in both treatments. Plants grown in the elevated temperature treatment tended to have higher heritability for size-related traits (rosette diameter and inflorescence height) and lower heritability for flowering time, though standard errors were fairly large (Table 3.2).

Univariate selection gradients are in the same direction in ambient and elevated plots for all traits (figure 3.1 and table 3.1). Elevated temperatures significantly relax selection for increased total branches and marginally relax selection for increased rosette diameter, whilst marginally intensifying selection for early flowering and taller inflorescences. Selection gradients are in the
Table 3.3: Mean standardised selection gradients ($\beta_\mu$) and evolutionary response vectors ($\Delta \bar{z}_\mu$) for experimental populations of *A. thaliana* MAGIC lines grown in ambient and elevated temperature treatments. Selection gradients in bold are statistically significant, based on regression of the trait on fitness.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Ambient</th>
<th>Elevated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta_\mu$</td>
<td>$\Delta \bar{z}_\mu$</td>
</tr>
<tr>
<td>Flowering time</td>
<td>-0.009</td>
<td>0.006</td>
</tr>
<tr>
<td>Rosette diameter</td>
<td><strong>0.181</strong>*</td>
<td>0.027</td>
</tr>
<tr>
<td>Inflorescence height</td>
<td><strong>0.308</strong>*</td>
<td>0.012</td>
</tr>
<tr>
<td>Branches on main stem</td>
<td><strong>-0.07</strong></td>
<td>0.054</td>
</tr>
<tr>
<td>Total branches</td>
<td><strong>0.937</strong>*</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Table 3.4: Fitness analysis of magnitude of phenotypic plasticity in *A. thaliana* MAGIC lines across temperature treatments. Partial regression coefficients. Ambient and Elevated column groups refer to the environment in which fitness was measured. Significant $\beta$ values are shown in bold.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Ambient treatment</th>
<th>Elevated treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$ (se)</td>
<td>t</td>
</tr>
<tr>
<td>Flowering time</td>
<td>-0.024 (0.03)</td>
<td>-0.8</td>
</tr>
<tr>
<td>Rosette diameter</td>
<td>0.0224 (0.0283)</td>
<td>0.8</td>
</tr>
<tr>
<td>Inflorescence height</td>
<td><strong>-0.023</strong> (0.008)</td>
<td>-2.8</td>
</tr>
<tr>
<td>Main stem branches</td>
<td>-0.007 (0.008)</td>
<td>-0.9</td>
</tr>
<tr>
<td>Total branches</td>
<td><strong>-0.039</strong> (0.017)</td>
<td>-2.3</td>
</tr>
</tbody>
</table>

same direction as the plastic responses observed in the elevated temperature plots, indicating that plastic responses are in an adaptive direction.

The selection analysis of trait plasticity indicates positive selection for plasticity in flowering time, rosette diameter, inflorescence height and total branches in the heated plots (table 3.4). In contrast, in the ambient treatment, genotypes with higher plasticity for inflorescence height and fruit numbers across environments are significantly less fit.

Using a multivariate approach, we found no significant difference in length of the selection gradient vectors (Table 3.5 $|\beta_\mu|$), but detected a significant angle between the direction of the vectors in ambient and elevated plots (Table 3.5 $\theta(\beta_\mu^{\text{ambient}}, \beta_\mu^{\text{elevated}})$). This means that there is no difference in intensity of selection in elevated temperatures, but a significant difference in the direction of selection. The direction of the response to selection in elevated temperature plots is significantly different from that in ambient temperature plots, whether we use the $G$ matrix from the elevated or ambient temperature with the selection gradients of the elevated plots. In other words, the difference in response is independent of the effects of phenotypic plasticity. However, the difference between responses in the two treatments is increased with the plastic response: The angle between selection and response vectors in elevated temperature plots is considerably shallower using the $G$ matrix without plasticity (Table 3.5 $\theta(\beta_\mu, \Delta \bar{z}_\mu)$). This suggests that plasticity reduces the ability of the population to respond in the direction of selection. Evolvability would be similar in the two treatments if there was no plastic response,
Figure 3.1: Mean-standardised Selection gradient ($\beta_\mu$) estimates for five traits in *A. thaliana* MAGIC lines across temperature treatments. Error bars represent 95% confidence intervals from residual bootstrapping. A $\beta_\mu$ overlapping zero shows no significant association of a trait with fitness.

but is significantly reduced in the elevated temperature plots with a plastic response (Table 3.5 $e(\beta_\mu)$). Together, these results suggest that although plasticity will help in the adjustment to elevated temperatures within a generation, it can compromise future response to selection.

### 3.4 Discussion

In previous work (Chapter 2) we demonstrated that a small increase in temperature can cause significant plastic phenotypic effects in *A. thaliana* and also that genotype-by-environment interactions on fitness are likely to cause changes in population genetic structure. Here, we found that the same small environmental changes were enough to cause changes in patterns of selection and in the population’s response to this selection. Furthermore we determined that, although plastic responses to elevated temperature are in the direction of selection (and therefore appear to be adaptive), observed plastic responses can reduce evolvability, and lead to a response to selection in the wrong direction, impairing future adaptation to environmental changes. Previous studies of the impact of climate change on populations persistence have used “climate envelope” models to infer species distributions given predictions of future climates (e.g. Thomas et al. (2004)). These approaches use current and predicted climate variables and link these to records of species presence and absence to infer future species ranges under predicted climate changes. However, these studies make the assumption that species have single, static environmental tolerance functions and do not take into account any of the evolutionary processes that may be involved in response to climate change. Such models are expected to overestimate the probability
Table 3.5: Comparisons of selection and of evolutionary response for A. thaliana MAGIC lines grown in ambient and elevated temperature treatment. Selection: $\theta(\beta_{\text{ambient}}^\mu, \beta_{\text{elevated}}^\mu)$ is the angle between $\beta_{\text{ambient}}^\mu$ and $\beta_{\text{elevated}}^\mu$. $|\beta_{\text{elevated}}^\mu|$ is the length of the vector $\beta_{\text{elevated}}^\mu$ in each environment. The cross-environment comparison is the difference between lengths of $\beta_{\text{ambient}}^\mu$ and $\beta_{\text{elevated}}^\mu$. Selection response scores are given for the case with plasticity ($\beta_{\text{ambient}}^\mu$ vs $G_{\text{ambient}}^\mu$ and $\beta_{\text{elevated}}^\mu$ vs $G_{\text{elevated}}^\mu$), without plasticity ($\beta_{\text{ambient}}^\mu$ vs $G_{\text{ambient}}^\mu$ and $\beta_{\text{ambient}}^\mu$ vs $G_{\text{elevated}}^\mu$) and just plasticity ($\beta_{\text{elevated}}^\mu$ vs $G_{\text{ambient}}^\mu$ and $\beta_{\text{elevated}}^\mu$ vs $G_{\text{elevated}}^\mu$). Evolvability, $\varepsilon(\beta_{\text{ambient}}^\mu)$ scores are shown in each environment, with a cross-environment comparison given by the ratio of $\varepsilon(\beta_{\text{ambient}}^\mu)$ and $\varepsilon(\beta_{\text{elevated}}^\mu)$. Angles in bold are significantly different from zero. 95% confidence intervals from residual bootstrapping are shown in parentheses.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>ambient</th>
<th>elevated</th>
<th>cross-environment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selection:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\theta(\beta_{\text{ambient}}^\mu, \beta_{\text{elevated}}^\mu)$</td>
<td></td>
<td></td>
<td>15.24° (5.69°, 26.79°)</td>
</tr>
<tr>
<td>$</td>
<td>\beta_{\text{elevated}}^\mu</td>
<td>$</td>
<td>1.01 (0.97, 1.06)</td>
</tr>
<tr>
<td><strong>Response (with plasticity):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\theta(\beta_{\text{ambient}}^\mu, \Delta z_{\mu})$</td>
<td>57.83° (55.00°, 60.58°)</td>
<td>59.85° (55.48°, 63.54°)</td>
<td></td>
</tr>
<tr>
<td>$\theta(\Delta z_{\text{ambient}}^\mu, \Delta z_{\text{elevated}}^\mu)$</td>
<td></td>
<td></td>
<td>5.72° (3.89°, 7.88°)</td>
</tr>
<tr>
<td>$</td>
<td>\Delta z_{\mu}</td>
<td>$</td>
<td>0.089 (0.085, 0.093)</td>
</tr>
<tr>
<td>$\varepsilon(\beta_{\mu})$</td>
<td>0.064 (0.058, 0.069)</td>
<td>0.054 (0.046, 0.064)</td>
<td>1.17 (0.98, 1.41)</td>
</tr>
<tr>
<td><strong>Response (without plasticity):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\theta(\beta_{\text{ambient}}^\mu, \Delta z_{\mu})$</td>
<td>57.83° (55.00°, 60.58°)</td>
<td>47.01° (44.08°, 49.95°)</td>
<td></td>
</tr>
<tr>
<td>$\theta(\Delta z_{\text{ambient}}^\mu, \Delta z_{\text{elevated}}^\mu)$</td>
<td></td>
<td></td>
<td>2.58° (0.94°, 4.63°)</td>
</tr>
<tr>
<td>$</td>
<td>\Delta z_{\mu}</td>
<td>$</td>
<td>0.089 (0.085, 0.093)</td>
</tr>
<tr>
<td>$\varepsilon(\beta_{\mu})$</td>
<td>0.064 (0.058, 0.069)</td>
<td>0.062 (0.057, 0.068)</td>
<td>1.00 (1.02, 1.03)</td>
</tr>
</tbody>
</table>
of extinction or range shift because they overlook the possibility that environmental tolerances might evolve or increase through phenotypic plasticity (Davis et al., 2005; Reed et al., 2011). Theoretical models (Chevin et al., 2010; Hoffmann and Sgro, 2011) and reviews (Davis et al., 2005; Reed et al., 2011) have begun to address the issue of integrating plastic and evolutionary response in predictions of response to climate change, but little experimental work has been done on this. These models underline a need to collect more empirical data on the environmental sensitivity of selection gradients, and better separation of plastic and genetic responses. Here we provide a first empirical approach to estimating species response to predicted climate warming in a natural environment without confounding environmental cues, taking into account both phenotypic plasticity and adaptive evolution. We use a novel combination of in situ simulation of climate warming, replicated lines of identical genotypes and statistical and quantitative genetic techniques previously used to estimate selection (e.g. Grant and Grant (2002)) or to compare evolvability (Hansen and Houle, 2008) in natural populations. Using these methods we were able to identify the strength and direction of selection, and response to a specific and ecologically relevant environmental change, isolate the genetic and plastic component of the response to this change and also predict the effect of this plastic response on future evolvability. The replication of genetically identical populations meant that we could use our estimation of the $G$ matrix in ambient and elevated temperatures to quantify the contribution of plasticity to response to selection. A plastic component of $G$ has rarely been considered in past studies, and the difference we identify between two genetically identical populations with only relatively small environmental changes further demonstrates that it is not sensible to assume that $G$ remains stable over evolutionary time for the purposes of making long term predictions of evolutionary change (Pigliucci, 2001; Walsh and Lynch, 2011).

One of the mechanisms by which plasticity is proposed to be beneficial in coping with new environments is by dampening the force of selection (Ghalambor et al., 2007). This means that plasticity moves the population towards the new phenotypic optimum associated with a new environment, reducing the intensity of selection and also the amount of evolutionary change that is needed to move towards that optimum. The population then experiences reduced exposure to the costs associated with strong selection, such as reduced phenotypic variation due to purging of unfit variants. However, plasticity is expected to be able to produce less extreme phenotypes than can adaptive evolution because plasticity is limited by the genetic and physiological constraints of the organism at that point (Gienapp, 2008). This may mean that, following plastic response to environmental change, an organism may be at the physiological limits of its ability to respond plastically and so be compromised if future environmental change occurs. Here, we show that plasticity is in an adaptive direction for all measured traits and that it contributes significantly to the vector of response to selection (Table 3.5, difference between $\theta(\Delta z^\text{ambient}_\mu, \Delta z^\text{elevated}_\mu)$ with and without plasticity). Plasticity should therefore reduce fitness loss due to new selection pressures because plastic response is in the direction of selection. However, although plasticity does not reduce the magnitude of response to selection, it does increase the angle between response and selection. Furthermore, plants grown under simulated climate change show reduced evolvability in the presence of plasticity than would be expected if there had been no plasticity. These results suggest that, although plasticity appears to
assist in moving the population towards a new phenotypic optimum, this immediate response is traded-off against potential response to future environmental change. The significance of this is that estimates of current genetic diversity and evolvability might wrongly estimate future response to selection because they do not account for the plastic response upon exposure to new environments. In Chapter 2 we found evidence for genotype-by-environment effects on fitness, suggesting that elevated temperatures can cause some fit genotypes to become maladapted, and that a change in population genetic structured is expected. It was not clear though if the change if fitness hierarchy was due to changes in selection gradients or the plastic response. Here, we observe positive selection gradients for plasticity in elevated temperatures, suggesting that plasticity is adaptive in elevated temperatures. However, there are also costs to being plastic in ambient temperatures. Therefore, it seems that plastic response contributes to the change in genotype fitness: genotypes that respond well to elevated temperature are not the same as the genotypes that are fittest under ambient conditions. Although the magnitude of selection was unaffected, under elevated temperature the direction of the selection vector is significantly different. This suggests that changes in selection as well as plastic responses are involved in the shift of genotype fitness.

Phenotypic plasticity is expected to have a role in assisting in species and population persistence, most commonly by buffering against strong selection pressures imposed by new and changing environments. In this study we have shown that plasticity in *A. thaliana* is in an adaptive direction when a population is exposed to simulated climate warming, and can assist plants to cope with this new environment. However, across generations, phenotypic plasticity can reduce the evolvability of the population (its ability to evolve in the direction of selection). The consequences of this are that the immediate ability to cope in a new environment may compromise future evolutionary response, and therefore be detrimental for a population’s long term persistence in the face of changing environments. The combination of climate manipulation in the field, replicated inbred lines and recent statistical techniques allow us to consider both plastic and evolutionary responses to predicted climate change and is a step towards the better understanding of plant response to environmental change under natural conditions, out of the lab and away from the computer simulation. This study demonstrates the importance of considering the interaction between plasticity and selection in predicting population persistence when exposed to changing climates.
Chapter 4

Correlated response in plasticity to selection for early flowering in Arabidopsis thaliana

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Abstract

Phenotypic plasticity is an important strategy for coping with changing environments. However, environmental change usually results in strong directional selection and little is known empirically about how this affects plasticity. If genes affecting a trait value also affect its plasticity, selection on the trait should influence plasticity. Outbred populations of Arabidopsis thaliana were selected for earlier flowering in simulated spring- and winter-annual conditions to investigate the correlated response of flowering time plasticity; and its effect on family-by-environment variance ($V_{gxe}$) within each selected line. We found that selection affected plasticity in an environmentally-dependent manner: under simulated spring-annual conditions selection increased the magnitude of plastic response but decreased $V_{gxe}$; selection under simulated winter-annual conditions reduced the magnitude of plastic response, but did not alter $V_{gxe}$ significantly. Since selection may constrain future response to environmental change, the environment for crop breeding and ex situ conservation programs should be carefully chosen. Models of species persistence under environmental change should also consider the interaction between selection and plasticity.
4.1 Introduction

Phenotypic plasticity is the property of a genotype whereby it can express different phenotypes depending on environmental cues. This can be an important factor allowing populations to colonize and persist in new and changing environments (Crispo, 2008; Ghalambor et al., 2007; Price et al., 2003; Yeh and Price, 2004). Plasticity may intensify or attenuate evolved responses in trait value, depending on the direction of the plastic response and its proximity to the phenotypic optimum (Ghalambor et al., 2007). Recent concerns about the ability of species to persist and adapt to new environmental conditions have renewed interest in the complex relationship between adaptive changes in trait mean and plastic responses, and their consequences for population mean fitness (Gienapp, 2008; Charmantier et al., 2008; Chevin and Lande, 2010). However, it remains unclear whether the plasticity of a trait can evolve independently of the trait mean and if so whether they constrain each others evolution (Pigliucci, 2005; Auld et al., 2010; Crispo, 2008).

The relationship between the value of a trait in one environment and its plasticity has been the subject of considerable debate (Via and Lande, 1985; Schlichting, 1986; Via et al., 1995; Pigliucci, 2005). Via and Lande (1985) proposed that plasticity is a function of the differential expression of the same genes in different environments. Under this model, the plasticity of a trait can have a strongly correlated response to direct selection on that trait (as long as the cross environment genetic correlation does not equal 1). Alternatively, the plasticity of a trait might be under separate regulatory control from the trait itself (Schlichting and Pigliucci, 1995). Under the second model, direct selection on a trait should have little effect on the plasticity of that trait. While these two mechanisms are not mutually exclusive, it is important to determine empirically whether correlated response to directional selection involves change in plasticity since this will enable us to ascertain if the current response of a trait to selection may constrain future evolution by reducing or increasing plasticity.

The most powerful approach to evaluate the evolutionary independence of trait value and its plasticity is the use of selection experiments (See Scheiner (2002); Callahan (2005); Garland and Kelly (2006) for extensive reviews on this topic). This approach allows for the investigation of how plasticity evolves as a correlated trait when selection is controlled to target the trait mean. While artificial selection experiments can never replicate field conditions exactly, and so cannot directly inform us about the evolution of traits in nature, they are helpful to understand the genetic relationship between the mean and plasticity. Despite their advantages, selection studies of plasticity in higher plants are relatively rare due to their large size and relatively long generation times (but see Mather and Jinks (1982) and Falconer (1990)). The use of the model organism *Arabidopsis thaliana* allows many plants to be grown in a short period of time. Previous studies using this species have mainly performed "line sorting" since *A. thaliana* reproduces primarily by self-fertilization. Here we take advantage of a set of lines selected for earlier flowering derived from a synthetic outbred population of *Arabidopsis thaliana* and hand-pollinated after selection (Scarcelli and Kover, 2009) to investigate the correlated response in plasticity. These lines have been subject to five generations of artificial selection under two different growth conditions, causing a reduction of mean flowering time of approximately two standard deviations.
4.2. Materials and Methods

4.2.1 Experimental design

The synthetic outbred population used in this study is described in detail in Scarcelli et al. (2007). Briefly, 19 natural accessions of *A. thaliana* from a wide geographical distribution were randomly inter-mated for 5 generations. In the first generation, 6 replicated founder populations were cultivated in growth chambers running either a simulated "winter-annual" (Winter treatment)
or a "spring-annual" program (Spring treatment). All 6 populations were initiated using full siblings from the same 200 families randomly chosen from the outbred population. Data from this first generation provides estimates of the relationship between mean and plasticity before selection. From each basal population a selected and a control line were derived, producing 6 lines selected for early flowering and 6 control lines. Every generation, plants were checked daily for germination and then for appearance of floral buds (bolting), flowering time was calculated as the number of days between germination and bolting. The selected lines were produced by randomly crossing the 50 earliest flowering plants after assigning them to 25 hand-pollinated crosses. Crosses were performed in floral buds emasculated prior to pollen maturation, to avoid any seeds being the result of self-fertilization. Control lines were produced similarly by crossing 50 randomly selected plants per generation. This protocol was repeated for 5 generations.

The "spring treatment" simulates conditions experienced in spring-annual life histories with transitions in temperature and day length reflecting changes from spring into summer (14°C : 10 °C day: night and 8h : 16h light : dark, transitioning to 21 °C: 18 °C day: night and 16h : 8h light : dark). The "winter treatment" simulates the light and temperature transitions expected in winter-annual life histories with the program starting with autumn-winter transitions (16 °C: 10 °C day: night and 8h : 16h light : dark transitioning to 4 °C: 4 °C day: night and 6h : 18h light : dark), followed by the same program as in the spring-annual treatment. This temporally varying environment was designed to give a range of environmental cues akin to that which A. thaliana may experience in the field. This is preferable to the uniform conditions used in many growth chamber experiments, although it is recognised that plants in natural conditions experience a far more complex environment.

At the end of the selection protocol, the 12 lines (3 control and 3 selected bred in both spring and winter treatments) were planted in a "reciprocal transplant" fashion: 3 full sibs from each of the 25 crosses performed in the fifth generation of selection were grown under each treatment (1200 plants in each) (for further details, see Kover et al. (2009a)). After the plants had senesced, the total number of fruits produced by each plant was counted, providing an estimate of fitness (fruit number and seed set have been shown to be highly correlated in A. thaliana (Mauricio and Raucher, 1997; Westerman and Lawrence, 1970).

4.2.2 Data analysis

This study considers both changes in the magnitude of population mean plasticity and changes in genotype x environment variation (V_{gxe}). Although they both tell us something about the response of the populations to different environments, they measure distinct aspects of plasticity. Mean plasticity shows the magnitude of the average response of the population of genotypes to these specific environments at a point in evolutionary time, while V_{gxe} indicates the range of possible plastic responses. Plasticity is most commonly estimated as the differences in mean for a given genotype when grown in different environments, and was here calculated as the family mean flowering time (FT) in winter treatment minus mean FT in spring treatment.

To determine the relationship between flowering time and flowering time plasticity in the population prior to selection, we calculated Pearson’s correlation coefficient and mean flowering time for the 200 full-sib families in spring- and winter-annual treatments. To determine the
existence of genotype by environment interaction prior to selection we used the linear model:

$$\text{FT} = \text{family} + \text{environment} + \text{family} \times \text{environment}$$

Family-by-environment interactions can arise from either changes in genetic variance across environments or a change in the rank order of genotypes across environments (crossing of reaction norms). We used the equations in Cockerham (1963, p.88) to identify the percentage of \( V_{gxe} \) that could be attributed to crossing following the method in Johnson (2007) for applying to a mixed effect model.

To determine whether selection on trait values affected the plasticity in flowering time we used the following linear model:

$$\text{Plasticity} = \text{Breeding environment} + \text{selection treatment} + \text{Breeding environment} \times \text{Selection treatment} + \text{Line} (\text{Breeding environment} \times \text{Selection treatment})$$

The same model factors were used to determine the effect of selection treatment on cross-environment mean flowering times (mean of spring and winter treatments). As a post-hoc test, to directly compare the mean plasticity and cross-environment mean for control and selected lines separately for each breeding environment, we used an exact permutation test (Ludbrook and Dudley, 1998). Observed differences between mean plasticity in control and selected treatments were compared to the distribution of differences obtained by randomizing all observed FT values in the 6 populations within each environmental condition 46,656 times (216 possible bootstrap permutations in first group x 216 possible bootstrap permutations in second group) into 2 groups of 3 populations (the maximum number of possible independent permutations) and calculating their plasticity.

To determine the effect of selection for early flowering on the genetic variance for plasticity, variance components for family x environment interactions (\( V_{gxe} \)) and total phenotypic variance (\( V_p \)) in each of the 12 populations was calculated using the R package \texttt{lme4} (Bates and Maechler, 2009). The following model was used:

$$\text{Flowering time} = \text{Family} + \text{Environment} + \text{Family} \times \text{Environment}$$

with Environment set as a fixed effect and Family as a random effect. Confidence limits (+/-95%) for each population’s \( V_p \) and \( V_{gxe} \) variance components were constructed from the 2.5 and 97.5 percentiles of the variance components from 1000 parametric bootstrap replicates. Differences in the variance components \( V_p \) and \( V_{gxe} \), for control and selected lines were tested separately in the spring and winter treatments using exact permutation tests. We compared the observed differences in values with a null distribution obtained by randomizing the observed variance components observed in the 6 populations into all possible permutations of 2 groups of 3 populations.

To determine the costs of flowering time plasticity, we estimated selection gradients (Lande and Arnold, 1983) as modified by Weinig et al. (2006) where the mean fruit count (fitness) of a family in a given environment is regressed onto the family mean flowering time and flowering time plasticity. The fitness analysis was performed only for the control lines, since strong artificial selection for early flowering could have affected the relationship between plasticity and fitness. Costs of plasticity are indicated by negative partial regression coefficients in the selection model (i.e. more plastic genotypes have lower fitness) while positive partial regression coefficients indicate that plasticity is adaptive or that homeostasis (maintaining the same
phenotype independent of the environment) is costly, i.e. more plastic genotypes have higher fitness. We estimate both the "local costs" (i.e., detected in only one environment), and the "global costs" (when they are detected in both treatments) (Sultan and Spencer, 2002). All data analyses were performed using R (R Development Core Team, 2011).

4.3 Results

4.3.1 Trait mean and plasticity prior to selection

An ANOVA for flowering time for the 200 full-sib families in the basal outcrossed population revealed a highly significant gxe interaction ($F_{187,653} = 1.8, P < 0.0001$). A plot of the reaction norms for the 200 families showed both non-parallelism and crossing, despite a much larger effect of treatment (Figure 4.1). Crossing of reaction norms accounted for 48.3% of the gxe variance, indicating that environmental changes can lead to changes in adaptive value.

We observed a strong negative relationship between family mean FT and plasticity under spring conditions (Figure 4.2), indicating that families that flower early in the spring treatment are also more plastic. However, the relationship between FT in winter and plasticity is positive and non-significant (Figure 4.2). Thus, we expect that selection for early flowering under the spring treatment should increase the magnitude of plasticity, while selection under the winter treatment should not affect the magnitude of plasticity, or only slightly reduce it.
Figure 4.2: Relationship between phenotypic plasticity and mean flowering time prior to selection in the spring ($r = -0.9$, $t_{186} = -28.11$, $P < 0.0001$) and winter ($r = 0.14$, $t_{186} = 1.96$, $P = 0.0517$) treatments. Best fit lines based on linear models are provided for better visualization of the trend.

Table 4.1: Mean (SE) for the magnitude of plasticity in flowering time and cross-environment means for each line; estimates for total phenotypic ($V_p$) and family x environment ($V_{gxe}$) variance components are also presented.

<table>
<thead>
<tr>
<th>Breeding environment</th>
<th>Selection treatment</th>
<th>line</th>
<th>Mean FT plasticity (SE)</th>
<th>Cross–environment mean FT</th>
<th>$V_p$</th>
<th>$V_{gxe}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>Control</td>
<td>1</td>
<td>33.78 (1.16)</td>
<td>54.29 (0.95)</td>
<td>46.36</td>
<td>9.37</td>
</tr>
<tr>
<td>Spring</td>
<td>Control</td>
<td>2</td>
<td>33.86 (1.21)</td>
<td>57.27 (1.03)</td>
<td>62.53</td>
<td>7.40</td>
</tr>
<tr>
<td>Spring</td>
<td>Control</td>
<td>3</td>
<td>31.28 (0.75)</td>
<td>52.90 (0.58)</td>
<td>25.45</td>
<td>2.00</td>
</tr>
<tr>
<td>Winter</td>
<td>Control</td>
<td>4</td>
<td>37.11 (0.77)</td>
<td>53.04 (0.70)</td>
<td>26.92</td>
<td>3.15</td>
</tr>
<tr>
<td>Winter</td>
<td>Control</td>
<td>5</td>
<td>35.80 (1.17)</td>
<td>55.23 (1.06)</td>
<td>68.14</td>
<td>1.87</td>
</tr>
<tr>
<td>Winter</td>
<td>Control</td>
<td>6</td>
<td>32.32 (0.98)</td>
<td>56.87 (0.81)</td>
<td>51.27</td>
<td>8.38</td>
</tr>
<tr>
<td>Spring</td>
<td>Selected</td>
<td>7</td>
<td>37.94 (0.65)</td>
<td>45.52 (0.36)</td>
<td>15.09</td>
<td>1.75</td>
</tr>
<tr>
<td>Spring</td>
<td>Selected</td>
<td>8</td>
<td>37.43 (0.48)</td>
<td>45.17 (0.35)</td>
<td>10.65</td>
<td>1.26</td>
</tr>
<tr>
<td>Spring</td>
<td>Selected</td>
<td>9</td>
<td>38.97 (0.56)</td>
<td>46.42 (0.26)</td>
<td>12.41</td>
<td>1.48</td>
</tr>
<tr>
<td>Winter</td>
<td>Selected</td>
<td>10</td>
<td>30.19 (1.07)</td>
<td>47.33 (0.68)</td>
<td>30.65</td>
<td>7.42</td>
</tr>
<tr>
<td>Winter</td>
<td>Selected</td>
<td>11</td>
<td>32.08 (0.48)</td>
<td>44.57 (0.30)</td>
<td>11.42</td>
<td>0.99</td>
</tr>
<tr>
<td>Winter</td>
<td>Selected</td>
<td>12</td>
<td>32.21 (0.79)</td>
<td>45.28 (0.48)</td>
<td>23.94</td>
<td>6.02</td>
</tr>
</tbody>
</table>
4.3.2 Correlated responses to selection in plasticity

Plasticity and cross-environment mean flowering time values for selected and control lines are shown in Table 4.1. The ANOVA for plasticity indicates that breeding environment significantly affected plasticity, with plants grown in the spring treatment being more plastic than plants bred in the winter treatment ($F_{1, 215} = 10.92, P = 0.001$). While plants under selection treatment were overall more plastic than plants in the control treatment, that difference is not significant ($F_{1, 215} = 2.96, P = 0.0869$). This is because selection has opposite effects on plasticity depending on the environment in which it was carried out (Figure 4.2), as confirmed by the presence of a significant environment by selection treatment interaction ($F_{1, 216} = 56.22, P < 0.0001$). A significant effect of lines nested within the interaction of environment and selection treatment ($F_{8, 223} = 2.53, P = 0.0118$) implies that there is heterogeneity among replicated lines. Exact permutation test show that among the plants bred under spring conditions, plants in selected lines were significantly more plastic than in the control lines (Table 4.2). In contrast, plants selected for early flowering in winter showed a significant decrease in plasticity relative to the controls. We also found that plants selected for early flowering in the spring treatment were significantly more plastic than those selected for early flowering in the winter treatment. While winter control lines were more plastic than spring control lines, this difference was not significant.

The cross-environment means showed no significant effect of breeding environment ($F_{1, 215} = 0.24, P = 0.6227$) or of an interaction between breeding environment and selection treatment ($F_{1, 215} = 0.002, P = 0.961$). However, a significant effect of selection was observed ($F_{1, 215} = 405.83, P < 0.0001$). Exact randomization tests indicate that selection significantly reduced the cross-environment mean flowering time by around 9 days, irrespective of whether the selection took place in spring or winter conditions (Table 4.2).

4.3.3 Effect of selection on gxe variance

Variance components and 95% confidence limits for each line are shown in Table 4.1 and Table B.1 in Appendix B. As expected, exact permutation tests show that selected lines have reduced total phenotypic variance ($V_p$) relative to the controls, independent of the environmental conditions under which selection was performed (Table 4.3). Plants selected for early flowering in winter conditions showed no significant change in $V_{gxe}$, but selection for early flowering in spring conditions significantly reduced $V_{gxe}$ relative to the control lines (Table 4.3).

4.3.4 Flowering time plasticity and fruit production (fitness)

Analysis of selection gradients found no significant partial regression coefficients for fitness on the plasticity of the plants in any of the control lines in either spring or winter treatments (Table 4.4).

4.4 Discussion

There is much interest in the importance of phenotypic plasticity as a coping strategy for organisms confronted with changing environments, and the need to include plasticity in evolutionary
Table 4.2: Results of exact permutation tests for differences in magnitude of plasticity and cross environment mean flowering time, between control and selected lines in the spring and winter treatments. P-values <0.05 are shown in bold.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Selected</th>
<th>Mean difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Magnitude of Plasticity:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>32.98</td>
<td>38.11</td>
<td>5.13</td>
<td>0.0084</td>
</tr>
<tr>
<td>Winter</td>
<td>35.08</td>
<td>31.49</td>
<td>-3.59</td>
<td>0.0293</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-2.10</td>
<td>6.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.0971</td>
<td>0.0071</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cross-environment mean:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>54.82</td>
<td>45.7</td>
<td>-9.12</td>
<td>0.0075</td>
</tr>
<tr>
<td>Winter</td>
<td>55.05</td>
<td>45.73</td>
<td>-9.32</td>
<td>0.0076</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-0.23</td>
<td>-0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.4352</td>
<td>0.4812</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.3: Results of exact permutation tests for differences in total phenotypic variance ($V_p$) and family x environment variance ($V_{gxe}$) for control and selected lines in the spring and winter treatments. P-values <0.05 are shown in bold.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Selected</th>
<th>Mean difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_p$:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>44.779</td>
<td>12.715</td>
<td>-32.06</td>
<td><strong>0.021</strong></td>
</tr>
<tr>
<td>Winter</td>
<td>48.774</td>
<td>22.002</td>
<td>-26.77</td>
<td><strong>0.042</strong></td>
</tr>
<tr>
<td>Mean difference</td>
<td>-3.9952</td>
<td>-9.28695</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.384</td>
<td>0.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{gxe}$:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>6.255</td>
<td>1.497</td>
<td>-4.76</td>
<td><strong>0.035</strong></td>
</tr>
<tr>
<td>Winter</td>
<td>4.467</td>
<td>4.810</td>
<td>0.34</td>
<td>0.438</td>
</tr>
<tr>
<td>Mean difference</td>
<td>1.7877</td>
<td>-3.3128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.262</td>
<td>0.070</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.4: Partial regression coefficients for fruit number on magnitude of plasticity in control lines. The slope is indicated by the partial regression coefficient, $\beta$, and the significance by the probability, $P$.

<table>
<thead>
<tr>
<th>Fitness measured in</th>
<th>Breeding environment</th>
<th>Magnitude of plasticity</th>
<th>$\beta$ (SE)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>Spring</td>
<td>-0.42 (1.6)</td>
<td>-0.26</td>
<td>0.797</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>Spring</td>
<td>-0.07 (0.95)</td>
<td>-0.08</td>
<td>0.941</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>Spring</td>
<td>0.16 (1.19)</td>
<td>0.14</td>
<td>0.894</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>Winter</td>
<td>1.54 (1.29)</td>
<td>1.19</td>
<td>0.249</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>Winter</td>
<td>0.77 (0.99)</td>
<td>0.77</td>
<td>0.451</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>Winter</td>
<td>-0.54 (1.02)</td>
<td>-0.53</td>
<td>0.606</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>Spring</td>
<td>-0.21 (0.12)</td>
<td>-1.74</td>
<td>0.098</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>Spring</td>
<td>-0.04 (0.1)</td>
<td>-0.35</td>
<td>0.728</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>Spring</td>
<td>0.12 (0.22)</td>
<td>0.54</td>
<td>0.596</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>Winter</td>
<td>&lt;0.01 (0.15)</td>
<td>-0.03</td>
<td>0.975</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>Winter</td>
<td>0.2 (0.12)</td>
<td>1.69</td>
<td>0.109</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>Winter</td>
<td>0.02 (0.17)</td>
<td>0.13</td>
<td>0.899</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.3: Effect of selection on population mean reaction norms for flowering time under the spring and winter treatments after six generations of selection or control breeding. Control populations are represented by solid lines; populations selected for early flowering are represented by dashed lines. Black lines represent populations bred under the spring treatment; grey lines represent populations bred in simulated winter-annual conditions.
models of species persistence under climate change has been highlighted (Chevin and Lande, 2010; Chevin et al., 2010; Nicotra et al., 2010; Reed et al., 2010). Maintenance of plasticity is generally considered as positive and this maintenance has been mainly evaluated against possible contemporary fitness costs (e.g. Relyea, 2002; van Tienderen, 1997; DeWitt et al., 1998; Ernande and Dieckmann, 2004). However, environmental change usually leads to strong directional selection, and little is known about how selection on trait value affects plasticity. We find that selection for earlier flowering time causes a correlated response in plasticity and that the direction and magnitude of this response depended on the growth conditions under which selection occurred. The direction of the observed effects is in agreement with trait correlation prior to selection; however, the observed correlated response in winter was not expected to be significant. In addition, changes in the magnitude of plasticity were not indicative of effects on $V_{gxe}$, which determines future responses to selection on plasticity. The relationship between trait value and plasticity is therefore complex, making it challenging to incorporate into general models. Nevertheless, our results indicate that response to directional selection can compromise plastic responses in the short and longer term (direct effect on plasticity or on $V_{gxe}$, respectively), depending on environmental conditions. Thus, the maintenance of plasticity in populations might be constrained not only by fitness costs, but also by its intrinsic relationship with the trait value.

Ideally, organisms would benefit from modifying trait values to better fit their environmental optima, without compromising their ability to respond plasticly to unpredictable contemporary changes in environmental conditions. Thus a genetic system where trait value and plasticity were independent will better allow adaptation and flexibility. However, the existence of "plasticity genes" has been much criticized in favour of a model where the same genes affect a given trait under different environments, but to different extents (allelic sensitivity). If the genes that contribute to flowering time variation were largely non-overlapping in the two environments, selection in one environment would have no effect on the phenotype in the other environment, resulting in changes in plastic responses and cross environment means (due to changes in the slope of the norm of reaction). Alternatively, if the same genes equally affect flowering time in two different environments, changes in mean flowering time would cause similar changes in the other environment and the evolution of flowering time would not affect the plastic response. Evidence for both types of genetic architecture exists for flowering time exists. For example Ungerer et al. (2003) found different QTL in plants grown under long and short day lengths while Schwartz et al. (2009) found that a single locus mediates flowering responses to photoperiod and temperature cues.

Figure 4.3 shows that in our study, much of the change in flowering time due to selection corresponds to changes in the height of the norm of reaction (as opposed to slope), indicating that a large proportion of the genetic changes involved loci with effects in both environments. Accordingly, the cross-environment mean flowering time is reduced by the same amount by selection in winter and spring treatments.

Although selection had the same overall effect on flowering time in both treatments, the slopes of the reaction norm changed in opposite directions in the two growth conditions. This result is partly consistent with the Jinks-Connolly rule (Jinks and Connolly, 1973; Falconer, 1990), which
proposes that selection away from the cross-environment mean (for early flowering in spring treatment) results in an increase in plasticity, whereas selection towards the cross-environment mean (for early flowering in the winter treatment) results in a decrease in plasticity. This rule holds because direct responses should always be greater than correlated responses, since genetic correlations are generally smaller than 1. However, this rule does not explain the difference in magnitude in the correlated response in the two treatments (larger in the spring treatment). Czesak et al. (2006) proposed that such asymmetrical correlated response in plasticity results from selection operating on a combination of genes with effects on both environments and genes with effects specific to one environment. Evidence of environment-specific selection in our selected lines was observed at the FRIGIDA locus, where non-functional alleles were significantly selected for in the simulated spring-annual conditions, but not in the winter (Scarcelli and Kover, 2009). It is likely that response to selection includes other genes with similar environment-specific effects.

Considering that fine-tuning of flowering time with environment cues is thought to be a critical life-history trait, it is surprising that we found no evidence for plasticity to increase fitness in this study. However, similar results have been previously observed for flowering time plasticity, and even when costs are detected they tend to be small (e.g. (Weinig et al., 2006; ?)). Nevertheless, our results only indicate that plasticity does not significantly affect fitness under the growth conditions in our experiment; it is possible that under natural conditions these genotypes would experience an advantage or a cost.

Dissection of the molecular pathways that regulate flowering time in Arabidopsis and other plants suggest a complex network which contains "receptor genes" that perceive environmental cues and "floral integrators" genes that actually promote floral induction (Michaels, 2009; Schwartz et al., 2009; Blazquez et al., 2003). The activity of the integrators is thought to be modulated by a signal cascade that starts with the receptors. Such a network suggests that evolution in flowering time can occur through changes in allele frequency in receptor genes with different perceptions of the environment. Response through such genes would affect flowering time only in environments with the appropriate cues and would be perceived as evolution in plasticity (changes in the difference in mean flowering between environments). Alternatively, changes in allele frequency in floral integrator genes which requires different amount of signaling from receptors can also cause changes in flowering time, but those would be perceived as independent of the environment. Our results suggest that much of the response to selection for earlier flowering involves core parts of the network, such as floral integrators, which reduce flowering times in both environments. It is also possible that the response could have involved receptors for cues that were present in both environments (such as changes in day length). However, some of the genetic factors selected have to be involved in specific responses to cues present in a single environment, since lines selected in one of the environments always flower earlier than the lines selected in the other environment (Kover et al., 2009a). Given that the largest change in plasticity is observed in the spring treatment, it is likely that there are more environmental specific changes that occurred in this environment than in the winter treatment.

According to theory, directional selection in a stable environment should reduce variation for plasticity when only a limited number of genotypes can express the selected phenotype.
Accordingly, selection for early flowering under both treatments reduced the variance for plasticity ($V_{gxe}$). However, the reduction in variation for plasticity in plants grown in winter conditions is smaller and not significant; suggesting that selection in winter should be less of a constraint to future plastic response to changing environments. Selection appears to have little effect on the amount of crossing of reaction norms in the population, suggesting that there will be little effect on the maintenance of the $V_{gxe}$ remaining in the population following selection. Although $V_{gxe}$ is reduced by selection, the proportion of the total phenotypic variance that is due to plasticity is increased. This suggests that although the variation in plasticity is reduced, it may become a more important avenue for future response to selection. A possible explanation for this fact is that the loci with larger effects on flowering (which are usually selected first), are the ones that affect flowering time similarly in both environment, and that future response to selection will be mainly through loci of smaller and environment-specific effects which affect the slope of the norm of reaction.

Changes in climate have already caused changes in phenology, and in particular in flowering time (Menzel et al., 2006; Fitter and Fitter, 2002; Franks et al., 2007). One of the predictions for anthropogenic climate change is that environments will become more extreme and less predictable (Meehl et al., 2000; IPCC, 2007), leading to the conclusion that plasticity would be a beneficial trait to cope with the uncertainty. While this expectation makes much intuitive sense, the definition of plasticity needs to be carefully considered in this context. Plasticity is most commonly defined as the difference in trait means across environments; species and populations with steeper norms of reaction are usually considered more plastic (e.g. Balaguer et al., 2001; Burns and Winn, 2006; Funk, 2008). However, it is unlikely that current norms of reaction would be still adaptive after significant climate changes (Visser, 2008). Thus, a population with more variation among norms of reaction (increased $V_{gxe}$) might be in a better position to cope with environmental shifts. In this study we find that the effect of selection on plasticity will depend on which estimate of plasticity is being considered (estimates of magnitude of plastic response or $V_{gxe}$). We argue that the latter definition should be favoured (unless the norms of reaction are for fitness) and conclude that selection under some environmental conditions can be detrimental for the ability of populations to cope with environmental uncertainty. Finally, our results suggest that breeders should consider carefully the environment in which artificial selection is to be carried out to enable the resultant varieties to be robust to unpredicted environmental variation. Equally, *ex situ* conservation programs needs to consider carefully which environment to keep organisms in to not adversely affect future re-introduction.
Chapter 5

The genetic basis of germination timing in *Arabidopsis thaliana* under field conditions

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Abstract

Plant fitness and crop yield are dependent on seed germination success and its coordination with optimal seasonal conditions for completion of its life-cycle. However, germination is a complex character composed of a variety of integrated traits, under the control of many genes and modulated by both maternal and climate environmental effects. Here, we investigated multiple aspects of germination under field conditions, using seeds from parents that were also grown in the same field, simulating the natural process of germination of seeds in the environment they would have encountered upon completion of their development. We used a set of *Arabidopsis thaliana* Multiparental Advanced Genetic InterCross (MAGIC) lines, and identified 11 QTL for 5 aspects of germination. Candidate genes identified have been previously associated with germination in mutation screens, but natural variation affecting germination phenotype has not been reported. Five out of those 11 QTL were phytochrome genes indicating the importance of environmental conditions in determining germination in the field. Furthermore, we identified a significant effect of maternal flowering time on germination, independent of maternal genotype, indicating that this relationship is likely due to the differential environment experienced during seed development. This cross-generational plastic response to maternal environment could have unpredictable effects on populations response to predicted climate changes.
5.1 Introduction

The long-term success of plant species depends on the successful germination of seeds. Germination is a composite trait that can be characterised by several different quantitative variables, such as the lag period of dormancy before any seeds germinates, the rate at which seeds germinate, the length of time that seeds remain viable, and the total number of seeds that do germinate. Germination timing, along with traits such as flowering time and dispersal, can be viewed as a mechanism of habitat selection and in annual plants germination at the wrong time can have highly detrimental effects on survival (Donohue, 2003; Huang et al., 2010; Seiwa, 1998). While the majority of previous studies have focused on the proportion of successful germinants (Huang et al., 2010; Alonso-Blanco et al., 2003; Silady et al., 2011), it is important to characterize multiple aspects of germination and their possible integration (Murren, 2012) because not only are the quantity of germinants important, but the timing and spread of germination can also significantly affect fitness (Verdú and Traveset, 2005; Childs et al., 2010).

Germination at the appropriate time is important not only to match ideal conditions for early seedling growth, but also to allow the subsequent life-history transitions to occur at an appropriate time. For example, germination late in the summer may induce flowering during winter, resulting in zero fitness (Donohue et al., 2005a). Recent work has shown that variation in germination timing can constrain later developmental transitions (Bentsink et al., 2010; Weitbrecht et al., 2011), for example explaining a large proportion of variation in flowering time (Wilcezek et al., 2009). Likewise, there is evidence that the environment experienced by the maternal plant while the seeds are developing can affect the germination characteristics of the seeds (Mousseau and Fox, 1998; Gutterman, 2000; Munir et al., 2001; Schmuths et al., 2006; Galloway and Ettersen, 2007; Galloway and Burgess, 2009). There are three distinct (but not mutually exclusive) explanations for correlations between germination and other developmental traits (such as flowering time): A correlation may be due to a common genetic pathway (where traits share some of their genetic controls, i.e. pleiotropy), through environmental effects (where the timing of one transition affects the environment experienced by the next stage in the life-history (Donohue, 2009)) or through maternal effects (e.g. seeds from earlier flowering plants may spend more time in the fruit on the plant, or may receive fewer nutrients since the plant may have laid down fewer vegetative resources (Roach and Wulff, 1987)).

As both germination and flowering respond to many of the same environmental cues (light quality, day length, temperature, etc.) it is reasonable to expect them to share common genetic regulatory elements. There is some genetic evidence for this relationship: The family of PHYTOCHROME genes which control light signal perception in plants have been strongly implicated in both flowering timing (Devlin et al., 1998; Balasubramanian et al., 2006a) and germination timing (Pons, 2000; Heschel et al., 2007, 2008; Laserna et al., 2008). Also, the transcription factor, FLOWERING LOCUS C, initially found to control flowering time epistatically with the FRIGIDA gene in Arabidopsis thaliana (Caicedo et al., 2004), has since been found to promote temperature-dependent seed germination, largely controlled by the maternal genotype (Chiang et al., 2009). It is also conceivable that germination and flowering are associated because the both respond to internal factors that control development such as circadian clock genes and hormones (Mizoguchi et al., 2005; Yakir et al., 2007; Santer and Estelle, 2009).
5.1. INTRODUCTION

It has been shown that environmental factors such as temperature and light quality experienced by the maternal plant during seed development can affect both the proportion and the timing of germination (Schmuths et al., 2006; Barua et al., 2012; Donohue, 2009). Parental environment has been shown to affect germination (Fenner, 1991; Donohue, 2009), and high temperatures and short days during seed development have been associated with increased proportion of germinants. A study of cohorts of Campanulastrum americanum that were manipulated to flower early showed a much higher prevalence of annual rather than biennial growth forms (Galloway and Burgess, 2009). Simulated climate warming experiments also found that early initiation of flowering due to warming resulted in a shorter germination time (Sherry et al., 2007; Post and Pedersen, 2008). In A. thaliana, a short-day photoperiod has been shown to cause increased responsiveness to cold-stratification, with higher germination percentages and rates in stratified seeds (Munir et al., 2001).

Although these studies all show a relationship between maternal flowering time and germination, it remains unclear if this relationship is mostly due to pleiotropy, maternal or environmental factors. This is because past experiments used a species that has low mapping resolution (e.g. Galloway and Burgess (2009)), assessment of flowering and germination is performed in different environments (Galloway and Burgess, 2009; Donohue et al., 2007), or both are performed under laboratory conditions (Munir et al., 2001; Schmuths et al., 2006). Because both flowering and germination are affected by a range of environmental cues, it is important to estimate both properties under field conditions. Because maternal environment and the environment experienced during seed development can affect seed germination properties (Galloway, 2005; Kendall and Penfield, 2012), it is also crucial to use seeds that were produced in the same environment in order to get a true estimate of maternal environmental effects.

Here, we studied germination traits under field conditions, and determine the genetic, maternal and environmental contribution to natural variation in germination traits. We used a set of Multiparent Advanced Genetic InterCross (MAGIC) lines of the model plant Arabidopsis thaliana (Kover et al., 2009b), where the seed’s parents were grown in the same field as the seeds are being tested for germination, simulating what would happen when the seeds dehisce and reach the soil. A. thaliana is a useful system for assessing genetic and environmental maternal effects because it has a wealth of genetic data and genotyped lines that enable the quantification of genetic effects in natural environments (Wilczek et al., 2009; Moyers and Kane, 2010), and extensive natural variation in germination has been previously observed (Donohue et al., 2005c; Alonso-Blanco et al., 2003; Clerkx et al., 2004). We measure the germination curve and calculate a range of indices by direct measurement of quantitative aspects of germination and by fitting survival models to the curves. By collecting the data on the entire cumulative germination curve, we are able to separate different aspects of germination and determine if they appeared to be under the same genetic control. We map QTLs for germination traits to determine the genetic basis of germination, and whether different aspects of germination appear to be under different genetic control. By collecting phenotypic data on the maternal plants (including size and flowering time, Chapter 2) we were able to test whether maternal traits explain variation in germination traits.
5.2 Materials and Methods

Three replicates of each of 152 *A. thaliana* MAGIC lines were planted outdoors in the Manchester Botanical experimental field (Manchester, UK) in May 2009 (See Chapter 2). Plants were monitored daily and flowering time (days to first appearance of white flower) was recorded. Following senescence, selfed seeds from each individual maternal plant were collected and briefly brought into the lab where 100-200 seeds were transferred into a watch-glass and photographed for subsequent counting before being transferred to Eppendorf tubes and transported back to the outdoor plots. All seeds within each tube were sowed into a 3” pots filled with John Innes #3 compost. Pots were randomised within 56 trays and placed outside in nursery trays on tables in the Manchester University Botanical research station, under the same fruit cage as maternal plants were grown. The number of seeds in the photographs for each sample taken before planting were then counted using the cell counter plugin in imageJ. Germinants in each pot were counted and removed with forceps approximately twice per week until no germination had been observed in any pot for 4 weeks. In total, germination was monitored for 13 weeks.

5.2.1 Data analysis

From the raw germination data, we calculated the final proportion of successful germinants (germination success), time to modal germination, the slope of the time to maximum germination and the area under the cumulative germination curve (AUC) for each pot. These indices are direct measures that can be calculated without fitting a regression model to the data. The indices values were averaged for each MAGIC line to give genotypic values for QTL analysis. We also estimated germination curves by fitting non-linear Weibull survival regression models to each pot data using the survreg function from The R package Survival (Therneau and Lumley, 2011) and extracted the shape and scale parameters. The shape parameter describes whether the curve is closer to a normal distribution, or skewed (right or left), and is also associated with a higher cumulative function, indicating that higher shape values are associated with higher final germination rate. The scale of a Weibull distribution indicates the value of the 63.2 percentile value, and can be thought in this case as indicative of the spread of germination times.

To determine the heritability of each germination indices and parameters, a mixed REML effect models was constructed using the R package lme4 (Bates and Maechler, 2009). The following model was used:

\[ \text{index} = \alpha + V_g + V_{tray} + \epsilon \]  

where where \( V_g \) is the genetic variance between MAGIC lines, \( V_{tray} \) is the variance associated with different trays in the experiment and \( \alpha \) and \( \epsilon \) are the intercept and error terms, respectively. Both \( V_g \) and \( V_{tray} \) were set as random effects. Variance components were extracted and significance of \( V_g \) and \( V_{tray} \) variances were determined by likelihood ratio tests. Heritability was determined as \( V_g/V_p \) where \( V_p \) is the total phenotypic variance in the model. Standard errors for the heritabilities were determined by jackknife using the R package Bootstrap (Tibshirani and Leisch, 2012).
5.3. RESULTS

Pairwise trait correlations were calculated between line means for the observed germination parameters, Weibull coefficients and maternal flowering time.

Since many of the germination traits were correlated to each other, we also performed a principal components analysis (PCA) using singular value decomposition on all of the measured germination indices and the Weibull coefficients to reduce the dimensionality of the data to a smaller number of uncorrelated variables. Variables were zero centred and scaled to unit variance before PCA was performed. Principal components with a standard deviation greater than 1 were retained as composite traits explaining the variation in germination. This ensured that the composite traits explained more variation than any of the original measured traits individually. Confidence intervals for the principal component loadings were generated using the 95% quantiles from 10000 bootstrap replicates (Peres-Neto et al., 2003).

To determine whether variation in environmental conditions during seed development caused by differences in flowering time affected the germination traits, independent of the genetic factors that contribute to flowering and germination, we extended the above model to include maternal flowering time:

\[ PC = \alpha + V_g + V_{tray} + \beta_{MatFT} + \text{cov}(V_g(matFT)) + \epsilon \] (5.2)

Here, \( \beta_{MatFT} \) is the fixed effect of maternal flowering time and \( \text{cov}(V_g(matFT)) \) is the genetic covariance with maternal flowering time. \( \beta_{MatFT} \) gives the effects of these maternal phenotypes once genotype effects have been accounted for and therefore represents the environmental effect of maternal flowering time, rather than correlated genetic effects of parental flowering time on germination. Independent random effects were specified for the correlation structure of \( \text{cov}(V_g(matFT)) \).

To investigate the genetic basis of the germination we performed a QTL analysis on all germination indices and Weibull coefficients using the R package happy.hbrem following the methodology in (Kover et al., 2009b) and Chapter 2. We also performed QTL analysis on the first three principal components because it is possible that the PCs (by identifying a common mechanism that affects different aspects of germination) would allow the identification of a higher order trait. Significant QTL locations were further investigated using the atEnsembl A. thaliana online genome browser (atensembl.arabidopsis.info/).

All data analyses were performed using R v2.14.1 R Development Core Team (2011)

5.3 Results

Significant genetic variation was found for all traits measured (Table 5.1). A significant tray effect was observed on time to modal germination, slope to modal germination and the AUC of the cumulative germination curve (See figure 5.1). Heritability for most traits was moderate, ranging from 2% in time to mode germination to 35% for the Weibull shape coefficient. The number of seeds planted in a pot had no significant effect on any of these indices and was removed from further analysis.

Germination traits are generally positively correlated to each other (Figure 5.2), indicating a lack of trade-off between components of germination. Both of the shape and the scale of the
Figure 5.1: Germination curves (a) by MAGIC line and (b) by tray

Table 5.1: Variance and heritability of Germination traits in *A. thaliana* grown in the field. Likelihood ratio tests are all based on a $\chi^2$ distribution with 1 degree of freedom.

<table>
<thead>
<tr>
<th>Index</th>
<th>MAGIC line var</th>
<th>P</th>
<th>tray effect var</th>
<th>P</th>
<th>Residual var</th>
<th>Heritability (se)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prop. successful germinants</td>
<td>$35.385$</td>
<td>&lt;0.0001 ($\chi^2=31.1$)</td>
<td>$1.44$</td>
<td>0.3443 ($\chi^2=0.2$)</td>
<td>94.602</td>
<td>0.27 (0.07)</td>
</tr>
<tr>
<td>Time to mode germination</td>
<td>$5.499$</td>
<td>&lt;0.0001 ($\chi^2=18.3$)</td>
<td>$4.754$</td>
<td>&lt;0.0001 ($\chi^2=30.9$)</td>
<td>16.755</td>
<td>0.2 (0.07)</td>
</tr>
<tr>
<td>Slope to modal germination</td>
<td>$0.254$</td>
<td>0.0167 ($\chi^2=4.5$)</td>
<td>$0.124$</td>
<td>0.0022 ($\chi^2=8.1$)</td>
<td>1.923</td>
<td>0.11 (0.07)</td>
</tr>
<tr>
<td>AUC</td>
<td>$3.366$</td>
<td>&lt;0.0001 ($\chi^2=38.5$)</td>
<td>$2.008$</td>
<td>&lt;0.0001 ($\chi^2=32.9$)</td>
<td>6.163</td>
<td>0.29 (0.05)</td>
</tr>
<tr>
<td>Weibull shape</td>
<td>$0.088$</td>
<td>&lt;0.0001 ($\chi^2=46.5$)</td>
<td>$0.006$</td>
<td>0.1587 ($\chi^2=1$)</td>
<td>0.158</td>
<td>0.35 (0.07)</td>
</tr>
<tr>
<td>Weibull scale</td>
<td>$0.004$</td>
<td>0.0004 ($\chi^2=11.5$)</td>
<td>&lt;0.001</td>
<td>0.5 ($\chi^2=0$)</td>
<td>0.017</td>
<td>0.18 (0.06)</td>
</tr>
</tbody>
</table>
Weibull distribution were strongly correlated with the proportion of successful germinants and with each other, indicating that genotypes with high rates of germination also tend to germinate more rapidly. No significant correlations were observed between any of the germination traits and maternal flowering time, indicating that genes that affect flowering time do not seem to have a clear effect on germination traits.

The principal components analysis shows that >95% of the variation among the different germination traits is captured in the first three principal components (Table 5.2). PC1 accounts for 55% of variation in germination traits and is most strongly correlated to the Weibull shape and proportion of germinants (Figure 5.2). PC2, which accounts for 33% of the variation in seed traits, is dominated by time to mode germination and the Weibull scale parameter. This PC probably represents the lag to germination. PC3 accounts for 9% of variation and represents the slope of the cumulative germination curve. Since PC1 and PC2 are the only principal components with a standard deviation > 1 (i.e. they explain more variation than would be explained by a raw germination index) and that together they explain 88% of the variation among the different germination indices, we use these only for further analysis.

The mixed effects model shows that there was is a highly significant effect of genotype on both PCs and a smaller but still significant tray effect (Table 5.3). The genetic covariance term between flowering time and PCs was found to be insignificant by likelihood ratio test (PC1: ,P =0.635; PC2: ,P >0.99 ) and was dropped from the final model, but there is a significant negative relationship between the fixed effect of flowering time across lines and PC1. This means that early flowering plants produce seeds with significantly higher values of PC1, but that this effect must be a component of the environment, suggesting that maternal flowering time affects the environment experienced by the developing seeds, independently of any shared genetic control. This is expected to result in increased germination success, since PC1 is most strongly correlated with proportion of germinants and the Weibull shape parameter (table 5.2) which are in themselves strongly correlated (figure 5.2). It may also result in a shorter lag time to germination, but these effects are expected to be weaker, since mode time to germination and slope to mode germination are less strongly correlated with PC1.

In total, we found four QTL for the individual germination traits (Weibull shape, time to mode, and AUC), and seven for the first three principal components (table 5.4). All significant QTL were found on chromosomes 4 and 5. On chromosome 4, two of the QTL for PC1 overlapped for QTL for mode germination and AUC. On Chromosome 5, the QTL for PC overlapped with the QTL for Weibull shape. The QTL for PC2 and PC3 did not overlap with any others (Figure 5.3). The estimated effects for the haplotypes of each the of the 19 parental accessions at the identified QTL (table 5.5) suggests that QTL identified for each trait is not due to the same genetic variant because there is little systematic correspondence between haplotypic effects across QTL. Five of the 11 QTL identified map to less than 200kb from phytochrome genes Of these, the peak of the QTL for AUC is directly overlapping the gene for PHYTOCHROME E (table 5.6). The transcription factor AGL19 is also close to QTL for AUC and PC1. This locus codes for a MADS-box transcription factor. MADS-box genes, such as the floral regulator FRIGIDA (Which interacts epistatically with FLC), are involved in controlling embryo and seed development as well as root, flower and fruit development Becker and Theißen (2003).
Figure 5.2: Correlations between genotype means for germination traits in A. thaliana. Univariate trait distributions are shown on the diagonal. Pearson correlations and indicators of significance are shown right of the diagonal. Scatter plots of the correlations with LOESS smoothers are shown left of the diagonal.
Table 5.2: Principal components analysis by singular value decomposition of germination indices and Weibull coefficients. Variables are zero centred and scaled to unit variance. Loadings are medians from 10000 bootstrap replicates. 95% quantile confidence intervals are shown in parentheses. Correlations $\geq 0.5$ are in bold.

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
<th>PC6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prop. Germinants</td>
<td>0.482</td>
<td>-0.305</td>
<td>0.225</td>
<td>-0.115</td>
<td>0.323</td>
<td>-0.711</td>
</tr>
<tr>
<td></td>
<td>(0.469, 0.497)</td>
<td>(-0.343, -0.267)</td>
<td>(0.188, 0.263)</td>
<td>(-0.161, -0.072)</td>
<td>(0.257, 0.382)</td>
<td>(-0.739, -0.678)</td>
</tr>
<tr>
<td>Mode time to germination</td>
<td>-0.37</td>
<td>-0.489</td>
<td>0.046</td>
<td>-0.771</td>
<td>-0.159</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>(-0.394, -0.342)</td>
<td>(-0.528, -0.448)</td>
<td>(-0.027, 0.116)</td>
<td>(-0.791, -0.743)</td>
<td>(-0.24, -0.082)</td>
<td>(0.006, 0.047)</td>
</tr>
<tr>
<td>Slope to mode germination</td>
<td>0.399</td>
<td>0.145</td>
<td>-0.843</td>
<td>-0.327</td>
<td>-0.023</td>
<td>-0.016</td>
</tr>
<tr>
<td></td>
<td>(0.379, 0.416)</td>
<td>(0.082, 0.198)</td>
<td>(-0.873, -0.806)</td>
<td>(-0.401, -0.251)</td>
<td>(-0.062, 0.019)</td>
<td>(-0.027, -0.004)</td>
</tr>
<tr>
<td>AUC</td>
<td>0.393</td>
<td>0.437</td>
<td>0.398</td>
<td>-0.313</td>
<td>-0.628</td>
<td>-0.031</td>
</tr>
<tr>
<td></td>
<td>(0.362, 0.421)</td>
<td>(0.387, 0.489)</td>
<td>(0.346, 0.449)</td>
<td>(-0.387, -0.232)</td>
<td>(-0.668, -0.581)</td>
<td>(-0.092, 0.036)</td>
</tr>
<tr>
<td>Weibull Shape</td>
<td>-0.506</td>
<td>0.209</td>
<td>-0.239</td>
<td>0.149</td>
<td>-0.363</td>
<td>-0.699</td>
</tr>
<tr>
<td></td>
<td>(-0.52, -0.495)</td>
<td>(0.174, 0.242)</td>
<td>(-0.27, -0.205)</td>
<td>(0.103, 0.189)</td>
<td>(-0.437, -0.288)</td>
<td>(-0.729, -0.661)</td>
</tr>
<tr>
<td>Weibull Scale</td>
<td>-0.246</td>
<td>0.641</td>
<td>0.136</td>
<td>-0.403</td>
<td>0.584</td>
<td>-0.067</td>
</tr>
<tr>
<td></td>
<td>(-0.295, -0.186)</td>
<td>(0.606, 0.671)</td>
<td>(0.071, 0.202)</td>
<td>(-0.464, -0.343)</td>
<td>(0.531, 0.629)</td>
<td>(-0.121, -0.019)</td>
</tr>
</tbody>
</table>

- Standard deviation: 1.817, 1.402, 0.731, 0.376, 0.204, 0.125
- Proportion of variance: 0.551, 0.328, 0.089, 0.024, 0.007, 0.003
- Cumulative proportion: 0.551, 0.878, 0.967, 0.991, 0.997, 1.000
Table 5.3: Mixed-effect models for Principal components 1 and 2. Random effects are MAGIC line, $V_g$, and tray effects, $V_{tray}$ (With residual error term, $\epsilon$). Fixed effects are of the intercept, $\alpha$, and maternal flowering time, $\beta_{MatFT}$ (From equation 5.2). Observations: 354, MAGIC lines: 146, Trays: 56. Random effect significance determined by likelihood ratio tests, fixed effect significance determined by MCMC resampling.

<table>
<thead>
<tr>
<th></th>
<th>PC 1 random effects</th>
<th>PC 2 random effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Var</td>
<td>SD</td>
</tr>
<tr>
<td>$V_g$</td>
<td>1.038</td>
<td>1.019</td>
</tr>
<tr>
<td>$V_{tray}$</td>
<td>0.225</td>
<td>0.474</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>2.096</td>
<td>1.448</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>PC 1 fixed effects</th>
<th>PC 2 fixed effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>2.567</td>
<td>0.915</td>
</tr>
<tr>
<td>$\beta_{MatFT}$</td>
<td>-0.049</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Table 5.4: QTL identified for germination traits. P is the genome-wide P value. % $V_p$ is an estimate of the percentage of the total variation explained by that QTL.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>chr</th>
<th>SNP</th>
<th>position</th>
<th>logP</th>
<th>p-value</th>
<th>% $V_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>mode.germination</td>
<td>chr4</td>
<td>PHYD_3094</td>
<td>9197205</td>
<td>4.26</td>
<td>0.008</td>
<td>13.0</td>
</tr>
<tr>
<td>PC1</td>
<td>chr4</td>
<td>PHYD_1815</td>
<td>9198484</td>
<td>3.99</td>
<td>0.023</td>
<td>13.4</td>
</tr>
<tr>
<td>AUC</td>
<td>chr4</td>
<td>PHYE_2771</td>
<td>10043931</td>
<td>3.56</td>
<td>0.05</td>
<td>23.8</td>
</tr>
<tr>
<td>PC1</td>
<td>chr4</td>
<td>MN4_10482087</td>
<td>10482077</td>
<td>3.94</td>
<td>0.026</td>
<td>23.9</td>
</tr>
<tr>
<td>AUC</td>
<td>chr4</td>
<td>MN4_11878394</td>
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Table 5.5: Estimated effects of different QTL. Figures are the estimated variance explained by that founder ecotype (Kover et al., 2009b). Positive values are in green while negative values are in red. Maximum and minimum values for each QTL are bordered.

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<td>involved in gibberellin signalling pathway.</td>
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5.4 Discussion

In this study, we were able to demonstrate significant natural variation for different aspects of germination under ecologically relevant field conditions. Previous work has shown variation in dormancy and proportion of germinants, but not in the whole germination curve over the longer term. We show that maternal flowering time affects germination, and that this effect appears to be a plastic response to the different conditions experienced by seeds produced at different flowering times rather than a correlated genetic effect of germination and flowering. This suggests that the environment during flowering has important cross-generational plastic effects, and hints at the possibility that the environment seeds experience can be manipulated by mothers in a non-genetic way. Analysis of the genetic basis of germination traits reveals that several of the underlying QTL appear to be related with genes that have been previously linked to regulation of both germination and flowering. The implications of environmental effects on flowering affecting offspring germination are a cause for concern due to predicted climate change.

Germination timing can explain a significant proportion of the variance in flowering time under natural conditions (largely due to different environments experienced by plants that germinate at different times (Bentsink et al., 2010; Weitbrecht et al., 2011; Wilczek et al., 2009) and is a major determinant of the expression of and selection on post-germination traits (Weinig, 2000; Donohue, 2002; Korves et al., 2007; Huang et al., 2010). However, germination is also highly responsive to environmental conditions both during seed maturation and after dispersal (reviewed in Baskin and Baskin (1998); Donohue (2009)). Artificial warming and forced early flowering...
experiments (Munir et al., 2001; Sherry et al., 2007; Post and Pedersen, 2008; Schmuths et al., 2006; Galloway and Burgess, 2009) have shown that early flowering can cause significant shifts in germination phenology and in the proportion of successful germinants. Although these studies have attributed this cross-generational response to phenotypic plasticity, their experimental designs have not always allowed them to effectively differentiate between correlated genetic effects between maternal flowering time and offspring germination and environmental effects on the developing seed. In this study we found no genetic covariance between flowering time and any aspect of germination, no evidence of shared genetic control of natural variation in flowering and germination, and no QTL identified in this study overlapping with QTL for flowering time (See Chapter 2). However, a significant environmental effect of flowering time on the first principal component of germination (which is more strongly associated with germination success than with phenology) was observed. This shows that the differential response in germination timing for early flowering plants appears to be a purely plastic one. These cross-generational responses to environment could have significant effects on community and ecosystem response to changing environments. If, as has been demonstrated in several cross-species studies (Fitter and Fitter, 2002; Sparks et al., 2000; Menzel et al., 2006), and in our work on A. thaliana (Chapter 2) climate change is expected to cause plants to flower earlier, then this is likely to result in a plastic response of earlier germination. This may have a positive effect on fitness by allowing the completion of an extra generation within a growing season. However, it may also be detrimental if seeds end up being dispersed at a less optimal time. An increase in proportion of successful germinants with earlier flowering plants would be expected to be adaptive, so the lack of an observed genetic covariance may be due to this plastic effect of flowering time on germination not being adaptive in all environments.

Germination is a complex character which relies on several related traits working together to be able to function properly. It is therefore expected to be a highly integrated trait with tight associations between the traits involved in germination (Murren et al., 2005; Galloway and Burgess, 2009). Though the different germination traits do exhibit clear genetic integration (a positive genetic correlation was observed among most germination traits), there does appear to be some separation between the control of germination success (PC1), germination lag (PC2) and germination rate (PC3), indicated by the different weightings of the orthogonal principal components. This separation is further supported by the fact that there is no overlap between the QTL identified for each of the PCs.

Surprisingly, we were not able to detect any QTL for proportion of successful germinants, which has been detected before (Alonso-Blanco et al., 2003; Clerkx et al., 2004). However, previous studies considered total number of germinants within a small period of time (in general less than 2 weeks). It is noted that if, for logistical reasons, only a single measure of germination was able to be recorded, proportion of successful germinants would be the most acceptable choice since it is so closely correlated with Weibull shape and scale and the first principal component of germination. However, if we had just looked at total germination in this study, we would have not seen an effect of maternal flowering time and would have not identified a QTL for germination. These results highlight the importance of considering multiple aspect of the germination process. Heritability of germination traits is considerably lower than previously
observed in laboratory controlled experiments (e.g. Clerkx et al. (2004) report heritabilities for germination traits between 60% and 90%). This could reflect the more complex environment experienced by the seeds in this experiment, and also could partially explain the lack of observed QTL for proportion of successful germinants.

QTLs for germination traits have been identified in various previous studies, mostly under greenhouse (Alonso-Blanco et al., 2003; Bentsink et al., 2010; Kover et al., 2009a) or growth chamber conditions (Clerkx et al., 2004). Although the candidate genes identified in this study have been previously associated with germination in mutation screen studies, the QTLs identified here have not been found in previous studies of natural variation affecting germination phenotype. Despite their clear importance to the germination process (Pons, 2000), PHYTOCHROME genes have not previously been identified in QTL studies, although they have been shown to have important influences on germination timing using knockout mutant lines (Donohue et al., 2007, 2012). PHYA and PHYD are both involved in pathways to flowering, although natural variation in flowering time due to these genes has not been identified (Weigel, 2012).

PHYA has previously been shown to be important to germination response late in the season (Donohue et al., 2012). In six of the eleven QTL, the haplotype with the most extreme value came from the parental accession Ws (Table 5.5). This accession, originating from Russia, has been shown to be late flowering under growth chamber conditions (Kover et al., 2009a). This suggests that it adopts a winter-annual strategy (overwintering as rosette, before flowering the following spring) in the wild, so it makes sense that it is adapted to germinate in late summer, which is when the experiment was conducted. Haplotype from this accession confer the most rapid time to mode germination and the greatest area under the curve, as well as larger values for PC1 and PC2. The latter suggests that this accession also have a high proportion of germinants, or the rate of germination would not be able to decrease along with an increase in AUC.

We have demonstrated that earlier flowering plants in an experimental population of an annual plant under natural conditions display a small but significant increase in the first PC of germination, likely resulting in higher seed germination and potentially accelerated phenology. Our previous work has shown that a small increase in ambient temperature results in earlier flowering and changes in direction of selection gradients for phenotypic plasticity. These cross-generational plastic effects are likely to increase due to climate change and may well have unpredictable effects on future plant community structure. Finally we have demonstrated genes important for germination in the field, particularly from the PHYTOCHROME family. These genes have not previously been associated with variation in germination in lab experiments, despite their known importance in germination pathways.
Chapter 6

Rapidly varying environments select for evolvable generalists

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Abstract

Classic evolutionary theory shows that environmental change selects for ecological generalism. More recent work suggests that changing environments should also select for increased evolvability. Here, we use the \textit{Avida} digital evolution platform to investigate the effects of different rates of environmental change on generalism and on three measures corresponding to three main definitions of evolvability in the literature: diversity, phenotypic sensitivity to mutation and fitness recovery following environmental change. First we generated populations of digital organisms that had been evolved in environments experiencing varying rates of change and recorded population diversity and mean sensitivity to mutation. In a second experiment we transferred these evolved populations to a different environment and measured their ability to recover fitness. We found that high rates of environmental change selected for a high degree of ecological generalism and high levels of all three measures of evolvability. However, we were unable to detect a relationship between any of these measures independent from environmental change. This suggests that evolvability and generalism assist the response of populations to changing environments but play little role when conditions are stable.

6.1 Introduction

The ability of organisms to tolerate and respond adaptively to changing environments is crucial for their persistence, particularly in the face of human-mediated climate change. Ecologists and evolutionary biologists studying this problem have focused on the evolution of ecological strategies that allow organisms to respond to short and long-term to environmental change,
examining, for example, relative costs or benefits of generalist or specialist phenotypes as a function of the period and intensity of environmental change (van Tienderen, 1991; Kassen, 2002; Futuyma and Moreno, 1988; Dykhuizen and Davies, 1980). More recently, another group of evolutionary biologists have explored how an organism’s underlying genetic architecture influences its capacity to adapt to environmental change (its evolvability Pigliucci (2008); Lenski et al. (2006); Houle (1992); Hansen and Houle (2008); Gilchrist and Lee (2007); Colegrave and Collins (2008); Aldana et al. (2007)). It has become increasingly clear that these approaches should not be considered in isolation, as the the processes underlying generalism and evolvability may be interdependent. The structure of environmental change may influence the evolution of genome architecture, which in turn may modify the form and rate of an organismal response to subsequent environmental fluctuations (evolvability) or its ability to respond across diverse environments (generalism). Here we examine evolution of digital organisms using the Avida digital evolution platform to examine this potential interdependence in detail.

Evolutionary ecology theory predicts natural selection in changing environments to lead to the evolution of a generalist strategy (Kassen and Bell, 1998; Kassen, 2002; van Tienderen, 1991), by which an organism increases mean fitness across environments. Organisms that are exposed to more heterogeneous environments or more rapid changes between environments are expected to exhibit increased generalism.

The quantification of evolvability is a complex problem, partly because the term has various definitions describing a family of related concepts rather than single idea (Pigliucci, 2008). Here, we investigate three aspects of evolvability. At the population level, Hansen (2006) defines evolvability as the ability to maintain “potentially adaptive genetic variants”, thus facilitating the responsiveness to environmental change (Houle, 1992). At the genome level, evolvability has been described as phenotypic sensitivity to mutation, or the capacity to evolve new phenotypes, irrespective of the action of natural selection (Dichtel-Danjoy and Felix, 2004). By this definition, a genome that is more able to respond phenotypically to mutation is more evolvable. This is the opposite to mutational robustness, which is defined as the ability of a genome to maintain constant phenotypes in the face of mutation (Wagner, 2005b). Organisms are expected to reach a balance between these two forces, whereby they can withstand generally deleterious mutations while still maintaining enough variation to allow for evolutionary adaptation and to cope with heterogeneous environments. Draghi et al. (2010) predict that intermediate levels of evolvability will promote adaptation, given that the number of phenotypes in the phenotypic neighbourhood is below the total number of possible phenotypes. Evolvability can also be defined retrospectively (i.e. after a selection event) as “the ability of a population to respond to natural selection” (Flatt, 2005). In this case, the rate at which a population can recover fitness is a direct measure of evolvability in the direction of selection.

The evolution of generalism in changing environments is well established in evolutionary ecology theory (for example, van Tienderen (1991); Gilchrist (1995)), and has been illustrated in the Avida system (Li and Wilke, 2004) but the connection has not yet been made with the evolution of evolvability (Dawkins, 1987). Here we use the Avida digital life platform to disentangle to role of environmental change on generalism and each of the three measures of evolvability described above. Specifically we address the following questions: Do rapidly
varying environments lead to the most generalist and evolvable populations and genotypes or do intermediate levels of environmental fluctuation result in the most successful generalists? Are generalist populations more evolvable? Is evolvability due to increased rates of environmental change expressed in the genetic architecture as increased mutational instability or just at the population level as increased diversity. If generalism and evolvability are found to be correlated, how do they interact to facilitate response to environmental change? We address these questions in a two stage experiment: First producing populations that have evolved on a range of different rates of environmental change, second taking the populations at the end points of the first experiment and transplanting them onto the opposite environment to the one they last experienced to measure their rate of fitness recovery. This approach uniquely permits the various views of evolvability to be evaluated in a single study.

6.2 Materials and Methods

6.2.1 The *Avida* platform

*Avida* is a software platform for the study of the evolution by mutation, selection and random drift of self-replicating computer programs (digital organisms Adami (1998); Ofria and Wilke (2004)). Avida is an ideal computational model system to investigate evolutionary questions since it provides a bridge between “wet” experimental biology and pure simulation. “Avidians” have a simulated metabolism but they undergo true Darwinian evolution by mutation, drift and selection. Experiments can be carried out in a fraction of the time of even the smallest microbes and the level of complexity is far higher than could effectively be processed in analytic simulations. The system has been used to investigate a range of evolutionary phenomena including mutational robustness (Wilke et al., 2001), adaptive radiation (Chow et al., 2004), ecological specialization (Ostrowski et al., 2007) and the evolution of complexity (Lenski et al., 2003). Avidian genomes are a circular set of instructions taken from a possible set of 25 instructions. To replicate, digital organisms must copy their genomes line by line and then divide the resulting genomes to yield two newly independent organisms. Replication is subject to point mutations, insertions and deletions. For point mutations, a random instruction is written in place of the original one, whereas for insertion or deletion mutations, a random instruction is either added to or removed from the genome. The success of a digital organism depends on its replication rate relative to other organisms in its population. An individual’s fitness in a given environment depends on the rate at which it can acquire energy from the environment and its efficiency in converting that energy into progeny. When an organism replicates, it replaces a random other member of the population, with no spatial relationships between parent and offspring.

In the *Avida* system, phenotypic traits are determined by the ability to perform specific logical computations (e.g. AND, NOT) on random input numbers and return the result. The individual is rewarded upon completion of a task with energy (extra CPU cycles), allowing it to reproduce more quickly (Lenski et al., 2003; Wilke and Adami, 2002). This is analogous to organisms performing metabolic reactions to obtain energy. The set of computations producing a reward defines the environment and is set by the investigator. More complex tasks yield higher rewards to organism performing that computation (trait), allowing for the evolution of genomic
6.2. MATERIALS AND METHODS

Table 6.1: Avida resources and their reward values for the two environments. Computations with the same reward values are broadly similar in terms of complexity. Rewards increase exponentially with computational complexity and represent number of CPU cycles per unit time (update).

<table>
<thead>
<tr>
<th>Env a</th>
<th>Env b</th>
<th>Reward for performing computation</th>
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<tbody>
<tr>
<td>NOT</td>
<td>NAND</td>
<td>$10^1$</td>
</tr>
<tr>
<td>AND</td>
<td>ORN</td>
<td>$10^2$</td>
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<tr>
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<td>ANDN</td>
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<tr>
<td>NOR</td>
<td>XOR</td>
<td>$10^4$</td>
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Complexity, since there is also a cost to maintaining a larger genome. Because of this trade-off between genome size and replication rate, traits that are unrewarded in a particular environment carry a direct cost and are therefore likely to be selected against. Unrewarded traits would also be expected to disappear due to adaptive decay (Ostrowski et al., 2007).

Fitness in Avida is measured by dividing the number of CPU cycles it can perform per update by the number of instructions that it must execute to produce an offspring (Adami, 1998). Updates are units of Avida time, set to an arbitrary number of instructions (in this case 30) to be run on average in organisms unable to perform any rewarding tasks. Generation time is typically around 10 updates, although this varies depending upon an individual’s fitness.

Resources are unlimited and equally available to all organisms, resembling the conditions in a well-stirred chemostat. In all experiments reported here, the resources associated with each trait were unlimited. Population size was constant in each run with the birth of a new individual replacing a random member of the existing population. No explicit selection regime is imposed (as opposed to a genetic algorithm), rather selection is dependent on the interaction between the phenotypes in the population and the environment. We used Avida Version 2.9.0, freely available from devolab.cse.msu.edu/software/avida/.

In any given experimental treatment, each replicate evolving population had identical initial conditions except for the random-number seed. All runs were seeded with a single hand-written ancestor (See appendix B) that was 100 instructions in length and could self-replicate but was unable to perform any tasks. See (Ofria and Wilke, 2004) and the above website for more details about the Avida system. All experiments ran under the Linux operating system on two dual core Dell Optiplex 745 machines.

6.2.2 Experimental treatments

The experiment was set up in two phases (Figure 6.1 on the following page). The first allowed populations to evolve on environments which fluctuated over a range of rates. The second phase then transferred these evolved populations into a different environment to measure their rate of fitness recovery.
6.2. MATERIALS AND METHODS

Figure 6.1: Schematic of the experimental process. Green boxes represent environmental change treatments (number of replicate experimental runs x the rate of change treatment). Yellow boxes are treatments affecting the “physics” of the Avida world (Are genome lengths free to vary or fixed?). Orange shows the length of time (in updates) of experimental runs and blue shows the data collected.

6.2.2.1 Phase 1: Adaptation to fluctuating environments

A single ancestor was seeded at the start of each run. This ancestor was able to reproduce, but not able to perform any tasks to earn increased energy. All experiments ran for 100000 updates and population size was limited to 3600 individuals. Updates are used as the unit of time, rather than generations, because of the variability in each individual’s reproductive rate. Each treatment consisted of a particular rate of alternation between two pre-defined environments, labeled Env a and Env b.

Each of the two environments was composed of four resources, yielding energy to individuals performing a given logical computation, with reward values for each task as given in table 6.1. The reward increased with the complexity of the task, providing an impetus for the evolution of increased complexity. The value of tasks scaled in the same way in both environments, with tasks of similar complexity netting the same rewards.

We evolved populations over five orders of magnitude of rates of environmental change, from 0 (no change) to 1 (environment changes on every update). 100 populations were evolved in each environmental change treatment the starting environment, Env a or Env b, was randomized at the start of each simulation. For the unchanging treatments, 100 populations were evolved on each environment. The experiment was repeated for fixed and unconstrained genome lengths. In the standard runs genome length was free to vary and could change during evolution, whereas in the fixed genome-length runs length was fixed at the ancestral value of 100 instructions.
Most Avida experiments are performed using fixed genome lengths to control for variation in genomic mutation rate due to different length genomes (e.g. Li and Wilke (2004); Ostrowski et al. (2007); Wilke et al. (2001)), but we were also interested in determining if adaptation to changing environments also occurred at the level of the genome.

Following evolution, in order to quantify mean generalism of each population, we calculated a generalism index, \( G \), by comparing the number of resources that an individual could utilise from one environment to how many it could utilise in the other:

\[
G = 1 - \frac{\sum_{i=1}^{N} \left( \frac{|R_a^{(i)} - R_b^{(i)}|}{R_a^{(i)} + R_b^{(i)}} \right)}{N}
\]  

(6.1)

where \( N \) is the population size, and \( R_a^{(i)} \) and \( R_b^{(i)} \) are the number of resources utilised by the \( i \)th individual in Env \( a \) and Env \( b \) respectively. From this equation, a score of \( G = 1 \) for a population means that all individuals can use an equal number of resources in each environment (i.e. they are perfect generalists), while a score of \( G = 0 \) means that all individuals can only use resources from one environment (i.e. they are pure specialists). This index provides an aggregate of the generalism scores of all organisms in the population.

Due to the small genome size of Avida organisms (compared to living organisms), mutation rates must be high (0.0075 mutations per instruction copy) to maintain mutation-selection balance. This means that measuring genetic diversity directly cannot identify differences in diversity between populations subjected to different rates of environmental change because the signal is swamped by the high mutation rate. However, because phenotypes in Avida are determined entirely by the genotype, measuring phenotype diversity gives an accurate diversity measure that can differentiate between treatments. Diversity was calculated using the Shannon-Weaver diversity index: \( H' = -\sum_{i=1}^{S} (p_i \ln p_i) \) where \( S \) is the number of distinct phenotypes (richness) and \( p_i \) is the relative abundance of the phenotype. The phenotypes in our experiments are defined by the distinct set of resources which an individual can use. For example, two individuals which can utilize NAND and XOR but cannot utilize any other resources would be classed as having the same phenotype. Alternatively, an organism utilizing NOT, AND and NOR would have a different phenotype to one utilizing NOT, OR and NOR.

### 6.2.2.2 Phase 2: fitness response to environmental change

In the second phase of the experiment, we transferred each evolved population in its entirety from stage 1 to the alternative environment from the last one it experienced. That is, if a population had last experienced Env \( a \) it was switched to Env \( b \) in Phase 2 and vice versa. Five replicates of each transferred population were then allowed to evolve for another 1000 updates, during which time we recorded change in fitness through time. Control populations were transferred to the alternative, previously unexperienced, environment. By transferring the entire population from Phase 1 to the new environment in Phase 2, both standing genetic variation in the population and genetic architecture could potentially contribute to the response to environmental change. To discriminate between these demographic (resulting from standing genetic variation) and evolutionary (ability of the genome to evolve) responses, we ran these
experiments both with and without the inclusion of new mutations. These experiments were also repeated with the differing genome length regimes detailed above. In total, 10,000 simulations (100 populations x 5 fluctuation rate environments x 5 replicates x 2 genome length treatments x 2 mutation treatments) were performed.

**6.2.3 Quantifying evolvability**

Evolvability was estimated at the end of both phases of this study. The diversity index, $H$, (defined above) measures evolvability *sensu* Hansen (2006). We also calculated an index of evolvability *sensu* Dichtel-Danjoy and Felix (2004), which we classify as genome sensitivity, $S_g$. This is a measure of the proportion of one step mutations from each genotype which will cause a phenotypic change. *Avida* has a landscape analysis tool which quantifies the fitness effects of all 1-step mutations from an individual genotype by replacing every instruction with every possible alternative. Genome sensitivity, $S_g$ is defined as:

$$S_g = 1 - \frac{\sum_{i=1}^{N} \left( \frac{\eta^{(i)}_a + \eta^{(i)}_b}{2} \right)}{N} \quad (6.2)$$

where $N$ is the number of individuals in the population, $\eta^{(i)}_a$ and $\eta^{(i)}_b$ are the proportions of all possible one-step mutations of the $i$th individual that are neutral (having a zero fitness effect) in $Env a$ and $Env b$ respectively. From this equation, we see that increasing values of $S_g$ mean that we should expect more mutations to have phenotypic effects. $S_g$ is very similar to 1-mutational robustness (as defined by Elena and Sanjuan (2008)). In our study, however, we take into account the genomes of every individual in the population, not just the dominant genotype. This index quantifies how evolving with different periods of environmental fluctuations influences the the probability that mutating an individuals genetic architecture will elicit a phenotypic response. This metric does not consider whether that response is adaptive. It takes no account of fitness consequences, just the capacity of a genome to allow for phenotypic change.

Following phase 2 of the experiment, evolvability was also measured directly as a fitness response to environmental change. Depending on the degree of evolved generalism during the first phase of this study, fitness is expected to decline after populations are transferred to a new fixed environment and then gradually recover as the population adapts to the new environment.

For each population, the fitness response index to environmental change, $\Delta \bar{\omega}$, was defined as the observed increase in log mean population fitness at the end of the experiment relative to the start:

$$\Delta \bar{\omega} = \log_{10} \left( \frac{\bar{\omega}_{phase2} - \bar{\omega}_{phase1}}{\bar{\omega}_{phase1}} \right) \quad (6.3)$$

where $\bar{\omega}_{phase1}$ and $\bar{\omega}_{phase2}$ are the mean population fitness at the end of phase 1 and phase 2, respectively. The difference between $\bar{\omega}_{phase1}$ and $\bar{\omega}_{phase2}$ is scaled by $\bar{\omega}_{phase1}$ to control for the effect of different starting fitness values. The logarithm is taken because fitness in Avida increases exponentially as more complex tasks are performed. A population with a relatively higher value for this index is considered more evolvable than one in which the fitness had
increased only slightly. This measure of evolvability, the ability of a population to recover fitness upon exposure to environmental change, is closest to that of Flatt: “the ability of a population to respond to natural selection”. This is similar to evolvability as defined by Elena and Sanjuan (2008), but we are looking at mean fitness of a population, rather than that of the dominant organism in a population. Because the “dominant” genotype often comprises well under 1% of the total population, examining evolvability of the entire population is a more representative measure of evolvability as a population level effect.

The difference in this index between a starting population with mutations turned on and turned off provides an estimate of the contribution of the genetic architecture to evolvability, since demographic effects are factored out. This is the potentially heritable component of evolvability.

### 6.2.4 Statistical analyses

Since the rate of environmental fluctuation was varied over several orders of magnitude, for regression the log of the rate of fluctuation per update was used. In all cases, a constant, \( \frac{1}{100000} \) (1 divided by the length of time of the experiment in updates) was added to the rate before the log was taken to normalise the log fluctuation rate.

At the end of phase 1, regressions were performed for the effect of log fluctuation rate on generalism, \( G \), Genome sensitivity, \( S_g \) and Diversity, \( H \) (Figure 6.2). Because a sigmoidal shape was observed in the data, in all cases, a non-linear logistic curve was compared with a linear regression by RSS ratios and AIC. A better fit of a logistic curve indicated that there were two regimes around a certain cutoff fluctuation rate.

In phase 2, the relationship between fitness response, \( \Delta \bar{\omega} \) and \( G \), \( S_g \) and \( H \) was determined by a two-way ANCOVA with mutations (on/off) and environmental fluctuation treatment set as factors. \( G \) and \( S_g \) were arcsinh transformed so they were not bounded by 0 and 1. The means of all traits for the five replicates of each population were taken to reduce stochastic noise. This model allowed us to investigate the effects of the covariates independently of fluctuation time to identify if they have a direct effect on fitness recovery, or if they just co-vary with fluctuation rate.

All analyses were performed using R version 2.14.1 (R Development Core Team (2011)). Example Avida configuration and analysis files and the genome of the ancestor are shown in appendix C.

### 6.3 Results

#### 6.3.1 Effect of fluctuating environments on generalism, genetic diversity and genome sensitivity

Rapidly fluctuating environments result in phenotypically diverse populations exhibiting generalist strategies where individual phenotypes are more likely to be affected by mutation (Figure 6.2). Furthermore, generalism, \( G \), and diversity, \( H \), appear to split into two regimes. The inflection point for \( G \) is between log fluctuation rate -2 and -3 (fluctuation every 100 and every
Figure 6.2: Generalism, $G$, diversity, $H$, and genome sensitivity, $S_g$ in populations of Avidians evolved on a range of rates of environmental fluctuation. Fixed genome size:-  
$G$: $y = \frac{0.893(1+e^{-2.893-2.7x/0.687})}{0.687}$, $R^2 = 77.4\%$, $H$: $y = \frac{2.445(1+e^{-4.523-2.8x/1.803})}{1.803}$, $R^2 = 50.4\%$,  
$S_g$: $y = 0.036x + 0.512$, $R^2 = 38.8\%$. Free-to-vary genome size:-  
$G$: $y = \frac{0.869(1+e^{-2.7-2x/0.487})}{0.487}$, $R^2 = 79.5\%$,  
$H$: $y = \frac{2.064(1+e^{-4.65-2x/1.852})}{1.852}$, $R^2 = 40.8\%$,  
$S_g$: $y = 0.028x + 0.513$, $R^2 = 20.9\%$. Each point represents a single replicate of 100 total replicates at each fluctuation rate. All curves are significant at $P<0.00001$. 

Fixed genome size 

Free to vary genome size
6.3. RESULTS

Table 6.2: Correlations between generalism ($G$), Genome sensitivity ($S_g$) and Diversity ($H$) within environmental fluctuation treatments following 100000 updates of evolution. Bold type indicates significance at $P<0.05$ (Holm-Bonferroni corrected)

<table>
<thead>
<tr>
<th>Correlation</th>
<th>log fluctuation rate</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No change</td>
</tr>
<tr>
<td>Fixed genome length</td>
<td></td>
</tr>
<tr>
<td>$H$ $G$</td>
<td>0.37</td>
</tr>
<tr>
<td>$H$ $S_g$</td>
<td>0.51</td>
</tr>
<tr>
<td>$G$ $S_g$</td>
<td>0.1</td>
</tr>
<tr>
<td>Free genome length</td>
<td></td>
</tr>
<tr>
<td>$H$ $G$</td>
<td>0.29</td>
</tr>
<tr>
<td>$H$ $S_g$</td>
<td>0.21</td>
</tr>
<tr>
<td>$G$ $S_g$</td>
<td>0.07</td>
</tr>
</tbody>
</table>

1000 updates). $H$ shows a peak at fluctuation rate -2 (fluctuation every 100 updates). Genome sensitivity, $S_g$, increases linearly with log fluctuation rate. This means that populations evolving on more rapidly fluctuating environments have individuals with, on average, a higher proportion of single-mutant neighbours causing a phenotypic effect. In all cases, there is little obvious difference between populations of Avidians with genome sizes fixed at 100 instructions and those with genome sizes that were free to vary.

$G$, $S_g$ and $H$ are all fairly strongly correlated (df=1405, $H$-$G$: $r$=-0.71, $p<0.0001$; $H$-$S_g$: $r$=0.62, $p<0.0001$; $G$-$S_g$: $r$=0.56, $p<0.0001$). However, this effect could be due to both being correlated with log fluctuation rate. To control for this, correlations between the three traits were tested within environmental fluctuation treatments (Table 6.2). The correlation between $G$ and $H$ at lower fluctuation rates seems to reflect that some populations here are dominated by generalists, and are also more diverse (See the top panel in figure 6.2). At higher rates of fluctuation, no specialist populations can be maintained, so there is no correlation because of reduced variation in $G$. In the fixed genome length case, populations with higher $S_g$ also tend to be more diverse, but this only holds at higher rates of change when the genome length is free to vary.

6.3.2 Fitness response to environmental change (phase 2)

Following the first phase of evolution on a range of rates of environmental fluctuation, populations were evolved for 1000 generations in the alternate environment to the one last experienced. The shorter period of time used in this phase is to gauge the immediate fitness response following environmental change. Fitness in Phase 2 was measured before and after the environmental shift. Populations that had evolved in rapidly changing environments in the past were expected to better recover fitness, because these populations are comprised of ecological generalists that are more phenotypically diverse and sensitive to mutation. Only populations in which genome lengths were free to vary were examined, since populations with fixed genome lengths showed almost no fitness recovery (Median fitness at end of phase 1: 244.97 (fixed genomes), 151.74 (free to vary genomes), $W = 255429$, p-value $= 0.2948$. Median fitness at end of phase 2: 1.85
6.3. RESULTS

Figure 6.3: Effect of evolution at different rates of environmental fluctuation on fitness recovery following transferal of whole populations to a new environment (control lines from phase 1 excluded). Lines represent the ANCOVA model $\Delta \omega \sim \log_{10}(\text{fluctuation}) \ast \text{mutations(on/off)}$ where $\Delta \omega$ represents the fitness change index in equation. With mutations $\alpha = -0.47 \pm SE0.05$, $\beta = 0.3 \pm SE0.01$. Without mutations $\alpha = -0.85 \pm SE0.06$, $\beta = 0.11 \pm SE0.03$. Effect of mutations(on/off): $F_{(1,1)} = 0.0015, P = 0.9689 \text{ns}$. Interaction of mutations and log(fluctuation rate): $F_{(1,1)} = 50.2, P < 0.0001$. $R^2 = 23.1\%$.

(fixed genomes), 93.69 (free to vary genomes), $W = 8108773$, $p << 0.0001$). This result strongly justifies allowing genome size to vary in Avida experiments, as the inability to vary constrains evolutionary response.

On initial analysis (and contrary to expectations) we found that populations that had only experienced a single environment for the entire long term evolution phase (the control lines from phase 1) appeared to have a much higher ability to recover fitness than any of the populations which had experienced other environments. However, we also found that log fitness values at the start and end of phase 2 were 2-4 orders of magnitude lower in these populations than the rest. Hence, the large relative fitness gain is due to their fitness being so low in the first place. For this reason, these control lines were excluded from further analysis.

As predicted, populations that previously evolved in more rapidly fluctuating environments are better able to recover fitness following environmental change (Figure 6.3). This effect is more pronounced in populations with mutations turned on, suggesting that individuals in these populations are adapting to their new environments by fixing new mutations rather than relying solely upon standing genetic variation to drive the response. Genetic diversity steeply declines in populations with mutations turned off (Figure 6.4), suggesting that a small number of fitter variants in the populations are increasing to fixation, accounting for the small increase in relative fitness even in these populations.
6.3. RESULTS

Figure 6.4: Genetic diversity after 1000 updates on a new environment in populations with mutations turned on and off. Here, diversity is measured as the Shannon index, H, of all genotypes in a population.

6.3.2.1 Overall effect of diversity, generalism and genome sensitivity

There are positive overall relationships between fitness recovery, $\Delta \omega$, following transfer of populations to a new environment and generalism, $G$, genome sensitivity, $S_g$, and diversity, $H$, (Figure 6.5, table 6.3). In all cases, the overall means of populations with mutations on and off are not different, but there are strong effects of the covariate ($G$, $S_g$ or $H$) and of the interaction between the covariate and the mutation state (Table 6.3 covariates:mutation).

6.3.2.2 Effect of diversity, generalism and genome sensitivity within treatments

Since we know that $G$, $S_g$ and $H$ are all well correlated with environmental fluctuation rate (Figure 6.2), we are also interested in determining the effects of these covariates within fluctuation rate treatments, to identify if these genetic and population traits affect fitness recovery following environmental change. $H$ shows a significant three-way interaction with the mutation and environmental fluctuation treatment (table 6.3). This shows up most obviously in the bottom middle panel of figure 6.6, where in the most rapidly fluctuating environment, more diverse populations recover fitness better with mutations off and worse with mutations on. There is no significant interaction of the mutation treatment and $G$ and $S_g$ on $\Delta \omega$ within environmental fluctuation treatments, despite the strong interaction between the traits and mutation treatment overall. The trait:mutation:treatment interaction terms for $G$ and $S_g$ were therefore dropped from the model and do not show up in table 6.3.

With diversity, $H$, there is no relationship with $\Delta \omega$ at fluctuation rates 10 and 100, but at the slower rates 1000 and 10000, more diverse populations are better able to recover their fitness. The effect is most pronounced at fluctuation rate 10000.

Table 6.4 compares the percentage of variance in $\Delta \omega$ explained by the covariates in a model with only the covariate on the right hand side and in the full model from table 6.3. The reduction in $R^2$ after including the co-factors shows that $G$ and $H$ only explain 1% and 5% of the variance in $\Delta \omega$ respectively. Much of the variance explained by the trait covariates is in fact a correlated
Figure 6.5: Effects of generalism, $G$, diversity, $H$, and genome sensitivity, $S_g$, on fitness recovery, $\Delta \bar{\omega}$, following transferal of populations to a new environment. $R^2(G) = 13.6\%$, $R^2(H) = 12.5\%$, $R^2(S_g) = 3.1\%$. 
Table 6.3: 2-factor ANCOVA tables for the effects of generalism, $G$, genome sensitivity $S_g$, and diversity, $H$, on fitness recovery, $\Delta \bar{\omega}$, given fluctuation rate treatment and mutations (on/off). Non-significant terms have been dropped from the model. $R^2 = 32.5\%$

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H$</td>
<td>1</td>
<td>51.86</td>
<td>51.86</td>
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</tr>
<tr>
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<td>12.22</td>
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<tr>
<td>$S_g$</td>
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<td>3.48</td>
<td>3.48</td>
<td>11.36</td>
<td>0.0008</td>
</tr>
<tr>
<td>mutation</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.002</td>
<td>0.9669</td>
</tr>
<tr>
<td>treatment</td>
<td>4</td>
<td>37.03</td>
<td>9.26</td>
<td>30.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$H:G$</td>
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<td>7.22</td>
<td>7.22</td>
<td>23.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$H$:mutation</td>
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<td>2.34</td>
<td>2.34</td>
<td>7.63</td>
<td>0.0058</td>
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<tr>
<td>$G$:mutation</td>
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<td>5.82</td>
<td>18.99</td>
<td>0.0001</td>
</tr>
<tr>
<td>mutation:treatment</td>
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<td>11.48</td>
<td>2.87</td>
<td>9.36</td>
<td>&lt;0.0001</td>
</tr>
<tr>
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<td>2.65</td>
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<td>0.0034</td>
</tr>
<tr>
<td>$H$:mutation:treatment</td>
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<td>6.98</td>
<td>0.87</td>
<td>2.84</td>
<td>0.004</td>
</tr>
<tr>
<td>Residuals</td>
<td>956</td>
<td>292.95</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6.4: Variance in fitness recovery, $\Delta \bar{\omega}$, explained by $G$, $H$ and $S_g$, with and without controlling for mutation and fluctuation treatments.

<table>
<thead>
<tr>
<th></th>
<th>$R^2%$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G$</td>
</tr>
<tr>
<td>Just covariate</td>
<td>11.8</td>
</tr>
<tr>
<td>Covariate controlling for mutation and fluctuation treatments</td>
<td>4.9</td>
</tr>
</tbody>
</table>

effect of fluctuation rate, rather than a direct effect of these traits on fitness recovery.

6.4 Discussion

Using the Avida digital evolution platform we have investigated how evolution in fluctuating environments influences ecological generalism and evolvability. We first generated a series of populations that had evolved from a common ancestor on a range of rates of environmental fluctuation, from 1 fluctuation per update to 1 fluctuation every 10,000 updates. We then determined how fluctuation rate affected “potential” evolvability (at the population and genotype level, $H$ and $S_g$ respectively) and generalism ($G$). In the second stage, to estimate realised evolvability, we measured the fitness response ($\Delta \bar{\omega}$) following movement of the populations to a different environment. The power of this approach is that we were able to examine evolvability at these two distinct scales: the first, where we infer evolvability of an individual or population as a product of evolution under different ecological scenarios, and the second, where we directly measure evolvability in response to an environmental change.

In agreement with evolutionary ecology theory, we found that evolution in more rapidly
Figure 6.6: Effects of generalism, $G$, Diversity, $H$ and genome sensitivity ($S_g$) on fitness recovery, $\Delta \bar{\omega}$, within fluctuation rate treatments (-4: slowest, 0: fastest) following transfer of populations to a new environment. Results are for mutations on treatment.
fluctuating environments results in populations of generalists. In the current experiment, we observed a cutoff beneath which individuals tend to be highly specialised and above which they tend to be highly generalised, indicated by the significantly better fit of a logistic model over a linear model (Figure 6.2). Although a few generalist individuals are able to survive in more slowly fluctuating environments, no specialised genotypes are present at all at the highest rates of environmental change.

Our results show that more rapidly fluctuating environments select for increased evolvability, by any of three main definitions given in the literature (Hansen (2006), Dichtel-Danjov and Felix (2004), Flatt (2005)): Populations exposed to increased rates of environmental fluctuation are more diverse, have genomes that are more susceptible to phenotypic change following mutation and are better able to recover their fitness following environmental change.

Fixing the genome length, as is common in *Avida* studies (e.g. Elena et al. (2007); Ostrowski et al. (2007); Wilke et al. (2001)), has limited effect over the long term evolution experiments in phase 1, but it drastically slows down response to environmental change to outside that of the range of the experiment in phase 2. Presumably this is because fixing the genome removes any selection to optimise performance by “streamlining” the genome. The consequence of this is that, when populations are transferred to a new environment in which they are unable to perform rewarding tasks, their reproductive rate drops to very low levels. It seems, therefore, that genome size is a selectable trait imparting added evolvability in *Avida*, as in biology.

The relationship between generalism and these different measures of evolvability is complex. Although generalism, diversity and genome sensitivity are all well correlated, much of this correlation is due to covariance with the rate of environmental fluctuation. Within treatments, generalism and diversity are significantly correlated only in relatively slowly fluctuating environments. This is because slowly changing environments are able to sustain both generalist and specialist populations and generalist populations are more diverse. Populations with higher diversity also are more responsive to mutation, and this correlation seems to be independent from that of diversity and generalism, since genome sensitivity is uncorrelated with generalism in most cases. The correlation between diversity and genome sensitivity is seen in all cases with a fixed genome length but only in more rapidly changing environments with a free to vary genome. It is not possible to determine if there is a causal relationship, or if diversity and genome sensitivity are just responding independently to the fluctuating environment.

Generalism, diversity and genome sensitivity all strongly affect fitness recovery. However, within fluctuation treatments, only diversity has a significant effect (Table 6.3 $H$:mutation:treatment), indicating that most of these effects are indirect correlated effects with fluctuation rate. The effect of diversity itself accounts for only 5% of the variance in fitness recovery when environmental fluctuation is controlled for (Table 6.4). It is surprising that only about 33% of fitness recovery is explained by all of the treatments and covariates (Table 6.3), even after some stochastic noise was controlled for by replicating the same populations.

Genome sensitivity is more commonly referred to in its inverse as mutational robustness (Draghi and Wagner, 2008; Wagner, 2008; Elena et al., 2007; Elena and Sanjuan, 2008). Elena and Sanjuan (2008) found that genetic robustness favours evolvability in the long term because relaxed selection facilitates the accumulation of genetic diversity. They found little effect, or
even detrimental effects in the short term, since robustness reduces the intensity of selection. Our results show no significant effect of genome sensitivity on fitness recovery when controlling for fluctuation rate (non-significant $S_g$:mutation:treatment term removed from the model in table 6.3). Draghi et al. (2010) show that intermediate levels of robustness (and so genome sensitivity) promote adaptation, given that the number of phenotypes in the direct phenotypic neighbourhood is below the total number of potential phenotypes. All of our results show intermediate sensitivity levels from 0.2 to 0.6 (e.g. see right panel of figure 6.5) and most genotypes have 3-5 different phenotypes accessible by one-step mutations and a genome length typically of 80-120 instructions. Given this, our results for the effect of sensitivity on fitness recovery across treatments (figure 6.5) are in agreement with the predictions in figure 2 of Draghi et al. (2010), i.e. within these ranges, less sensitive genomes will adapt more quickly. However, the lack of a clear relationship between genome sensitivity and fitness recovery within treatments suggests that the increased sensitivity to mutation may have evolved as a side effect of more rapid environmental fluctuation (the opposite expectation to that of Wagner (2005a)), although we cannot discount that sensitivity has an effect on adaptation between environmental treatments.

In all cases, effects of fluctuation rate are much higher on measures of “potential” evolvability ($H$ and $S_g$) than on “realised” evolvability. This is unsurprising since they are directly measured properties of populations and genomes whilst fitness recovery is an indirect effect and populations rely on stochastic mutations to move through phenotype space towards an adaptive peak.

So what then is the true measure of evolvability? More rapid environmental fluctuation directly or indirectly selects for increased evolvability in all of our measures, but the fitness response to environmental change is the only direct measure of the ability of the population to evolve. The predictive powers of diversity and genome sensitivity on fitness recovery are limited, explaining less than 15% of the variation. Within treatments, only diversity has a significant effect at all, and still only explains 5% of the variance in fitness recovery. Diversity is obviously very important for the capacity of a population to evolve (for example the bottleneck effect in small populations), but it doesn’t seem to represent evolvability conceptually. In addition, the clear improvement in fitness recovery in populations that have been allowed to evolve genetically over those that have not shows that there are more than just demographic effects at play (Although we note that figure 6.6 shows a striking increase in variance of fitness recovery when mutations are allowed). Clearly there is a genetic component to evolvability that may well comprise genome sensitivity, although it is not limited to it. It is interesting that genome sensitivity does not seem to have an effect on the response to selection, despite the clear effect of environmental change on it. It may be that the mean genome sensitivity for a population is too coarse a measure, and clearer results may be found by considering the distribution of sensitivities in a population as a mathematical function (i.e. a function-valued trait (Aston et al., 2012)) and identifying if different rates of environmental change produce different sensitivity functions.

What is the relationship between evolvability and ecological generalism? If a generalist organism is moved to an environment in which it is already able to perform fitness tasks, it seems that it may be quicker to increase fitness by losing the tasks rewarded in the previous
environment (and their associated costs) than it would be for another organism to evolve the ability to perform new tasks de novo (Ostrowski et al., 2007). This assumes that the “genes” for these tasks are uncoupled. Preliminary results (not shown) suggest that modularity between tasks may also be selected for at high fluctuation rates. Although generalism is selected for at high fluctuation rates (as predicted by classical ecological theory), it is not well correlated with either genome sensitivity or diversity within treatments, and has no significant effect on fitness recovery, controlling for treatment.

In one sense, the concept of evolvability is a diversion from the real issue that organisms strive to maintain fitness in the face of perturbation. It is therefore not sensible to speak of “evolvability of fitness” and so any seeming evolution of evolvability in some other trait is really just an indirect response to organisms maximising their own fitness (Wagner, 2005a). Furthermore, evolvability is a general property of an organism, not of a trait (Hansen et al., 2011). In this sense, evolvability is itself a trait like any other that is tuned by its environment, albeit one that is complicated by demographic effects and self-organisation at the population and ecosystem level (Wagner, 2005a).

In this paper we have shown that environmental fluctuation selects for not only generalism (as predicted from the evolutionary ecology literature), but also for increased evolvability, in three main forms it is commonly referred to. However, the relationship between these measures is unclear, and further work needs to be done to determine how the genetic architecture of trait modularity affects these measures. It may be that generalism and evolvability respond to the same cues but are not interdependent.

The Avida world, although vastly simplified, is subject to the same fundamental evolutionary processes that exist in “real” biology. It is therefore not unrealistic to expect similar responses in real ecosystems to rapid environmental change. We are currently undergoing a period of rapid climate change, largely mediated by human actions, and this change is predicted to deepen over the coming decades. One of the main predictions of this is that current environmental conditions will become much more unpredictable (IPCC, 2007). Our work suggests that this unpredictability will select for generalist organisms with labile genomes that are able to quickly adapt to new environmental conditions. These are properties commonly associated with invasive and weedy plant species (Lee et al., 2008). Should climate change continue at its predicted rate, we should expect significant invasions of affected habitats. The more rapid and unpredictable this change, the more invasions we should expect to see.
Chapter 7

General discussion and concluding remarks

In this thesis, I have used two very different model systems, one natural and one in silico, to investigate the roles of plasticity and adaptation in response to environmental change. First, I used the model plant Arabidopsis thaliana and a combination of inbred and outbred lines, climate manipulations, quantitative genetics and ecological genomics. These allowed me to investigate the plastic and genetic response to simulated climate warming, the effect of artificial selection for early flowering on phenotypic plasticity and the cross-generational plastic effects of maternal flowering time on germination traits. Second, I used the artificial life platform Avida to investigate the effect of different rates of environmental fluctuation in resource availability on the diversity and evolvability of populations of digital organisms.

In this final chapter, I will discuss the major findings of my research and their implications for evolutionary biology, conservation biology and crop science. Next, I will highlight some of the questions arising from my research and consider some avenues for further research. Finally I will evaluate the contribution of this thesis to the study of environmental change.

7.1 What do we mean by plasticity?

This thesis raises important questions about the meaning phenotypic plasticity and exactly how it should be used. Plasticity has featured prominently in four of my five research chapters, but in the literature the term has been used almost interchangeably to mean either the magnitude in differences in average phenotype across environments or the degree of genotype-by-environment interaction variance. There is also confusion about what level plasticity operates on: Is it a property of a population (Gianoli and Valladares, 2012), an individual (Adams and Collyer, 2009; Dennis et al., 2011) or a trait (Pigliucci, 2005; Richards et al., 2006)? To complicate things further, there are a host of different ways to measure plasticity, and no one way is universally agreed to be the best (Valladares et al., 2006).

Looking at the population as a whole, we can investigate the mean plastic response of the population (Chapter 3) or gxe (Chapter 2, 3, 4). In chapters 2 and 3 we show that overall
population level plasticity is in an adaptive direction (i.e. mean plastic responses for a range of traits to elevated temperature are in the same direction as the selection gradients for those traits), but some traits showed no gxe despite a clear plastic response and in the case of rosette diameter, there was a lack of gxe but clear evidence of genetic control of plasticity being separate from control of the trait itself. Likewise, in chapter 4, we see that selection can both increase magnitude of plasticity and reduce variance for gxe ($V_{gxe}$). The magnitude is useful when talking about direction of adaptive plasticity in a particular organism, but gxe is more important if we are discussing the statistical properties of the population and whether plasticity will be able to assist in response to future and unpredictable environmental change (Chapter 4; Pigliucci (2005)). Conservation biologists should be more concerned about measuring gxe of at risk species than the magnitude of plasticity. Environmental change may quickly render current norms of reaction no longer adaptive (Visser, 2008). The maintenance of a range of different norms of reaction means that a population is likely to be better able to respond to unpredictable future change.

7.2 Arabidopsis and the interaction of plasticity and selection

In this thesis I have observed plasticity in flowering time and gxe interactions in fitness to elevated temperatures, evolutionary responses in flowering time plasticity to artificial selection for early flowering and cross-generational plastic effects in germination due to maternal phenotype. Theoretical studies (e.g. Chevin et al. (2010)) are beginning to recognise the importance of considering both plasticity and adaptive change in models of response to changing environments and my work here is an important step in applying these ideas experimentally.

Despite its reputation as a lab-based genetics and physiology model, *A. thaliana* has proved useful in evolutionary ecology research (Pigliucci, 2002; Mitchell-Olds, 2001; Wilczek et al., 2010). Apart from its ease of experimental manipulation, small and well-characterised genome and self-compatibility (making it amenable to the construction of recombinant inbred lines), *A. thaliana* has some key advantages as a model for the study of response to environmental change. It has a wide geographical distribution, variable life history patterns and variation for plasticity (reviewed in Pigliucci (2001)). Recent work has also shown that it has a highly evolvable genome that is particularly amenable to local adaptation (Fournier-Level et al., 2011). This flexibility has allowed it to be a successful coloniser. Studies of *A. thaliana* response to environmental change should aid our understanding of plant response in general and also they should be applicable to other weedy colonising species, which are likely to be significant threats under current predictions of climate change (Drenovsky et al., 2012; Gilchrist and Lee, 2007; Gurevitch and Padilla, 2004; Richards et al., 2006). It is difficult to draw conclusions about the adaptive significance of phenotypic plasticity unless the fitness consequences of plastic phenotypic changes are measured (Richards et al., 2006; Nicotra et al., 2010). This is where the real strength of our methods can be seen, particularly in chapters 2 and 3. Because we are able to quantify fruit number in *A. thaliana*, which is known to be an accurate proxy of fitness, we can test if other traits such as flowering time are adaptive, predict evolutionary response
to environmental change as well as map quantitative trait loci to understand the genetic basis of fitness responses. Through these methods I was able to go beyond simply identifying and measuring plastic responses and actually determine how plasticity and adaptation interact. In chapters 2 to 5 I observe that selection and plasticity do in fact interact in unpredictable ways: In chapter 2, plants showed different relative fitnesses under elevated temperatures to ambient temperatures, resulting in a significant genotype-by-environment (gxe) interaction for fitness. If this interaction were found in natural populations, it could lead to unpredictable effects on community structure under conditions of climate warming, potentially leading to populations that are maladapted to their environment because of their response to elevated temperature. In addition, the phenological changes caused by climate change could create powerful selection pressure on species at higher trophic levels that depend on these plants, initiating changes that over time might affect community organization and have far-reaching consequences for ecology and evolution (Sherry et al., 2007). Concerns about population maladaptation as a consequence of climate change have been raised before (Yang and Rudolf, 2010; Visser, 2008), but this is the first example of gxe in response to climate warming causing changes in fitness rank order.

In chapter 3 I found that plastic responses to elevated temperatures, though in an adaptive direction, can significantly alter the direction of selection gradients and also hamper evolvability and longer term evolutionary response to environmental change. I also found that plastic responsiveness can be detrimental in ambient conditions and yet beneficial in elevated temperatures, which may partly explain the gxe observed in chapter 2. Climate change is expected to result in more extreme and less predictable environments (Meehl et al., 2000; IPCC, 2007), and this is likely to favour the evolution of plasticity. From this study, we can infer that plasticity may assist with immediate response to climate change and allow organisms to survive in a new environment to begin with, thus potentially allowing adaptation to take place (Pigliucci et al., 2006). However, there may be a trade off in that the plasticity that allowed for persistence in the first place may have negative effects on future adaptation.

In chapter 4 I found that not only can plasticity affect selection, but that the reverse is also true: Strong, directional selection for early flowering time can significantly change both the magnitude of and variation for phenotypic plasticity. This is an important result both because climate change is likely to impart strong directional selection on traits such as flowering time (although our results suggest that phenology changes are also plastic), and because a reduction in $V_{gxe}$ due to directional selection may have negative effects on adaptive plastic responses. If these selection pressures affect plasticity, they can also affect persistence. Here, we also show that the effect of direct selection on a trait’s plasticity are environment dependent: Artificial selection for early flowering in simulated spring conditions increased the magnitude of plastic response but reduced $V_{gxe}$. Selection for early flowering in winter conditions, on the other hand, reduced the magnitude of plastic response but left $V_{gxe}$ unaffected. These results have implications for both conservation biology and plant breeders: Ex situ conservation programs needs to consider carefully which environment to keep organisms in to not adversely affect future re-introduction, while plant breeders should consider carefully the environment in which artificial selection is to be carried out in to enable the resultant varieties to be robust to environmental variation.

Chapter 5 does not explicitly deal with plasticity, but one of the main results from this chapter
is that non-genetic environmental effects on a developmental trait (flowering time) can have effects on another developmental trait (germination) in plants in the next generation. Since there is no significant genetic component to this response, offspring appear to be responding to plasticity in flowering time to micro-environmental changes in the maternal generation. The presence of non-genetic inheritance like the trans-generational plasticity observed here complicates further our understanding of evolutionary response to environmental change (Bonduriansky et al., 2012; Jablonka and Lamb, 2005). Various other studies have observed plasticity acting across generations, particularly in artificial selection experiments (Sherry et al., 2007; Munir et al., 2001; Post and Pedersen, 2008; Galloway and Burgess, 2009). It has been suggested that plastic responses may even be a precursor to sympatric speciation. For example, in a study of two species of palm known to have diverged on a single remote oceanic island (Savolainen et al., 2006), a proposed mechanism for this speciation event is that plasticity to different soil conditions may have affected phenology, which in turn created a barrier to reproduction leading to speciation. Today, the two species have different phenologies and one grows almost exclusively on acidic volcanic soils while the other grows on basic limestone soils.

7.3 Avida and selection in fluctuating environments

Artificial life studies like Avida occupy a curious niche somewhere between experimental biology and “pure” simulation. While the metabolism of Avidians is clearly synthetic, they evolve under pure Darwinian natural selection and are capable of exhibiting levels of complexity much higher than would be observed in an analytic simulation. This has led some researchers to go as far as to say that experiments with digital organisms represent instantiations of life on the computer, rather than being merely simulations (Ray and Hart, 1999; Adami, 2006). Because Darwinian principles are universal and not necessarily tied to any particular genetic inheritance system, Darwinian systems can therefore be realised on a computer. Whether or not the vastly simplified digital organisms in Avida represent life itself, this system is inherently different from a simulation such as a genetic algorithm because the fitness function is not defined a priori by the user. Instead, as with biochemical life, the lineages that manage to survive to reproduce in the face of resource competition are, retrospectively, the fittest (Adami, 2006; Wilke and Adami, 2002). Repeatable, statistically powerful experiments under fully controllable conditions, with a perfect fossil record, that are impossible or impractical in the lab can be designed using Avida. However, artificial life experiments have important limitations, and many questions concerning the causes and consequences of evolution still require conventional evolutionary genetics. Avida can obviously tell us nothing about genetics, mutation, transcription etc., and digital organisms have only limited means of expression regulation and no developmental phase or epigenetic modifications. Finally, digital genomes are small compared with free-living organisms and even most viruses. Consequently, although the mutation rate per genome is usually comparable to that of an RNA virus, the rate per gene is likely to be much higher (Adami, 2006).

It has been suggested that an environment that fluctuates in time between states like in this experiment is the most biologically relevant and realistic model of climate change (Boyce et al., 2006; Lundberg et al., 2000; Schreiber et al., 2011). The Avida experiment in chapter 6 shows that
in the long run, populations exposed to changing environments are more phenotypically diverse and better able to cope with future environmental change. Selection in fluctuating environments has also been shown to facilitate the evolution of phenotypic plasticity. Hallsson and Bjoerklund (2012) demonstrated the effect of rapid, continuous and fluctuating temperature changes in the seed beetle *Callosobruchus maculatus*. They found that genetic variation decreased after fluctuating selection but that there was an increase in gxe after selection. The authors concluded that the evolution of phenotypic plasticity is strongly dependent on the selective past and on whether selection involved fluctuations in the environment or not. Others found that a history of phenotypic plasticity increases the rate of adaptation to a new environments via genetic assimilation (Fierst, 2011). However, our work with *Avida* in chapter 6 also achieved an increase in evolvability in an environment where plasticity is impossible (although work has been done with a “plasticity gene” coded into *Avida* (Clune et al., 2007)). The mode of that response was constrained by what is available to the system in question, which is the shortcoming of the *Avida* system: If plasticity were available as a mechanism to cope with environmental change, what would the circumstances be under which these responses would evolve above, for example, a less robust genome or increased phenotypic diversity? This is in itself an interesting result: We show that evolvability behaves in the same way that classical ecological theory predicts generalism will. So, if generalism, bet hedging, plasticity and evolvability are all ways of coping with environmental change and can arise in similar conditions, then maybe the evolution of one over another is due to an “accident of history”: genetic or developmental constraints present before exposure to that change, rather than necessarily different environmental conditions. Despite this, I have shown that the *Avida* system is useful for uniting classical ecological theory of generalism with more recent concepts of evolvability.

### 7.4 Final thoughts and directions for future research

Phenotypic plasticity is generally considered to assist organisms in coping with changing environments. My work in part agrees with this hypothesis. I have shown that plastic responses to elevated temperatures are in the same direction as selection gradients and that there is evidence for selection for increased plasticity under elevated temperatures. However, I also find that plastic responses can have negative effects on future evolvability. The paradox here is that plastic responses may allow an organism to survive in a new or changed environment, but at the expense of being able to improve the fit to that environment. It should be noted from chapter 6, however, that evolvability may be measured in several different ways and if evolvability (in the sense of being able to respond to that specific selection gradient) is impeded, it may be aided by another mechanism. For example, phenotypic plasticity can facilitate the accumulation of cryptic genetic variation, which is hidden variation that is only expressed under certain environmental conditions (Pfennig et al., 2010; Rouzic and Carlborg, 2008). This can increase the number of potentially adaptive genetic variants in the population (Evolvability sensu Houle (1992)), thus facilitating the responsiveness to future environmental change. There is huge potential for work that empirically tests for the relationship between different measures of evolvability and how they affect evolutionary dynamics (Pigliucci, 2008).
Thermal models predict that plant phenology will respond to elevated temperatures in a linear fashion. Many demographic models also rest on this assumption. However, others also expect climate warming to have complex and non-linear effects on crops and populations. Some of this non-linearity is expected to be due to different rates of response in different species. We also found that a linear response in phenology, predicted by a thermal model and a similarly linear response in vegetative size resulted in genotype-by-environment interactions for fitness. This shows that linear developmental responses to climate warming are also capable of causing changes in fitness rank order within a species, as well as between species. This should be of much concern to conservation biologists. Until recently, conservation biologists had not taken evolutionary processes into account. There has been much progress in species distribution and vegetation models but most models do not even take into account evolution, lat alone plasticity and the potential complex interactions between plasticity and selection (Nicotra et al., 2010; Chevin et al., 2010). The contribution of plasticity to species persistence will be most important at range boundaries where the greatest and most rapid environmental changes will be felt. Maintenance of variation for plasticity will be important for rapid initial response to these changes. Conservation biologists should be less concerned with measuring the magnitude of plasticity per se, because individual reaction norms can quickly become maladapted. Measuring gxe in natural populations is a great challenge, although some progress has been made in small populations with known pedigrees (Husby et al., 2010; Charmantier et al., 2008) and by estimating mean population ecological plasticity (Gianoli and Valladares, 2012). Theoretical evolution models are beginning to integrate information from paleoecological observations, recent phenological and micro-evolutionary responses, climate manipulation experiments and computational models in order to provide accurate predictions and effective solutions to the challenges associated with climate change (Hoffmann and Sgro, 2011; Dawson et al., 2011; O’Connor et al., 2012; Reed et al., 2010). Conservation biologists are also starting to realise that evolutionary theory must be taken into account when designing reintroduction programmes and other conservation projects (Hellman and Pfrender, 2011). There is a need for more research into effectively incorporating plasticity and adaptation in these models if they are to make accurate predictions about population responses to climate change.

I have shown that combining climate manipulation, a model organism and sophisticated statistical techniques can be used to effectively study response to predicted climate change. Given a large enough experiment, even more complex interactions can be investigated. Grime et al (2000 and 2008) included droughted and watered treatments with elevated temperatures to determine the effect of climate change on grasslands. Using these extra treatments in combination with a large RIL population would allow for much greater insight into the effects of future climate change and specifically the interaction of different climatic variables. Another direction would be to investigate the relationship between developmental instability (within-genotype variation) and phenotypic plastic response to elevated temperatures. The two are thought to be closely linked because one path to increased plasticity is via a less stable development (Debat and David, 2001). This is a relationship that is strikingly reminiscent of that between robustness and evolvability (Wagner, 2005a, 2012), where a genome that is less buffered to mutation may be more phenotypically responsive. Experiments such as these can become prohibitively large
as the number of replicates and treatments increases. There will therefore also be a role for computer simulations and artificial life models to predict the long term effects of interactions between plasticity and evolvability.

The work in this thesis represents a significant contribution to the study of environmental change. I have provided rigorous empirical tests of theoretical predictions and illustrated that both selection and plasticity are critical to the effective investigation of the response of populations to climate change and that interactions between selection and plasticity are likely to be complex, non-linear and unpredictable. Phenomena such as evolvability, bet-hedging and range shifting as well as the effects of other environmental factors such as habitat fragmentation, pollution and invasive species will add extra complexity to models of climate change response and will require careful investigation by a large body of researchers.
Appendix A

Supporting material for Chapter 2

Table A.1: Mean trait values for four traits in *Arabidopsis thaliana* MAGIC lines grown in ambient and elevated temperature treatments. Subset with density = 1 (individuals with a single plant in each planting). 95% Confidence intervals are shown in parentheses.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Ambient temperature mean</th>
<th>Elevated temperature mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering time</td>
<td>53.432 (0.52)</td>
<td>49.32 (0.54)</td>
</tr>
<tr>
<td>Photothermal flowering time</td>
<td>541 (6.37)</td>
<td>495.48 (6.49)</td>
</tr>
<tr>
<td>Rosette diameter</td>
<td>37.33 (1.82)</td>
<td>49.21 (2.02)</td>
</tr>
<tr>
<td>Number of fruits</td>
<td>801.87 (57.5)</td>
<td>986.38 (63.44)</td>
</tr>
</tbody>
</table>
Table A.2: Treatment effects, variance components and heritabilities. Subset of density = 1 (individuals with a single plant in each planting).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Control mean (se)</th>
<th>Elevated mean (se)</th>
<th>MS</th>
<th>F (P)</th>
<th>Vg (P)</th>
<th>Vgxe (P)</th>
<th>% Vgxe due to crossing</th>
<th>h^2 (se)</th>
<th>r_g (se)</th>
<th>Flushing time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering time</td>
<td>53.51 (0.57)</td>
<td>49.33 (0.74)</td>
<td>787.75</td>
<td>32.81 (0.0004)</td>
<td>12203.34 (0.0001)</td>
<td>0.34127 (0.4047)</td>
<td>99.57</td>
<td>0.33 (0.04)</td>
<td>0.11 (0.06)</td>
<td></td>
</tr>
<tr>
<td>Rosette diameter</td>
<td>37.18 (2.52)</td>
<td>48.05 (3.4)</td>
<td>4150.82</td>
<td>11.43 (0.0122)</td>
<td>10918764 (0.0001)</td>
<td>0.43644 (0.491)</td>
<td>72.48</td>
<td>0.22 (0.04)</td>
<td>0.04 (0.22)</td>
<td></td>
</tr>
<tr>
<td>Number of fruits</td>
<td>780.16 (58.06)</td>
<td>981.49 (78.57)</td>
<td>2524041</td>
<td>6.57 (0.0020)</td>
<td>296604.4 (0.0427)</td>
<td>71105.7 (0.0032)</td>
<td>74.87</td>
<td>0.06 (0.04)</td>
<td>-0.08 (0.24)</td>
<td></td>
</tr>
</tbody>
</table>

Figure A.1: Full genome scans for QTLs for Flowering time traits in ambient and elevated temperature treatments
Figure A.2: Full genome scans for QTLs for rosette size and fitness in ambient and elevated temperature treatments
Figure A.3: Full genome scans for QTLs for phenotypic plasticity in ambient and elevated temperature treatments

Flowering time plasticity

Photothermal flowering time plasticity

Rosette diameter plasticity

Fruit number plasticity
## Appendix B

### Supporting material for Chapter 4

Table B.1: Line means and standard errors for the magnitude of plasticity in flowering time and cross-environment means; REML estimates for total phenotypic ($V_p$) and family x environment ($V_{gxe}$) variance components; Bootstrap confidence limits for variance components; percentage of $V_{gxe}$ that can be attributed to crossing of reaction norms.

<table>
<thead>
<tr>
<th>Breeding environment</th>
<th>Selection treatment</th>
<th>line</th>
<th>Mean magnitude of plasticity (se)</th>
<th>Cross-environment mean FT (se)</th>
<th>$V_p$ bootstrap</th>
<th>$V_{gxe}$ bootstrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring Control</td>
<td>1</td>
<td>33.78 (1.16)</td>
<td>54.29 (0.95)</td>
<td>46.36</td>
<td>31.58</td>
<td>66.83</td>
</tr>
<tr>
<td>Spring Control</td>
<td>2</td>
<td>33.86 (1.21)</td>
<td>57.27 (1.03)</td>
<td>62.53</td>
<td>38.22</td>
<td>90.97</td>
</tr>
<tr>
<td>Spring Control</td>
<td>3</td>
<td>31.28 (0.75)</td>
<td>52.9 (0.58)</td>
<td>25.45</td>
<td>19.67</td>
<td>31.88</td>
</tr>
<tr>
<td>Winter Control</td>
<td>4</td>
<td>37.11 (0.77)</td>
<td>54.04 (0.7)</td>
<td>26.92</td>
<td>21.19</td>
<td>33.84</td>
</tr>
<tr>
<td>Winter Control</td>
<td>5</td>
<td>35.8 (1.17)</td>
<td>55.23 (1.06)</td>
<td>68.14</td>
<td>34.02</td>
<td>117.12</td>
</tr>
<tr>
<td>Winter Control</td>
<td>6</td>
<td>52.32 (0.98)</td>
<td>56.87 (0.81)</td>
<td>51.27</td>
<td>31.2</td>
<td>77.87</td>
</tr>
<tr>
<td>Spring Selected</td>
<td>7</td>
<td>37.04 (0.65)</td>
<td>45.52 (0.36)</td>
<td>15.09</td>
<td>11.83</td>
<td>18.84</td>
</tr>
<tr>
<td>Spring Selected</td>
<td>8</td>
<td>37.43 (0.48)</td>
<td>45.17 (0.35)</td>
<td>10.65</td>
<td>7.63</td>
<td>14.4</td>
</tr>
<tr>
<td>Spring Selected</td>
<td>9</td>
<td>38.97 (0.56)</td>
<td>46.42 (0.26)</td>
<td>12.41</td>
<td>10.1</td>
<td>15.3</td>
</tr>
<tr>
<td>Winter Selected</td>
<td>10</td>
<td>30.19 (1.07)</td>
<td>47.33 (0.68)</td>
<td>30.65</td>
<td>23.21</td>
<td>40.27</td>
</tr>
<tr>
<td>Winter Selected</td>
<td>11</td>
<td>32.69 (0.48)</td>
<td>44.57 (0.3)</td>
<td>11.42</td>
<td>7.79</td>
<td>16.64</td>
</tr>
<tr>
<td>Winter Selected</td>
<td>12</td>
<td>32.21 (0.79)</td>
<td>45.28 (0.48)</td>
<td>23.94</td>
<td>18.79</td>
<td>30.77</td>
</tr>
</tbody>
</table>
Appendix C

Configuration files for chapter 6

1. An example Avida configuration file (avida.cfg). Non-relevant and unchanged groups are omitted for the sake of brevity.

```
#############################################################
# This file includes all the basic run-time defines for Avida.
# For more information, see doc/config.html
#############################################################
VERSION_ID 2.9.0    # Do not change this value.
### GENERAL_GROUP ### # General Settings
ANALYZE_MODE 0   # 0 = Disabled VIEW_MODE 1
CLONE_FILE # data/clone1 #- # Clone file to load
VERBOSITY 1     # 0 = No output at all
### ARCH_GROUP ###
# Architecture Variables
WORLD_X 60     # Width of the Avida world
WORLD_Y 60     # Height of the Avida world
WORLD_Z 1      # Depth of the Avida world
WORLD_GEOMETRY 2 # 2 = Torus
RANDOM_SEED 0   # Random number seed (0 for based on time) HARDWARE_TYPE 0  # 0 = CPUs
CPU SPECULATIVE 1 # Enable speculative execution
TRACE_EXECUTION 0 # Trace the execution of all organisms
BCAST_HOPS 1    # Number of hops to broadcast an alarm
ALARM_SELF 0    # 0=no
IQ_EXPIRE 1     # Is the expiration function enabled?
### CONFIG_FILE_GROUP ###
# Configuration Files
DATA_DIR data
INST_SET instset-classic.cfg
INST_SET_FORMAT 0 # 0 = Default
EVENT_FILE events.cfg # File containing list of events during run
```
APPENDIX C. CONFIGURATION FILES FOR CHAPTER ??

ANALYZE_FILE analyze.cfg   # File used for analysis mode
ENVIRONMENT_FILE environment.cfg   # File that describes the environment
START_CREATURE default-classic.org #default
### REPRODUCTION_GROUP ###
# Birth and Death
BIRTH_METHOD 4   # Which organism should be replaced on birth?
    # 0 = Random organism in neighborhood
    # 1 = Oldest in neighborhood
    # 2 = Largest Age/Merit in neighborhood
    # 3 = None (use only empty cells in neighborhood)
    # 4 = Random from population (Mass Action)
    # 5 = Oldest in entire population
    # 6 = Random within deme
    # 7 = Organism faced by parent
    # 8 = Next grid cell (id+1)
    # 9 = Largest energy used in entire population
    # 10 = Largest energy used in neighborhood
PREFER_EMPTY 1   # Give empty cells preference in offsping placement?
ALLOW_PARENT 1   # Allow births to replace the parent organism?
DEATH_METHOD 0   # 0 = Never die of old age.
AGE_LIMIT ALLOC_METHOD 0   # (Original CPU Only)
DIVIDE_METHOD 1   # 0 = Divide leaves state of mother untouched.
    # 1 = Divide resets state of mother
EPIGENETIC_METHOD 0   # Inheritance of state information other than genome
INJECT_METHOD 0   # 0 = Leaves the parasite thread state untouched.
RESET_INPUTS_ON_DIVIDE 0   # Reset environment inputs of parent upon successful divide.
REPRO_METHOD 1   # Replace existing organism: 1=yes
### DIVIDE_GROUP ###
# Divide Restrictions
CHILD_SIZE_RANGE 1.0   # Maximal differential between child and parent sizes.
MIN_COPIED_LINES 0.5   # Code fraction which must be copied before divide.
MIN_EXE_LINES 0.5   # Code fraction which must be executed before divide.
MIN_GENOME_SIZE 0   # Minimum number of instructions allowed in a genome.
MAX_GENOME_SIZE 0   # Maximum number of instructions allowed in a genome.
REQUIRE_ALLOCATE 1   # (Original CPU Only) Require allocate before divide?
REQUIRED_TASK -1   # Task ID required for successful divide.
IMMUNITY_TASK -1   # Task providing immunity from the required task.
REQUIREDREACTION -1   # Reaction ID required for successful divide.
REQUIRED_BONUS 0.0   # Required bonus to divide.
IMPLICIT_REPRO_BONUS 0   # Call Inst_Repro to divide upon achieving this bonus.
APPENDIX C. CONFIGURATION FILES FOR CHAPTER ??

IMPLICIT_REPRO_CPU_CYCLES 0 # Call Inst_Repro after this many cpu cycles. 0 = OFF
IMPLICIT_REPRO_TIME 0 # Call Inst_Repro after this time used. 0 = OFF
IMPLICIT_REPRO_END 0 # Call Inst_Repro after executing last instruction
IMPLICIT_REPRO_ENERGY 0.0 # Call Inst_Repro if this amount of energy.

### MUTATION_GROUP ###

# Mutations
POINT_MUT_PROB 0.0 # Mutation rate (per-location per update)
COPY_MUT_PROB 0.0075 # Mutation rate (per copy)
COPY_INS_PROB 0.0 # Insertion rate (per copy)
COPY_DEL_PROB 0.0 # Deletion rate (per copy)
COPY_UNIFORM_PROB 0.0 # Uniform mutation probability (per copy)
COPY_SLIP_PROB 0.0 # Slip rate (per copy)
DIV_MUT_PROB 0.0 # Mutation rate (per site, applied on divide)
DIV_INS_PROB 0.0 # Insertion rate (per site, applied on divide)
DIV_DEL_PROB 0.0 # Deletion rate (per site, applied on divide)
DIV_UNIFORM_PROB 0.0 # Uniform mutation probability (per site, applied on divide)
DIV_SLIP_PROB 0.0 # Slip rate
DIVIDE_MUT_PROB 0.1 # Mutation rate (per divide)
DIVIDE_INS_PROB 0.0 # Insertion rate (per divide)
DIVIDE_DEL_PROB 0.0 # Deletion rate (per divide)
DIVIDE_UNIFORM_PROB 0.0 # Uniform mutation probability (per divide)
DIVIDE_SLIP_PROB 0.0 # Slip rate (per divide)
INJECT_INS_PROB 0.0 # Insertion rate (per site, applied on inject)
INJECT_DEL_PROB 0.0 # Deletion rate (per site, applied on inject)
INJECT_MUT_PROB 0.0 # Mutation rate (per site, applied on inject)
SLIP_FILL_MODE 0
PARENT_MUT_PROB 0.0 # Per-site, in parent, on divide
SPECIAL_MUT_LINE -1 # If this is >= 0, ONLY this line is mutated
META_COPY_MUT 0.0 # Prob. of copy mutation rate changing (per gen)
META_STD_DEV 0.0 # Standard deviation of meta mutation size.
MUT_RATE_SOURCE 1 # 1 = Mutation rates determined by environment.
MIGRATION_RATE 0.0 # Uniform probability of offspring migrating to a new deme.

2. Environmental configuration file (environment.cfg)

REACTION NOT not process:value=1:type=pow requisite:max_count=1
REACTION NAND nand process:value=1:type=pow requisite:max_count=1
REACTION AND and process:value=2:type=pow requisite:max_count=1
REACTION ORN orn process:value=2:type=pow requisite:max_count=1
REACTION OR or process:value=3:type=pow requisite:max_count=1
REACTION ANDN andn process:value=3:type=pow requisite:max_count=1
REACTION NOR nor process:value=4:type=pow requisite:max_count=1
REACTION XOR xor process:value=4:type=pow requisite:max_count=1
3. Example events file (events.cfg) from Alternating_10000 (switch environment every 10000 updates)

```plaintext
# Print all of the standard data files...
u 0:100:end PrintAverageData   # Save info about they average genotypes
u 0:100:end PrintDominantData  # Save info about most abundant genotypes
u 0:100:end PrintStatsData     # Collect statistics about entire pop.
u 0:100:end PrintCountData     # Save organisms counts for each task.
u 0:100:end PrintTasksData     # Save organisms counts for each task.
u 0:100:end PrintTimeData      # Track time conversion (generations, etc.)
u 0:100:end PrintResourceData  # Track resource abundance.
u 0:100:end PrintTasksExeData  # number of times tasks executed
u 0:100:end PrintReactionData
u 0:100:end PrintReactionRewardData

# Setup the exit time and full population data collection.
u 5000:5000:end SavePopulation  # Save current state of population.
u 5000:5000:end SaveHistoricPopulation # Save ancestors of current population.
u 100000 exit # exit
u 100000 SaveClone clone1

##alternating_10000. Events generated on 2010-03-08 15:48:21
##Environment 1 on
u 0 SetReactionValue NOT 1
u 0 SetReactionValue NAND 0
u 0 SetReactionValue AND 2
u 0 SetReactionValue ORN 0
u 0 SetReactionValue OR 3
u 0 SetReactionValue ANDN 0
u 0 SetReactionValue NOR 4
u 0 SetReactionValue XOR 0

##Environment 2 on
u 10000 SetReactionValue NAND 1
u 10000 SetReactionValue NOT 0
u 10000 SetReactionValue ORN 2
u 10000 SetReactionValue AND 0
u 10000 SetReactionValue ANDN 3
u 10000 SetReactionValue OR 0
u 10000 SetReactionValue XOR 4
u 10000 SetReactionValue NOR 0

##Environment 1 on
u 20000 SetReactionValue NOT 1
u 20000 SetReactionValue NAND 0
u 20000 SetReactionValue AND 2
u 20000 SetReactionValue ORN 0
```

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u 20000 SetReactionValue OR 3
u 20000 SetReactionValue ANDN 0
u 20000 SetReactionValue NOR 4
u 20000 SetReactionValue XOR 0
## Environment 2 on
u 30000 SetReactionValue NAND 1
u 30000 SetReactionValue NOT 0
u 30000 SetReactionValue ORN 2
u 30000 SetReactionValue AND 0
u 30000 SetReactionValue ANDN 3
u 30000 SetReactionValue OR 0
u 30000 SetReactionValue XOR 4
u 30000 SetReactionValue NOR 0
## etc. --->>> update 100000

4. Genome of the ancestor organism used to seed all runs

```
h-alloc       # Allocate space for child
h-search      # Locate the end of the organism
nop-C         # No-operation
nop-A         #
mov-head      # Place write-head at beginning of offspring.
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
nop-C         # nop-C   # nop-C   # nop-C   #
```

APPENDIX C. CONFIGURATION FILES FOR CHAPTER ??
nep-C  #
nep-C  
hs-search  # Mark the beginning of the copy loop
h-copy  # Do the copy
if-label  # If we’re done copying....
nep-C  
nep-A  
h-divide  #  ...divide!
mov-head  # Otherwise, loop back to the beginning of the copy loop.
nep-A  # End label.
nep-B  

5. Avida analysis file used to generate genotype-phenotype maps (analysis.cfg).

FORRANGE  j  5000  100000  5000
  LOAD  data/detail-$j.pop
  FIND_GENOTYPE  RENAME $j
  MAP_TASKS  analysis task.0 task.1 task.2 task.3 //
  task.4 task.5 task.6 task.7
  MAP_MUTATIONS  analysis
  PURGE_BATCH  END
LOAD  data/detail-100000.pop
recaclulate  DETAIL  function.dat  id  length  merit  gest_time //
  fitness  num_cpus  viable  task.0  task.1  task.2 //
  task.3  task.4  task.5  task.6  task.7
PURGE_BATCH
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