

# **Rapid *in situ* Adaptation to Changed Precipitation in Annual Plant Species**

## **Dissertation**

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## Contributions

I declare that I carried out the experiments described in the three chapters by myself. Throughout the period of my thesis I was supervised by both, Prof. Dr. Tielbörger and Prof. Dr. Kigel, who advised me in practical problems and helped discussing the results. All three chapters were written by me, but proofread by both of my supervisors. All statistical analyses were carried out by myself. Additionally to the data collected by myself, I incorporated data of the onset of flowering and plant size of six species which were measured in the greenhouse season 2011/12 by Nicola Lechner within the scope of her bachelor thesis.

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“Life improves the capacity of the environment to sustain life.”

P. Kynes (F. Herbert 1965)

## **Abstract**

Global warming is expected to cause a severe decrease in precipitation over the Mediterranean Basin that might exceed the tolerance range of annual plant species in this region and cause local extinctions. Therefore, the ability of species to adapt to high rates of environmental changes is important for the persistence of populations under stress. We studied the ability of a set of common annual species to adapt within few generations to changes in precipitation *in situ*.

In a unique long-term rain manipulation experiment which was conducted in a semi-arid and a Mediterranean site in Israel, we simulated rainfall variation that corresponded to predictions by climate change models for the East Mediterranean Basin. The treatments included 30% reduction of rain by shelters, an unaltered control, and a 30% increase of rain by irrigation, without changing the frequency and seasonal distribution of rain events. We evaluated the direct impact of precipitation changes on the phenotypic variation of life-history traits and survival after eight years of rain manipulations in the field. To distinguish between plastic response to the immediate environment and adaptation due to a shift in genotypic composition, we raised the offspring of the individuals measured in the field over two generations under similar greenhouse conditions. We measured life-history traits such as phenology, plant size, above-ground biomass and reproduction related traits since they are an important indication for the future persistence of a population. We used the trends in trait variation between sites as a proxy to predict adaptive responses of life-history traits to the rain manipulation treatments within sites. We expected that a reduction in precipitation will lead to earlier flowering associated with smaller plant size, and reduced above-ground biomass but higher reproductive allocation at the end of the growth cycle.

Our results showed that survival in the field was barely affected by the rain manipulation treatments. Opposite to our expectations, the wet-treatment in the Mediterranean site had a greater negative impact on survival than the dry-treatment in the semi-arid site. Also, the observed trait responses to the treatments within sites did not resemble the trends between sites and could be attributed to stress tolerance strategies.

In contrast to our findings in the field, in the second greenhouse generation we found inherited trait shifts in five out of eight studied species. Decreasing water availability in the field caused adaptive shifts in the onset of flowering, a reduction in plant height, each in one species and an increase of reproductive allocation in two species. Furthermore, we found inherited shifts in growth related traits that indicate an adaptation to higher plant density caused by the wet-treatment. Our results also showed that the selection on life-history traits which was caused by increased drought was greater in relation to additional irrigation in the semi-arid site and vice versa in the Mediterranean site.

Among the two species that were examined under a greenhouse irrigation gradient, *Biscutella didyma* demonstrated that higher reproductive allocation and smaller diaspores contributed to higher fitness under drought. Although *Biscutella* plants originating from the semi-arid site had a significantly higher survival rate in relation to plants of Mediterranean origin under drought in the greenhouse gradient, survival was not affected by the rain manipulation history. Therefore, the observed adaptive shifts improved fitness but did not contribute survival in this species. We could also show that in *Hymenocarpus circinnatus* the range of plasticity was sufficient to cope with increased drought.

In this thesis we present evidence that *in situ* adaptation to precipitation changes after few generations is possible. We were able to show for the first time that these adaptive processes are a rather common response to precipitation change, by subjecting species to rain manipulations within their communities. Further, we could demonstrate that the range of plasticity covered the range of environmental changes. Our experiment provides a tool to distinguish between genetic response, plastic response as a successful strategy, and plastic response due to a lack of the ability to adapt. It is possible to assess the ability of species to persist under environmental change and therefore, to evaluate the actual risk of biodiversity loss under climate change.

## Contents

Declaration of working contribution	1
Acknowledgements	2
Abstract	4
Synopsis	
Introduction	7
Thesis objectives and organisation	11
Key results	12
Discussion	14
Chapter 1	20
Chapter 2	48
Chapter 3	72
CV	98



## Synopsis

### Introduction

Climate change is considered to be a threat to species (Pitelka 1997, Neilson et al. 2005, Araújo and Rahbek 2006) because climatic conditions change in a rapid pace (IPCC 2007, 2012, Loarie et al. 2009) resulting in a strong impact on species, communities and their biotic interactions (Walther et al. 2002, Defila and Clot 2005, Menzel et al. 2006, Parmesan 2006, Cleland et al. 2007, Jenouvrier and Visser 2011). Plant species are limited in their options to migrate to more favourable climatic conditions due to geological and anthropogenic barriers, limitations of dispersal abilities and already occupied habitats (Pitelka 1997, Neilson et al. 2005, Siewert and Tielbörger 2010, Corlett and Westcott 2013). As an alternative to migration, physiological adjustment to the changed conditions *in situ* can occur either by plastic response or adaptation which involves genetic changes (Davis et al. 2005, Gienapp et al. 2008). If the environmental changes are severe enough to cause differences in survival and fitness that are associated with different phenotypes and consequently genotypes, the result will be a shift in the genotypic composition favouring the better adapted genotypes (Fox 1990, Franke et al. 2006, Rathcke and Lacey 2007). Regarding the rate of global warming, the question is whether the rate of evolution can keep pace with the rapidly changing environment (Bradshaw and McNeilly 1991, Burger and Lynch 1995, Etterson and Shaw 2001, Berteaux et al. 2004, Rice and Emery 2008). Therefore, this thesis aims to answer the question: Are annual plants able to genetically adapt within few generations to changes in precipitation or is the response plastic?

Recent studies that dealt with the velocity of migration of plant populations were not able to answer the question whether the observed time lag of migration in response to climate change is due to a lack in ability or a lack in necessity to migrate (see review by Corlett and Westcott 2013). So far only few studies addressed *in situ* adaptation as an alternative option to migration and presented controversial findings, indicating that adaptive response is species-specific and depends on many additional factors (Billington and Pelham 1991, Etterson and Shaw 2001, Franks et al. 2007, Bradshaw and Holzapfel 2008). The rate of adaptation can be slowed down by antagonistic

genetic correlations between traits (Etterson and Shaw 2001), and by insufficient genetic variation in too small populations (Reznick and Ghalambor 2001, Jump et al. 2009). Other factors like biotic interactions and plasticity of species directly affect survival and fitness, and thus influence genetic variation, though the influence on evolvability is ambiguous. Plasticity contributes to survival which increases the chances of a population to persist (Lande 2009, Chevin et al. 2010) and can simultaneously decrease the selection pressure which in turn might slow the actual rate of adaptation (de Jong 2005, West-Eberhard 2005).

A precondition for selection to occur in the first place is genetic variation in the respective trait, as well as a difference in fitness and/or survival, caused by the changed phenotype (Falconer 1981, Lande 1982, Lande and Arnold 1983, Mousseau and Roff 1987). Therefore, the study of fitness-related traits was the focus of this thesis. Promising trait candidates for adaptation to a decreasing precipitation include phenology, growth and reproduction related traits. The response of such traits to drought is an important indication for the future persistence of a species, because growth is a limit to the development and the developmental timing is tightly connected with reproductive success. There is evidence that with a shortening of the growing season the development to reproductive phase accelerates (Aronson et al. 1992, 1993, Volis et al. 2002, Petrů et al. 2006, Franks et al. 2007, Kigel et al. 2011) and the reproductive allocation increases (Aronson et al. 1990, Petrů et al. 2006). Greenhouse experiments for multiple species have shown that these characteristics can be adaptive (Aronson et al. 1992, Peñuelas et al. 2004, Petrů et al. 2006, Volis 2006, Liancourt and Tielbörger 2009, Kigel et al. 2011).

Our study systems are located in the East Mediterranean Basin for which climatic projections predict a continued decrease in precipitation for the next decades (Cruz et al. 2007, Gao and Giorgi 2008). The unpredictability of rain events is a major factor determining these ecosystems, in which annuals are the dominating life-form (Zohary 1937). Under these conditions of great environmental variation species evolved a wide range of plasticity (Bradshaw 1965, Aronson et al. 1990). However, the range of plasticity might be insufficient to maintain population persistence in the case of sudden and high selection intensity (Relyea 2002, Valladares et al. 2007). Consequently,

these ecosystems might experience a loss of biodiversity because of climate change (Sala 2000).

An artificial selection experiment was set *in situ* to compare the phenotypic response to the actual environmental change and to test whether these responses were genetic or plastic. To simulate the projected climate change over the Mediterranean Basin, precipitation was manipulated over a period of eight consecutive years in a Mediterranean and a semi-arid field site in Israel. The trends in phenotypic variation between the two field sites were used as a proxy to predict the phenotypic shifts caused by the rain manipulation treatments within sites. The rain manipulation in the field sites included dry-treatments in which rain-out shelters kept off approximately 30% of the precipitation, unaltered control-treatments, and wet-treatments in which additional irrigation increased the precipitation after each rain event by ca. 30%. The frequency, and therefore the predictability of rain events and length of the growing season, remained unaltered. We manipulated precipitation while leaving the studied species within their natural community, therefore evolutionary processes include also the effects of changed biotic interactions (Sternberg et al. 2011). This approach is a realistic simulation of climate change that allows to gain reliable information regarding the evolutionary processes and the impact of changed precipitation on these populations.

Although selection acts directly on phenotypes, regardless of their genetic background, the adaptive response depends on the heritability of the trait characteristics to the offspring generation (Lande and Arnold 1983). In order to disentangle plastic from genetic responses, a three generation experiment was used. Subsequent to the *in situ* measurements in the season 2009-2010, the first greenhouse generation (G1) of the collected seeds was grown to produce seeds under the same optimum conditions in 2010-11. The purpose of this G1 was to equalise maternal effects due to which plastic responses to the field conditions can persist into the offspring generation (Stratton 1989, Rossiter 1996). In the second greenhouse generation (G2) in 2011-12, the remaining differences in the variation of life-history traits are assumed to be inherited. Additionally, a greenhouse experiment was conducted for two species in the G2 to compare plants from all rain manipulation treatments under an irrigation gradient to test whether the selection under the field

rain manipulation treatments resulted in differences in survival and fitness under drought in the greenhouse.

The main hypotheses were:

- After 8 years of rain manipulation *in situ* plants have adapted to the changes in water availability and the trends in phenotypic shifts due to the rain manipulation treatments resemble those observed along the climate gradient.
- Shifts in the phenotypic expression of life-history traits due to the rain manipulation are inherited and contribute to an enhanced fitness and survival under stress conditions.

## **Thesis objectives and organisation**

The aim of this thesis was to evaluate the ability of annual plant species to respond and to genetically adapt *in situ* to changes in precipitation within few generations in order to assess their potential to persist under climate change. The thesis is composed of three chapters, each prepared for publication in international journals, and with the following objectives:

- (I) To study the direct effects of *in situ* rainfall manipulations on the phenotypic variation of life-history traits and on survival in 16 common annual plant species in a semi-arid and a Mediterranean site. We expected the trends in response to the rain manipulations to resemble the trends between sites as an indication of an adaptive trait shift due to a change in the genotypic composition of the populations (Chapter 1).
- (II) To assess potential of the species to evolve and adapt to the changed precipitation in the field. We tested plants originating from the rain manipulation treatments in both field sites for inherited shifts in the life-history traits in the greenhouse under similar, well-watered conditions (Chapter 2).
- (III) To evaluate whether the adaptive shifts in life-history traits are an adaptation to the rain manipulation treatments. We tested plants originating from the rain manipulation treatments in both field sites for differences in survival and fitness in the greenhouse under different irrigation levels (Chapter 3).

## **Key results**

### **Chapter 1: Phenotypic response to simulated climate change in a long-term rain manipulation experiment - a multi-species study**

To evaluate the direct impact of water availability on survival and the phenotypic variation in life-history traits, we compared populations of common annual plants which were subjected to rain manipulation treatments for eight years in a semi-arid and a Mediterranean field site. The survival rates of the 16 studied species were barely affected by the rain manipulation treatments and overall survival was high. Opposite to our expectations, mortality increased in the treatment with the highest water availability (Mediterranean wet), but not under increased drought. Trends of phenotypic variation within site due to the rain manipulation treatments did not resemble trends between sites. Plants in the semi-arid site were more affected by drought compared to plants in the Mediterranean site. In the semi-arid site the dry-treatment had a strong negative effect on plant size and biomass production and caused a severe delay in the development, especially in species that flower early in the season. In contrast, species in the Mediterranean site benefitted from a reduced plant density in the dry-treatment and accumulated more biomass compared to the control. The observed response to the treatments within sites was most likely due to stress tolerance strategies, and between sites we found stress escape.

### **Chapter 2: Long-term *in situ* rain manipulation caused adaptive responses in life-history traits in a set of common annual plants**

We evaluated the inherited intraspecific variation in response to *in situ* rain manipulation treatments to assess the potential impact of changes in precipitation on life-history traits. Corresponding to our prediction, decreasing water availability in the field caused an acceleration in the onset of flowering, a reduction in plant height, each in one species, and an increase of reproductive allocation in three species. Our results also showed that the dry-treatment affected trait variation to a greater extent than the wet-treatment in the semi-arid site and vice versa in the Mediterranean site.

### **Chapter 3: Rain manipulation history affects phenotypic variation and fitness under different irrigation levels in annual plants**

We found evidence for *in situ* adaptation after eight years of rain manipulation. The rain manipulation treatments in the field led to adaptive shifts in life-history traits, altering fitness under drought in the greenhouse. A higher fitness in *Biscutella* plants originating from the semi-arid dry-treatments in relation to plants from the respective control-and wet-treatments was observed under all greenhouse irrigation levels. This improved fitness under drought was associated to an increase in reproductive allocation and a reduction in diaspore weight. Although *Biscutella* plants originating from the semi-arid site had a significantly higher survival rate in relation to plants of Mediterranean origin, survival under drought was not affected by the rain manipulation history.

## **Discussion**

The overall objective of this thesis was to test the ability of annual plant species to genetically adapt *in situ* to changes in precipitation within few generations in order to assess their potential to persist under environmental changes due to global warming.

The key finding of this thesis is the evidence that *in situ* adaptation in response to chronically decreased precipitation occurred after only few generations. A higher fitness under drought conditions was shown in *Biscutella didyma* as a result of selection under reduced water availability for genotypes that had a higher reproductive allocation and produced smaller diaspores (Chapter 3). These drought adapted genotypes were able to produce significantly more seeds, not just under resource limitation due to drought but also under well-irrigated conditions in the greenhouse. This adaptation is likely to be a compensation for fitness loss under decreasing amounts of rain.

Furthermore, we found inherited shifts in life-history traits that can result in survival and fitness advantages under drought in *Aegilops geniculata* and *Crupina crupinastrum* (Chapter 2). These inherited trait shifts in response to the dry-treatments in the field involved higher reproductive allocation and earlier onset of flowering in *Aegilops*, and shorter plant size at the onset of flowering in *Crupina*. In contrast to half of the other studied species in the semi-arid site, *Aegilops* had no delayed flowering time due to the dry-treatment (Chapter 1). We could show, that the ability to start flowering at an earlier stage of development was inherited, and therefore, it is a likely mechanism to avoid a delay in the field. In the greenhouse, *Aegilops* plants of dry-treatment origin also had a higher reproductive allocation and higher fitness compared to *Aegilops* plants originating from the control- and the wet-treatment, which is likely to be associated with an earlier onset of flowering that allowed a longer reproductive period under well-irrigated conditions (Chapter 2). In *Crupina*, the reduction in plant height at the onset of flowering was inherited. This finding implies that a reduced competition in the dry-treatments favoured genotypes that compensated the resource limitation under lower water availability by flowering at a smaller size. We suggest to test these shifts in phenology and plant size for a contribution to fitness under drought in these two species.



While our research aimed to study the effects of reduced precipitation, we also found clear signs that an increase of precipitation in the field resulted in adaptive shifts in life-history traits (Chapter 2). *Hymenocarpus* plants from the wet-treatment of the semi-arid site of origin produced significantly more biomass, which is likely an adaptation to increased competition under the additional irrigation. A similar response of a wider instead of taller growth was observed in *Aegilops* originating from the semi-arid wet-treatment which can as well be advantageous under conditions of high plant density. These findings indicate that competition is an important selective factor, and further tests that include this factor are needed to estimate to what extent these adaptive shifts can be a fitness advantage.

Our results also showed that the selection on life-history traits which was caused by increased drought, was greater in relation to additional irrigation in the semi-arid site and vice versa in the Mediterranean site. Interestingly, the additional irrigation in the wet-treatment in the Mediterranean site affected survival more than low water availability in the dry-treatment in the semi-arid site (Chapter 1). The biotic interactions are a stronger selective force in relation to the amount of precipitation in this environment (Petrů et al. 2006, Schiffers and Tielbörger 2006, Liancourt and Tielbörger 2009). This implies for more mesic areas that climate change projections need to be viewed in the light of possible interactions with biotic factors.

Plasticity is a successful strategy to cover the range of environmental changes, and allowed to survive until the reproductive stage in the studied species, but not necessarily to maintain fitness. The assumption that plasticity ensured survival was endorsed by the stable survival rates, under the dry-treatments in the field (Chapter 1). An additional support is the finding that survival rates under drought in the greenhouse irrigation gradient did not differ due to adaptation in relation to the rain manipulation treatment origin in the two examined species (Chapter 3). An indication that plasticity failed to prevent fitness loss could be shown in *Biscutella* that demonstrated how adaptive trait shifts contributed to fitness under drought in the greenhouse irrigation gradient. The finding of adaptive shifts in more than half of the studied species is a clear sign that plasticity alone can not maintain the population size under decreasing precipitation in these species.

In contrast to the cases described above, we could show for *Hymenocarpus* (Chapter 3) that plasticity maintained survival and fitness. The lack of inherited trait shifts in response to the rain manipulation treatments in the other studied species (Chapter 2) can imply that there is no need to adapt, but it can also indicate that such species are too slow or unable to genetically adapt. The studied species were found in a state, where the selection pressure by increased drought was still eased by plasticity, but selection already resulted in shifts in the genotypic composition. Species that can not genetically adapt to decreasing precipitation and compensate the fitness loss will face local extinction under future decrease in precipitation.

### Conclusion

We present evidence that eight years of precipitation change alone already led to adaptive responses that improve fitness under stress. Rapid *in situ* adaptation to precipitation changes after few generations is possible. We were able to distinguish between genetic and plastic responses and found inherited trait shifts in five out of eight studied species. Our experiment also allows to distinguish between plastic response as a successful strategy and plastic response due to a lack of the ability to adapt. Therefore, it provides a mean to assess the ability of species to persist under increasing drought, and estimate, until which degree of stress plasticity is a successful strategy and when increased mortality and fitness loss lead to either genetic adaptation or local extinction. By subjecting species to rain manipulations within their communities, we could show for the first time that these adaptive processes are a common response to precipitation change, and this approach can be an important tool to evaluate the actual risk of biodiversity loss under climate change.

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## Chapter 1: Phenotypic response to simulated climate change in a long-term rain manipulation experiment - a multi-species study

### Abstract

Species are threatened by the rapid change in precipitation due to global warming. To maintain fitness and prevent local extinction, populations need to genetically adapt to the changing conditions. We tested 16 annual species within their natural communities for phenotypic responses of life-history traits to long-term drought. Therefore, we simulated the predicted change in annual rainfall in a Mediterranean and a semi-arid field site ( $\pm 30\%$  of rain) and compared survival, phenology and growth related traits between sites and between treatments within sites. Under the assumption that, after eight years under rain manipulation, species have adapted to the changed precipitation, we hypothesised that the trends of trait response within sites resemble the trends between sites. The climatic difference between the sites resulted in a clearly distinct expression of life-history traits. With decreasing precipitation we found an accelerated phenology, and a reduced size and biomass production of plants. Survival rates were lower in the semi-arid site compared to the Mediterranean site. Within-site comparisons showed species-specific responses and contradicted our predictions. Opposite to our expectations, mortality increased in the treatment with the highest water availability (Mediterranean wet), but not under increased drought. Drought affected life-history traits the most in the semi-arid site. Here, the dry-treatment had a strong negative effect on plant size and biomass production, and resulted in a delayed phenology, especially in early flowering species. In contrast, species in the Mediterranean site benefitted from a reduced plant density in the dry-treatment and accumulated more biomass compared to the control. The phenotypic shifts between sites can be attributed to stress escape strategies, while differences in trait response within sites were expressions of plastic dehydration tolerance. In addition, we suggest that competition for light had a stronger impact on individual persistence than precipitation in the Mediterranean site. We found strong support that plasticity covered the here applied range of environmental changes in the studied set of common species. However, the impact of climate change will likely be larger if not just the amount but also the frequency of rain events decreases. Nevertheless, a decoupling of factors is a necessary and important contribution toward an understanding of the processes that affect and change these ecosystems.

## Introduction

Climate is a major factor, shaping the genetic architecture in natural populations (Linhart and Grant 1996, Joshi et al. 2001). Thus, the recent climate change is an important driver of selection processes (Walther et al. 2002, Hoffmann and Willi 2008). Furthermore, it can alter biotic interactions, de-stabilize communities and drive major biome shifts (Parmesan and Yohe 2003). The evidence for global warming and its effects on species distribution, abundance and phenotypes is numerous and increasing (Walther et al. 2002, Menzel et al. 2006, Parmesan 2006, Cleland et al. 2007, Jenouvrier and Visser 2011). Climate change is considered to be a threat to species, especially if the possibilities of migration are limited or slowed down by dispersal capacities, geographical barriers and already occupied habitats (Pitelka 1997, Thompson 1998, Neilson et al. 2005, Corlett and Westcott 2013). Plant populations have been shown to migrate only partly or not at all (Corlett and Westcott 2013). Additionally, the environmental tolerances of plant species might evolve too slowly (Bradshaw and McNeilly 1991, Etterson and Shaw 2001, Berteaux et al. 2004).

Predictions of the climatic changes in a region and how plants there already cope with recent climatic conditions allow us to draw conclusions about the future persistence of these populations. Climatic projections for the next decades predict a continued decrease in precipitation over the Mediterranean Basin (Cruz et al. 2007), with larger changes in the east-Mediterranean region (Gao and Giorgi 2008). This region is already experiencing increasing winter dryness for the last decades (Mariotti and Struglia 2002). Especially where water is limited, the increasing intensity and number of drought events is expected to have a strong negative impact on natural populations (Ackerly et al. 2000, Knapp et al. 2002, Chaves et al. 2003). In arid ecosystems water availability is a major constraint for species distribution and persistence, and therefore plant communities are dominated by ephemeral life forms (Noy-Meir 1973). Plants can withstand drought by combining several strategies, among them drought escape, dehydration avoidance and dehydration tolerance (Levitt 1972, Blum 2005). The distinction between plastic and adaptive responses is an important tool in the evaluation of a populations future persistence under changing environmental condition. Plasticity allows plants to tolerate drought periods at the cost of postponed reproduction and reduced growth rate, but increased survival rates, and is therefore particularly important

in unpredictable environments (Schlichting 1986). When resource limitation becomes a chronic condition which does not allow a compensatory growth, the reliance on plasticity to survive can result in fitness loss and decreasing population size as the threshold of the plastic range is approached and crossed (DeWitt 1998, Chevin et al. 2010). Therefore, it is necessary to shift reaction norms according to the new environment by genetic changes in order to maintain fitness under such circumstances. In this light, studies assessing the capacity of *in situ* adaptation are of increasing importance. Here, we tested if annual species are able to cope with decreasing precipitation *in situ* and whether the responses to short-term changes in precipitation resemble adaptations to long-term differences in precipitation.

As a consequence of a distribution over a wide range of climatic conditions species can vary considerably in the phenotypic expression of life-history traits (Bradshaw 1965, Schlichting 1986, Rathcke and Lacey 2007, Bolnick et al. 2011). These fitness related traits are widely used to compare intraspecific variation between differing climate regions, and to assess the adaptive potential of species (Aronson et al. 1990, 1992, 1993, Sultan 2000, Etterson and Shaw 2001, Volis et al. 2002a). They also allow us to evaluate the direct effects of changes in precipitation regime on natural populations. Therefore, we studied life-history traits such as phenology, vegetative growth, and reproductive allocation. Short-lived annuals in more arid environments flower and complete their life-cycle earlier compared to conspecific populations in more mesic environments, which enables them to escape the risk of death due to drought events toward the end of the growing season (Fox 1990a, Aronson et al. 1992, Peñuelas et al. 2004, Petru et al. 2006, Volis 2006, Liancourt and Tielbörger 2009, Kigel et al. 2011). Drought escape by shortened life-cycle and developmental plasticity is a strategy that is selected for when the growing season is shortening or the predictability of rainfall events decreases (Heschel and Riginos 2005). A reduction in water availability over the whole growing season might select for physiological adjustments to enhance dehydration tolerance rather than escape (Stanton et al. 2000, Chaves et al. 2003, Chaves and Oliveira 2004, Franke et al. 2006, Franks 2011).

In annual plants fitness-related life-history traits are well studied and allow us to make specific predictions about their response to changing climatic conditions. There is evidence that with the shortening of the growing season the development to



reproductive phase accelerates (Aronson et al. 1992, 1993, Volis et al. 2002a, Petrů et al. 2006, Franks et al. 2007, Kigel et al. 2011). Selection for smaller plant size might, directly or indirectly, also result in early flowering and seed set under the assumption that competition for light decreases with decreasing water availability (Petrů et al. 2006, Schiffers and Tielbörger 2006, Liancourt and Tielbörger 2009). If selection pressure is strong enough, changes in the genotypic composition due to selection for better adapted genotypes can result in shifts of the mean phenotype of a population (Fox 1990b, Franke et al. 2006, Rathcke and Lacey 2007). Greenhouse experiments for multiple species have shown that the phenotypic shifts observed between sites can be adaptive (Aronson et al. 1992, Peñuelas et al. 2004, Petrů et al. 2006, Volis 2006, Liancourt and Tielbörger 2009, Kigel et al. 2011). Additionally, the short life cycle of annuals and their tendency to autogamy (Aarssen 2000, Snell and Aarssen 2005) increases the probability of a shift in allele frequencies because individuals that can not successfully reproduce are excluded from the population (Hoffmann and Willi 2008).

For the purpose of testing the effects of chronically altered precipitation on the phenotypic response of a set of abundant annual species, a rain manipulation experiment was set up in a Mediterranean and a semi-arid site in Israel. Climate change was simulated *in situ* by reducing or increasing rainfall events, without changing the frequency of rain events, nor the duration of the rainy season. Thus, the complex and dynamic ecological interactions were maintained as a whole, and only the amount of rain was manipulated in each site (Sternberg et al. 2011). The chosen range of rain manipulation corresponds to the predictions for the Mediterranean Basin (Cruz et al. 2007). We consider the trends in the conspecific phenotypical differences which were previously observed across aridity gradients as a proxy for an adaptive response to climate change and test whether these trends can be found within sites.

We assume that the trait responses after eight years of exposure to the rain manipulation treatments are adaptive. Therefore, we predict a resemblance of the trends within climate stations due to the rain manipulation treatments with the trends observed between stations. As a result of reduced precipitation we expect:

- Shorter plants and reduced biomass
- Earlier onset of flowering and seed maturation
- The effect of decreased precipitation is greater in the semi-arid site

## Methods

### Study sites and rain manipulations

Our study was conducted in a Mediterranean and a semi-arid site which are located along a steep North-South climate gradient, in the transition zone between the mesic Mediterranean and the desert regions of Israel (Holzapfel et al. 2006, Metz et al. 2010, Sternberg et al. 2011). Both sites are situated on the same limestone bedrock, at a similar altitude, and on south exposed slopes (Petru et al. 2006). The soil in the semi-arid site is a stony Loess soil and Terra Rossa in the Mediterranean site. The long-term rainfall averages are 300 mm for the semi-arid and 540 mm for the Mediterranean site (Sternberg et al. 2011).

The experimental set-up to simulate the expected changes in precipitation was established in 2001, and rainfall manipulations were initiated in the 2002/3 growth season. The measurements for this study were taken in the 2009/10 growth season. The treatments were: 1) control - exposed to natural rainfall, 2) dry-treatment - simulating the long-term predicted decrease in the amount of annual precipitation, using rain-out shelters reducing natural rainfall by 30%, and 3) a contrasting wet-treatment, with additional 30% irrigation after each rain event. The frequency of rain events remained unaltered. Each treatment was replicated by five plots (10 × 25 m<sup>2</sup>) in both sites.

The germination inducing rain event occurred at the end of October 2009 in the Mediterranean site and 2 months later in the semi-arid site. The total precipitation of the study season was with 246 mm in the semi-arid site and 530 mm in the Mediterranean site slightly lower than the long-term average. The rainy season ended in late March 2010 in both sites, spanning 3 and 5 months in the semi-arid sites and in the Mediterranean, respectively.

### Study species

A total of 16 winter annual species were selected in both sites (Tab 1). Criteria for selection were: high seedling abundance, ability to identify species at the seedling stage, different taxonomic and functional groups and ideally occurrence at both sites.

Yet, due to the large differences in climate between sites, the latter criteria could be realized only for four species.

Tab. 1) Study species. (Zohary 1966, 1972, Feinbrun-Dothan and Zohary 1978, 1986)

The species' occurrence in the two sites are indicated by "+", (+) means that not all of the traits could be measured.

Species	Family	Occurrence in the study sites	
		Semi-arid	Medit.
<i>Aegilops geniculata</i> ROTH	<i>Poaceae</i>	+	
<i>Anagallis arvensis</i> L.	<i>Primulaceae</i>	+	(+)
<i>Atractylis cancellata</i> L.	<i>Asteraceae</i>	+	
<i>Avena sterilis</i> L.	<i>Poaceae</i>		+
<i>Biscutella didyma</i> L.	<i>Brassicaceae</i>	+	+
<i>Crupina crupinastrum</i> (MORIS) VIS.	<i>Asteraceae</i>		+
<i>Helianthemum salicifolium</i> (L.) MILL.	<i>Cistaceae</i>	+	+
<i>Hymenocarpus circinnatus</i> (L.) SAVI	<i>Fabaceae</i>	+	+
<i>Linum corymbulosum</i> DESF.	<i>Linaceae</i>		+
<i>Linum strictum</i> PALL.	<i>Linaceae</i>	+	
<i>Onobrychis crista-gallis</i> (L.) LAM.	<i>Fabaceae</i>	+	
<i>Plantago afra</i> L.	<i>Plantaginaeae</i>		+
<i>Scorpiurus muricatus</i> L.	<i>Fabaceae</i>		+
<i>Torilis tenella</i> (DELILE) RCHB.	<i>Apiaceae</i>		+
<i>Trifolium stellatum</i> L.	<i>Fabaceae</i>		+
<i>Urospermum picroides</i> (L.) SCOP. EX F.W.SCHMIDT	<i>Asteraceae</i>		+

### Sampling

A total of 50 seedlings per species were randomly chosen in open patches in each of the three treatments, in both sites. Shortly after the first effective rain event seedlings were labelled (Nov - Dec 2009 in the Mediterranean site, and Jan 2010 the semi-arid site). We started with a total of 2760 individuals. Of these 2348 survived until flowering and 1888 until seed-set. Phenological, morphological and reproduction related traits were measured until the end of the growth season in the beginning of May 2010.

### Trait measurements

The onset of flowering was recorded as the time between germination inducing event until the first flower at anthesis. In the case of grasses, anther emergence indicated the begin of anthesis. Diaspores and fruits were defined as fully mature, when dry and fully grown, and easily dehisce or detach from the plant. Time to maturation was recorded as the day to first mature diaspore or fruit by using the first effective rain event as a reference point. Phenology traits were measured every third day. Plant height (length if prostrate) was measured from the ground to the tip of the plant at first flower.

Labelled plants were individually harvested in paper bags after seed dispersal. Plants were oven dried at 70°C and weighed. Plant biomass at this late stage was not complete due to losses during the growth season, but we assume that relative losses were similar among individuals of the same species. We refer to vegetative biomass as all above-ground biomass except for biomass of diaspores or fruits, because these could not be completely collected for most species. Plant fitness was assessed from the number of diaspores produced per individual. Diaspore production was statistically analysed only in species that had not dispersed their seeds before harvesting.

### Statistics

The statistical software package Jmp7.1 (SAS) was used to carry out all analyses. The treatment effect on survival was tested per site by using a generalised linear model for Poisson distributed data and a log-link function. The analysis was based on the number of individuals that did not survive until seeding time. For within-site evaluations of treatment and species effects on trait variation, we used generalised linear models with Poisson error distribution and log-link function for phenology and with exponential error distribution and reciprocal link function for all other traits. These models were run for each site separately. Between-site comparisons of survival as well as life-history traits were carried out only for *Biscutella*, *Helianthemum* and *Hymenocarpos*, which occurred in both sites, using a full factorial generalised linear model with species, site and treatment as fixed factors. For these three species a two-sided t-test was used to compare trait means between the respective control populations in each species. For all other species, treatment effects within species and sites were analysed using generalised linear models with a posteriori pairwise likelihood ratio test using Holm's sequential Bonferroni test (Rice 1989) to correct the alpha. The significance levels used in all analyses are 0.05 (\*), 0.01 (\*\*), and 0.001 (\*\*\*).

## Results

### Survival analysis

We compared survival rates until the time of seed-set within each site in all studied species to determine the effect of rain manipulation treatments on mortality. The treatments had over all only a marginally significant effect on survival (Tab. 2a). Most notably, separate comparisons within sites of each the dry- and the wet-treatment to the control-treatment revealed a significantly reduced survival rate in the Mediterranean wet-treatment ( $p=0.0216$ ,  $\text{Chi}^2=7.673$ ). Within species, a negative effect was found for both *Biscutella* and *Avena* (Tab. 2c). *Linum corymbulosum* had even an increased survival rate in the dry-treatment. While the average survival rate in the semi-arid wet-treatment was highest, it was lowest in the Mediterranean wet-treatment.

We tested the effect of site on survival rates in the species which occurred in both sites (Tab. 2b), and found that the survival rate in the semi-arid site was lower compared to the Mediterranean, though the difference was only marginally significant. The trends of survival under the rain manipulation treatments differed between the two sites.

Tab. 2a) Treatment effect on survival over all species per site.

Source of variation	Semi-arid			Mediterranean		
	Chi <sup>2</sup>	p	d.f.	Chi <sup>2</sup>	p	d.f.
Species	42.738	<b>&lt;.0001</b>	6	59.702	<b>&lt;.0001</b>	10
Treatment	5.333	0.0695	2	4.866	0.0878	2
Treatment*Species	12.979	0.3706	12	10.113	0.9661	20

Tab. 2b) Effect of species, site and treatment on survival for species that occurred in both sites.

Survival Source of variation	Chi <sup>2</sup>	p	d.f.
Species	8.436	<b>0.0147</b>	2
Site	3.626	0.0569	1
Treatment	1.623	0.4442	2
Site*Species	2.124	0.3458	2
Treatm.*Species	4.436	0.3502	4
Treatm.*Site	6.935	<b>0.0312</b>	2
Treatm.*Site*Species	4.551	0.3365	4

Tab. 2c) Effect of rain manipulation treatments on the proportion of surviving individuals in the Mediterranean or the Semi-arid site, and in both sites (in gray), respectively.

<b>Survival ( in % )</b>									
<b>Semi-arid site</b>					<b>Mediterranean site</b>				
Species	Dry	Contr.	Wet	Av.	Species	Dry	Contr.	Wet	Av.
Bis	34.0	55.6	57.1	<b>48.9</b>	Bis	70.6	70.6	54.6*	<b>65.2</b>
Hel	63.6	60.0	87.1*	<b>70.3</b>	Hel	68.6	89.4	73.2	<b>77.0</b>
Hym	69.1	75.5	72.6	<b>72.4</b>	Hym	64.0	78.9	66.0	<b>69.6</b>
Aeg	63.8	73.8	72.0	<b>69.9</b>					
Ana	80.4	86.5	84.0	<b>83.4</b>					
Atr	52.1	43.6	42.6	<b>46.1</b>					
Li str	74.5	71.4	72.6	<b>72.8</b>					
Ono	62.3	73.2	73.6	<b>69.7</b>					
					Av	86.2	98.2	72.9**	<b>85.8</b>
					Cru	81.8	70.9	77.6	<b>76.8</b>
					Uro	56.0	54.0	50.0	<b>53.3</b>
					Li co	78.4*	63.2	60.9	<b>67.5</b>
					Sco	51.1	58.3	50.0	<b>53.1</b>
					Pla	92.0	88.0	88.2	<b>89.4</b>
					Tor	55.8	62.0	53.7	<b>57.2</b>
					Trif	78.6	82.8	65.8	<b>75.7</b>
<b>Average</b>	<b>62.5</b>	<b>67.5</b>	<b>70.2</b>		<b>Average</b>	<b>71.2</b>	<b>74.2</b>	<b>64.8</b>	

### Fitness

As a measure of fitness we compared the number of mature diaspores between rain manipulation treatments within sites of those species that had not dispersed before harvesting. The rain manipulation treatments affected the number of diaspores in both sites (Tab. 3), though the responses were species-specific (Fig. 1). The dry-treatment reduced diaspore production of *Linum str.* in the semi-arid site, and increased it in *Linum co.* and *Anagallis* in the Mediterranean site, compared to the controls. On the other hand, the wet-treatment led to a lower number of diaspores in *Atractylis* in the semi-arid site. Additional irrigation had no effect in the Mediterranean site on the diaspore production in the analysed species.

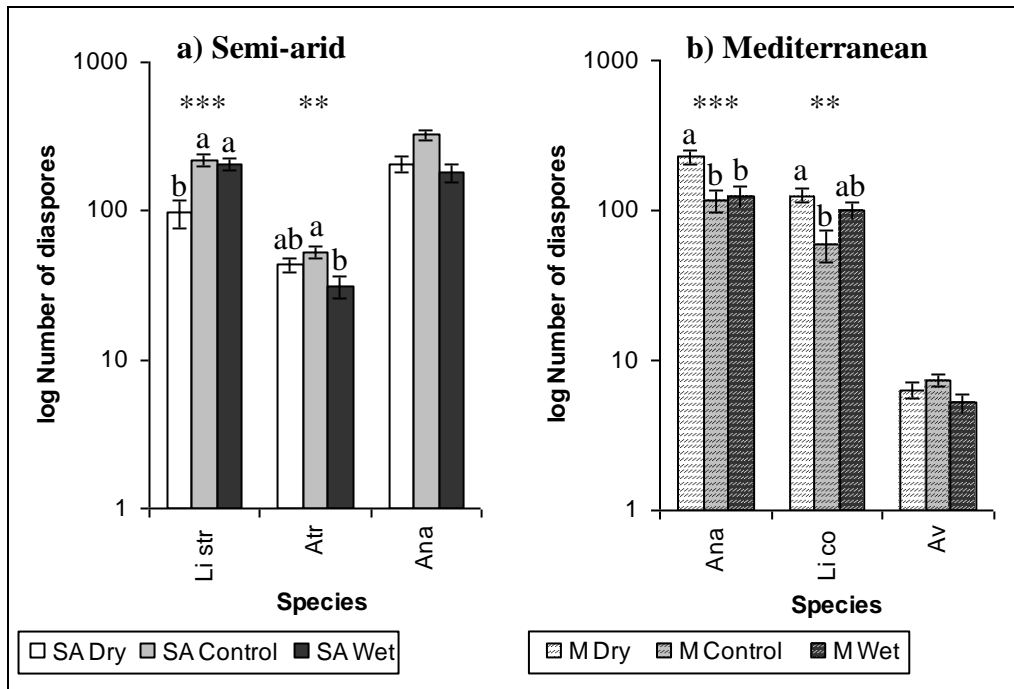


Fig. 1) Mean number of diaspores (+/- standard error) in the Semi-arid (a) and the Mediterranean (b) site. Different letters above the error bars indicate significantly different means.

### Phenology

We found species-specific significant differences between the rain manipulation treatments in time to flowering and time to seed maturation in both the semi-arid and the Mediterranean site (Tab. 3). In the semi-arid site the dry-treatment led to a significant delay in the onset of flowering in 4 of the 8 species examined. The early flowering species were more affected than later flowering species (Fig. 2a). In contrast, the wet-treatment had no significant effect on the flowering of both the early and late flowering species. Similar trends were observed for time to seed maturation (data not shown).

In the 11 species from the Mediterranean site trends were weaker and less consistent. Here, the dry-treatment led to advanced flowering in *Torilis*, *Hymenocarpus* and *Linum co.* (Fig. 2b). Seed maturation occurred in Mediterranean site at about the same time for most species, independently of their flowering time, except for *Biscutella* in which had an accelerated seed maturation in the dry-treatment (not shown).

Differences in the response of phenological traits between sites were analysed in the 3 species occurring in both sites (Tab. 4a). Flowering of *Biscutella*, *Helianthemum* and *Hymenocarpus* occurred earlier in the semi-arid site. The time to seed maturation

differed between stations and species. *Biscutella* and *Hymenocarpus* had an earlier seed maturation in the semi-arid site, while *Helianthemum* developed seeds faster in the Mediterranean site.

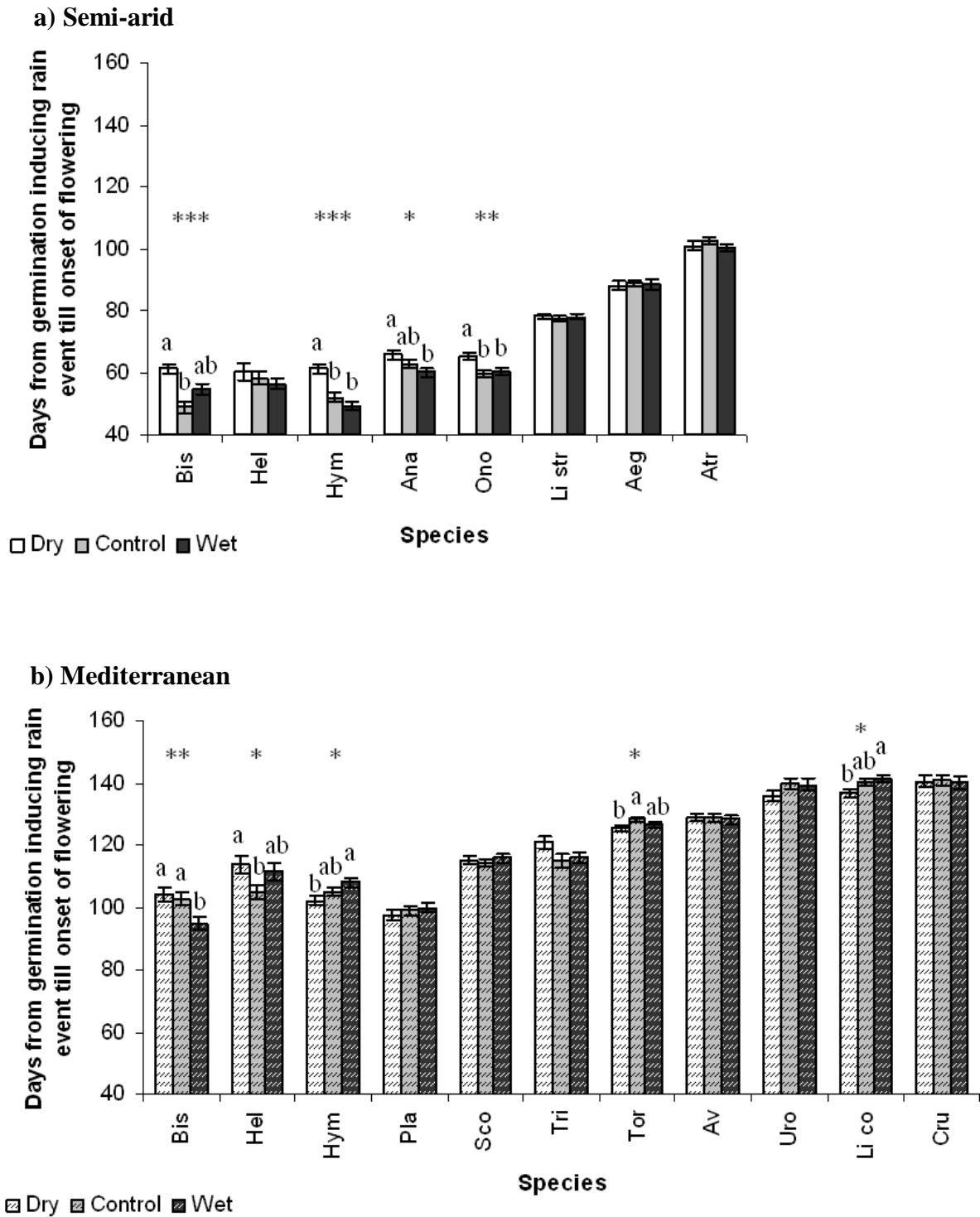


Fig. 2) Onset of flowering (+/- standard error) in the Semi-arid (a) and the Mediterranean (b) site.



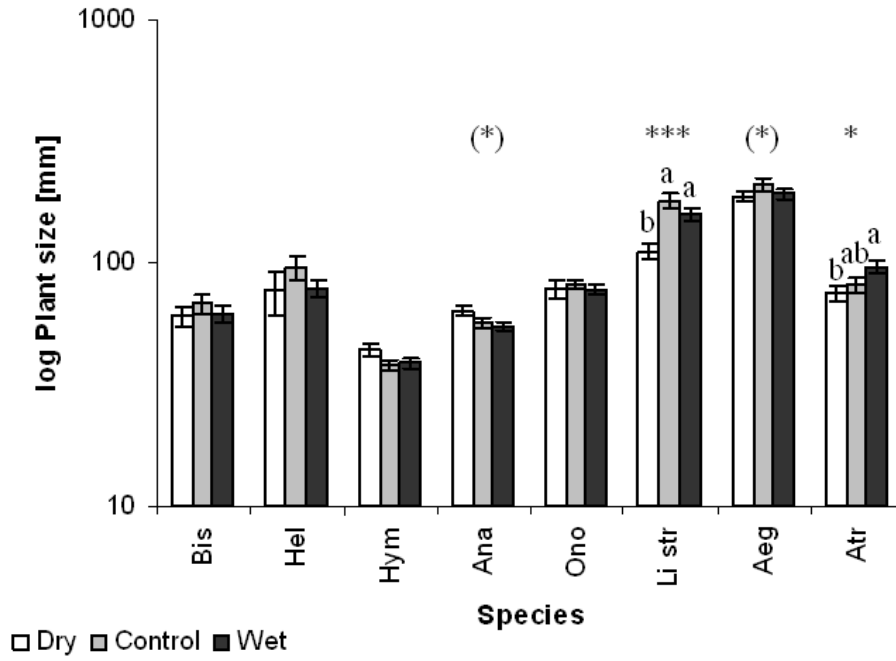
### Plant size and biomass

Response of plant size at the onset of flowering to the rain manipulation treatments was significant and species-specific in both sites. In the semi-arid site we found a significant reduction of plant size in the dry-treatment in two species (Fig. 3a). In the Mediterranean site, three species showed an increased size at the onset of flowering in the dry-treatment, while the wet-treatment did not result in an increased size (Fig. 3b).

The above-ground vegetative biomass was significantly affected by the treatments in both sites. In all but two (*Hymenocarpus* and *Anagallis*) species, biomass production in the semi-arid site was highest in the control-treatments (Fig. 4a). In the Mediterranean site, four species produced significantly more vegetative biomass in the dry-treatment compared to the control (Fig. 4b).

A comparison between sites showed that plants from populations in the Mediterranean site were significantly taller (at both the flowering and seed-set stages) than their counterparts in the semi-arid site. Biomass production differed significantly between stations, though the differences were species-specific. *Hymenocarpus* and *Biscutella* produced more biomass in the Mediterranean site, though this difference was significant only in *Hymenocarpus* ( $p < 0.0001$ ,  $\text{Chi}^2 = 26.181$ ).

## a) Semi-arid



## b) Mediterranean

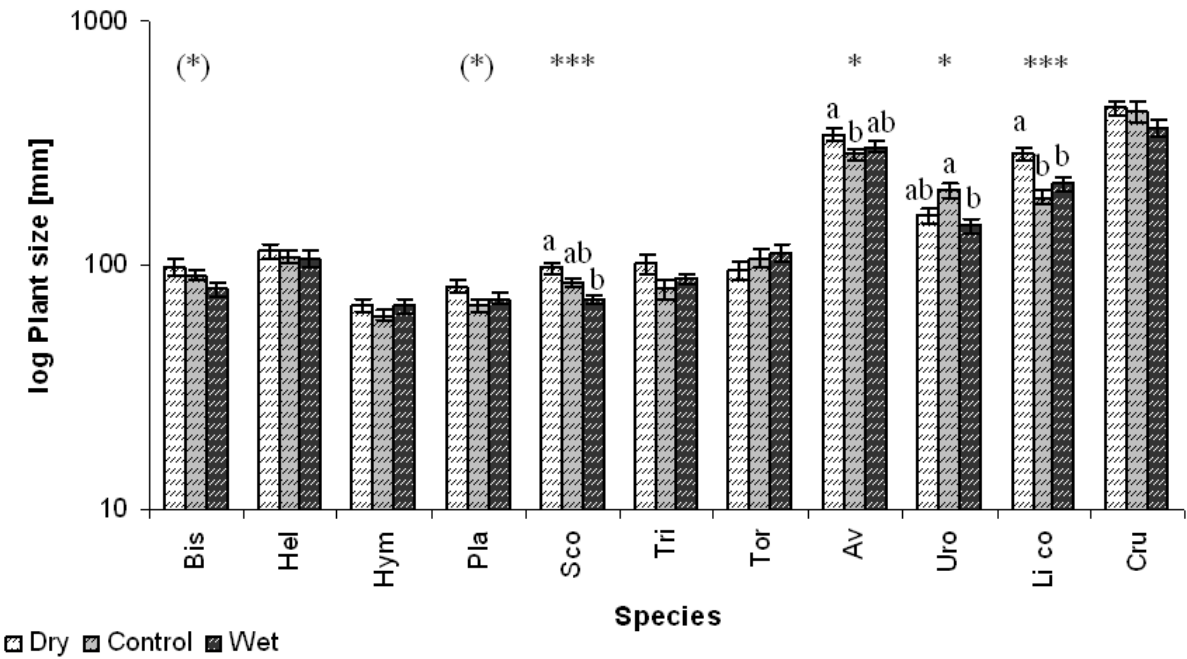
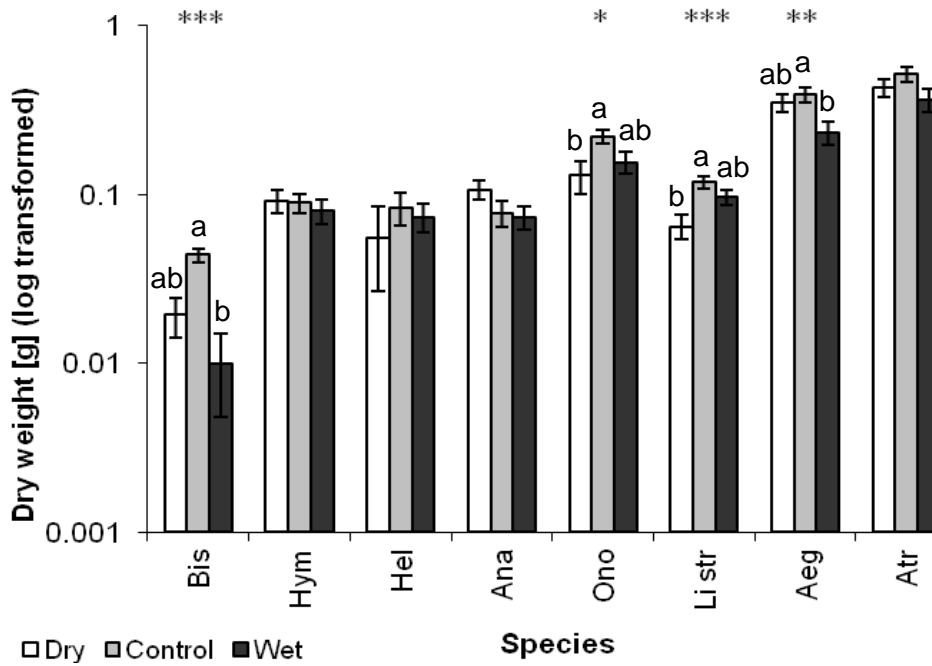


Fig. 3) Log-transformed plant size at the onset of flowering (+/- standard error) in the Semi-arid (a) and the Mediterranean (b) site.

## a) Semi-arid



## b) Mediterranean

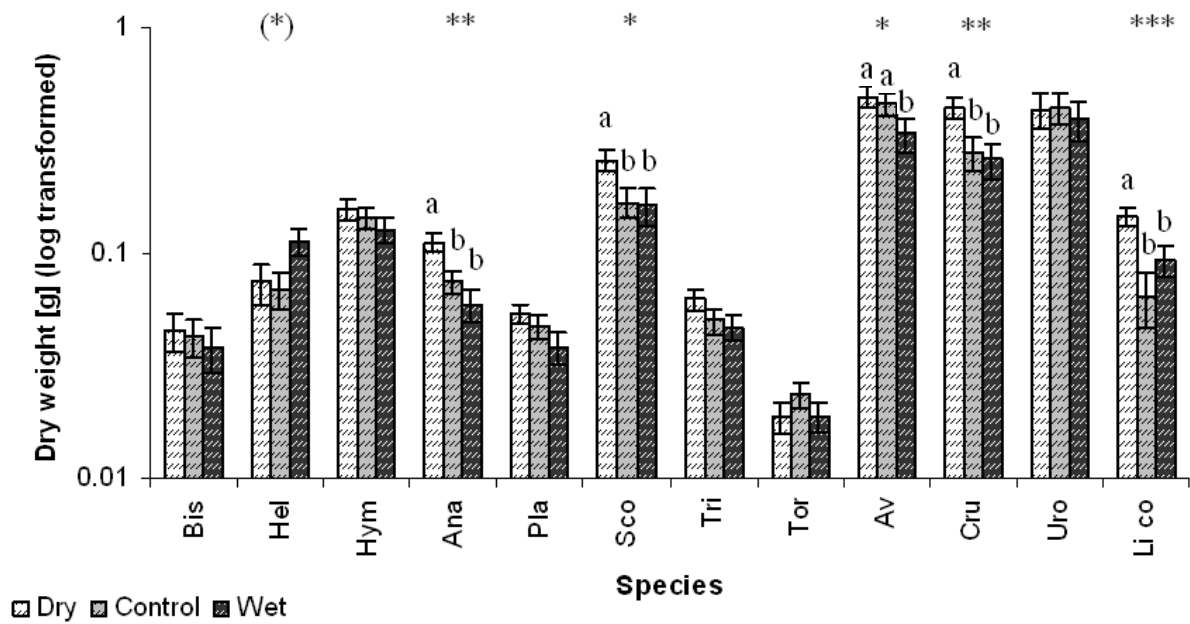


Fig. 4) Log-transformed above-ground vegetative biomass (+/- standard error) in the Semi-arid (a) and the Mediterranean (b) site.

Tab. 3) Results of generalised linear models for each trait including all species per site.

Trait	Source of variation	Semi-arid			Mediterranean		
		Chi <sup>2</sup>	p	d.f.	Chi <sup>2</sup>	p	d.f.
Number of diaspores	Species	134.03	<b>&lt;.0001</b>	2	423.36	<b>&lt;.0001</b>	2
	Treatment	20.09	<b>&lt;.0001</b>	2	14.67	<b>0.0007</b>	2
	Treatm.*Spec.	18.784	<b>0.0009</b>	4	12.54	<b>0.0138</b>	4
Time to onset of flowering	Species	1264.01	<b>&lt;.0001</b>	7	1306.24	<b>&lt;.0001</b>	10
	Treatment	42.71	<b>&lt;.0001</b>	2	0.57	0.7539	2
	Treatm.*Spec.	85.18	<b>&lt;.0001</b>	14	62.93	<b>&lt;.0001</b>	20
Time to onset of seed maturation	Species	526.89	<b>&lt;.0001</b>	4	2021.59	<b>&lt;.0001</b>	10
	Treatment	13.03	<b>0.0015</b>	2	0.46	0.7944	2
	Treatm.*Spec.	21.24	<b>0.0065</b>	8	47.82	<b>0.0005</b>	20
Plant size at onset of flowering	Species	977.81	<b>&lt;.0001</b>	7	1325.11	<b>&lt;.0001</b>	10
	Treatment	10.37	<b>0.0056</b>	2	15.69	<b>0.0004</b>	2
	Treatm.*Spec.	46.63	<b>&lt;.0001</b>	14	47.45	<b>0.0005</b>	20
Vegetative biomass	Species	454.48	<b>&lt;.0001</b>	7	1401.00	<b>&lt;.0001</b>	11
	Treatment	27.70	<b>&lt;.0001</b>	2	5.45	0.0655	2
	Treatm.*Spec.	34.74	<b>0.0016</b>	14	44.60	<b>0.0030</b>	22

Tab. 4) Results of full factorial generalised linear model for species occurring in both sites.

Tab. 4a)	Onset of flowering		d.f.	Seed maturation		d.f.
Source of variation	Chi <sup>2</sup>	p		Chi <sup>2</sup>	p	
Species	20.26	<b>&lt;.0001</b>	2	340.29	<b>&lt;.0001</b>	2
Site	1010.49	<b>&lt;.0001</b>	1	253.54	<b>&lt;.0001</b>	1
Treatment	43.20	<b>&lt;.0001</b>	2	14.50	<b>0.0007</b>	2
Site*Species	8.49	<b>0.0143</b>	2	904.21	<b>&lt;.0001</b>	2
Treatm.*Species	7.75	0.1012	4	12.94	<b>0.0116</b>	4
Treatm.*Site	23.22	<b>&lt;.0001</b>	2	14.43	<b>0.0007</b>	2
Treatm.*Site*Species	29.27	<b>&lt;.0001</b>	4	10.48	<b>0.0331</b>	4

Tab. 4b)	Height at flower			Vegetative biomass		
Source of variation	Chi <sup>2</sup>	p	d.f.	Chi <sup>2</sup>	p	d.f.
Species	167.44	<b>&lt;.0001</b>	2	134.95	<b>&lt;.0001</b>	3
Site	108.87	<b>&lt;.0001</b>	1	24.39	<b>&lt;.0001</b>	1
Treatment	3.63	0.163	2	16.02	<b>0.0003</b>	2
Site*Species	5.68	0.0585	2	26.33	<b>&lt;.0001</b>	3
Treatm.*Species	5.93	0.2043	4	31.31	<b>&lt;.0001</b>	6
Treatm.*Site	2.719	0.2568	2	17.17	<b>0.0002</b>	2
Treatm.*Site*Species	5.52	0.238	4	19.90	<b>0.0029</b>	6

## Discussion

Our results show that the observed pattern in phenotypical changes within sites does not resemble the trends found between sites. This implies that the plastic response to the immediate environment determined the phenotype rather than an adaptive response to long-term treatments.

We found in response to both rain manipulation treatments a trend toward a decreased fitness in the semi-arid site, while in the Mediterranean site fitness tended to increase compared to the respective controls. A significantly higher mortality was found only in the Mediterranean site, and surprisingly not due to the reduced water availability but under additional irrigation. This finding indicates the importance of other factors than drought, such as competition, that limit survival in this environment (Metz et al. 2010). An example is the increasing survival rate in response to lower water availability in *Linum corymbulosum* (Mediterranean site). It is likely, that this species benefitted from lower competition, which implies a higher dominance of this species under decreasing precipitation in the wake of climate change. A significantly higher mortality in the Mediterranean wet-treatments, is an indication that *Biscutella* and *Avena* are under selection and adaptive trait shifts in response to increase competition might be found.

As predicted, the trends found between sites were an earlier onset of flowering, and a reduction in plant size and biomass with decreasing precipitation. We assumed these trends to indicate an adaptive response to the decreasing water availability within sites. In the semi-arid site, the trend in onset of flowering contradicted the prediction. The finding of a delayed flowering was supported by the results of similar drought experiments (Fox 1990b, Blum 1996, Stanton et al. 2000, Peñuelas et al. 2004) and can be attributed to a severe growth limitation. In the Mediterranean site, on the other hand, two species showed an accelerated onset of flowering as a response to the dry-treatment. It is possible that this earlier flowering is an adaptive response in the course of a drought escape strategy (Geber and Dawson 1990, Stanton et al. 2000, McKay et al. 2003, Sherrard and Maherali 2006, Franks 2011). However, under a more mesic Mediterranean climate the vegetation is typically denser, and under higher levels of competition for light within the vegetation, the flowering time starts late compared to semi-arid sites (Petrú et al. 2006). In this context, the earlier onset of flowering as found

in the Mediterranean site in the dry-treatment is most likely a plastic response to a less competitive environment.

The response of growth differed greatly between the two sites. In the semi-arid site, the dry-treatment caused a reduction in plant size and vegetative biomass. Combined with the delayed flowering time, this reduction in growth can be interpreted as a plastic response to tolerate drought, and therefore contradicts our prediction. A lower plant productivity is a common result of reduced water availability (Grime et al. 2008, Miranda et al. 2009). Nevertheless, plant size and biomass production are known to show an inherited differentiation between the climatically differing sites along aridity gradients (Aronson et al. 1992, Volis et al. 2002b, Petrů et al. 2006, see also this thesis chapters 2 and 3). In the Mediterranean site, on the other hand, the dry-treatment caused plants to grow taller and produce more biomass compared to the control, whereas phenology was barely affected. This trend is again opposite to our expectations. We suggest for these findings in the Mediterranean site that the lower precipitation in the dry-treatment had a negative effect on the shrub cover over the years of manipulation. As a consequence, annual plants were able to benefit from the lower competition level.

The way life-history traits respond to drought gives an important indication for the future persistence of species, because growth is a limit to the development and the developmental timing is tightly connected with reproductive success. One of the most important features of vegetative growth traits is their wide range of plasticity, which enables plants to adjust according to their immediate environment (Bradshaw 1965, Schlichting 1986, Aronson et al. 1990). As long as the environmental changes are within the plasticity range a plant population can survive unfavourable periods without shifts in its genotypic composition. A passive dehydration tolerance has the benefit of survival at the cost of a reduced growth and a delayed onset of flowering, both of which might severely impair reproduction. Under a long-term decrease in precipitation, however, species need to adapt and accelerate the flowering and reproduction time in order to maintain high survival rates and simultaneously produce high numbers of offspring. A high biomass production is positively correlated to the reproductive output (Cohen 1971, 1976, Bazzaz et al. 1987, Schmid et al. 1995). Therefore, high biomass production is favoured in any environment. In this light, a plastic response to tolerate drought, such as pausing growth, can be a disadvantage because it results in the delay of reproduction

and limits the reproductive output. Even before climate change causes the environmental conditions to exceed the plasticity range a mere plastic response might result in severe fitness loss.

Our results lead us to three possible explanations for the phenotypic responses which opposed our predictions: Firstly, the environmental change was within the range of the species' plasticity. Secondly, we were not able to detect shifts that can be interpreted as adaptive response because the variation caused by the environment disguised such trends. And thirdly, these species are not able to adapt in so few generations or might not be able to adapt at all. An important hint was given by the result of the survival analysis. The survival rates have not been negatively affected by the dry-treatment, which confirms the first option. However, it does not necessarily negate the second or the third. To test the second option, to disentangle the genetic from the environmental influence on phenotypic variation, an additional experiment is needed. Whether the observed differences in the phenotypic reaction norms are inherited, can be tested by comparing plants derived from seeds which were produced under the same conditions to see if these differences persist (see Chapter 2).

We expected a larger impact of decreasing precipitation in the semi-arid site, because the limitation of water is stronger there. The extent to which the dry-treatments affected trait variation was indeed greater in the semi-arid site. However, the decreased precipitation had no significant negative effect on survival in the species in our study. Regarding the high between year fluctuations in precipitation recorded for these sites (see Fig. 7 in the Appendix and Metz et al. 2010), it can be argued that the studied species still persist within their tolerance limits and the stress applied by the treatments was not strong enough to exceed the impact of these fluctuations.

Regarding the future of this region, we expect the impact of climate change to be stronger compared to changes in precipitation alone. According to the predictions for the study region (Cruz et al. 2007, Gao and Giorgi 2008), it is likely that the variability of precipitation will increase in the wake of climate change. Increased rain fall variability and a change in frequency can have a strong impact on species composition and consequently on ecosystem functioning (Knapp et al. 2002, Fay et al. 2011). Our



approach to decouple these factors allowed an evaluation of the impact of a chronic resource alteration.

### Conclusion

We found strong support that plasticity covered the range of environmental changes in the studied set of common species. The phenotypic shifts between sites can be attributed to stress escape strategies, while differences within sites are due to stress tolerance which is likely to be a plastic response and not due to genetic adaptation.

On the one hand, our results imply that, especially in ecosystems with unpredictable conditions, species might be underestimated in their abilities to cope with increased stress. But on the other hand, the implication of a strictly plastic response for the long-term persistence of these species under decreasing precipitation levels might be an inability to maintain population size and finally local extinction. If these species are not able to accelerate their development and flower and reproduce earlier, the delay due to the dehydration tolerance strategy might prove to be fatal when late rain does not come. If there is no genotype that can reproduce in time, this population will go extinct. We wish to point out that rare species might be affected to a greater extent, and interfering factors such as competition might restrict phenotypic variation in a way that intervenes with the response to drought.

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## Appendix and supplemental material

Tab. 5) Multiple comparisons (GLM) of the mean onset of flowering (a), plant size (b) and vegetative biomass (c) per species and site with rain manipulation treatment as fixed factor. Given are the number of observations (n), mean trait values and standard error per species and rain manipulation treatment for each site.

Tab. 5a)

			Onset of flowering								
<b>Semi-arid site</b>			Mean	StErr	<b>Mediterranean site</b>			Mean	StErr		
<b>Bis</b>	p	<0.0001	Dry	61.8 ±	1.6	<b>Bis</b>	p	0.0018	Dry	104.2 ±	2.1
	Chi <sup>2</sup>	27.733	Con	48.8 ±	1.9		Chi <sup>2</sup>	12.65	Con	102.7 ±	1.9
	n	85	Wet	54.7 ±	1.9		n	135	Wet	94.9 ±	2.0
<b>Hel</b>	n	ns	Dry	60.3 ±	3.0	<b>Hel</b>	n	ns	Dry	114.0 ±	2.8
			Con	58.2 ±	1.9				Con	104.7 ±	2.4
			Wet	56.5 ±	1.6				Wet	111.4 ±	2.7
<b>Hym</b>	p	<0.0001	Dry	61.2 ±	1.3	<b>Hym</b>	p	0.0232	Dry	102.2 ±	1.5
	Chi <sup>2</sup>	45.163	Con	51.9 ±	1.3		Chi <sup>2</sup>	7.527	Con	105.0 ±	1.3
	n	142	Wet	49.2 ±	1.3		n	125	Wet	108.0 ±	1.5
<b>Ana</b>	p	0.0245	Dry	65.8 ±	1.5	<b>Pla</b>	n	ns	Dry	97.3 ±	1.7
	Chi <sup>2</sup>	7.418	Con	62.6 ±	1.3				Con	99.0 ±	1.5
	n	140	Wet	60.2 ±	1.3				Wet	100.0 ±	1.5
<b>Ono</b>	p	0.0019	Dry	65.4 ±	1.2	<b>Sco</b>	n	ns	Dry	115.1 ±	1.2
	Chi <sup>2</sup>	12.575	Con	59.9 ±	1.2				Con	114.1 ±	1.1
	n	128	Wet	60.4 ±	1.2				Wet	115.8 ±	1.2
<b>Li str</b>	n	ns	Dry	78.2 ±	1.0	<b>Tri</b>	n	ns	Dry	120.9 ±	2.1
			Con	77.3 ±	0.9				Con	115.1 ±	2.3
			Wet	77.9 ±	0.9				Wet	116.0 ±	1.8
<b>Aeg</b>	n	ns	Dry	88.0 ±	1.4	<b>Tor</b>	p	0.0337	Dry	125.5 ±	0.8
			Con	88.8 ±	1.0		Chi <sup>2</sup>	6.780	Con	128.4 ±	0.8
			Wet	88.7 ±	1.6		n	116	Wet	126.5 ±	0.8
<b>Atr</b>	n	ns	Dry	101.1 ±	1.2	<b>Av</b>	n	ns	Dry	129.0 ±	1.3
			Con	102.5 ±	1.0				Con	128.8 ±	1.2
			Wet	100.1 ±	1.2				Wet	128.3 ±	1.3
<b>Uro</b>	n	ns	Dry	135.8 ±	1.9	<b>Li co</b>	p	0.0280	Dry	136.7 ±	1.3
			Con	139.7 ±	1.8		Chi <sup>2</sup>	7.154	Con	140.3 ±	1.3
			Wet	139.4 ±	2.1		n	80	Wet	141.3 ±	1.2
<b>Cru</b>	n	ns	Dry	140.6 ±	1.8	<b>Cru</b>	n	ns	Dry	140.6 ±	1.8
			Con	140.9 ±	1.9				Con	140.9 ±	1.9
			Wet	140.1 ±	1.9				Wet	140.1 ±	1.9

Tab. 5b)

## Plant size

Semi-arid site			Mean	StErr	Mediterranean site			Mean	StErr
<b>Bis</b>	n	ns 83	Dry	60.7 ± 5.5	<b>Bis</b>	p Chi <sup>2</sup> n	0.0639 5.5 134	Dry	98.5 ± 7.1
			Con	67.9 ± 6.0				Con	91.3 ± 4.9
			Wet	62.2 ± 5.3				Wet	79.8 ± 5.0
<b>Hel</b>	n	ns 48	Dry	76.9 ± 16.4	<b>Hel</b>	n	ns 79	Dry	113.8 ± 7.9
			Con	96.1 ± 11.0				Con	108.3 ± 6.8
			Wet	79.0 ± 6.2				Wet	106.8 ± 8.5
<b>Hym</b>	n	ns 141	Dry	44.2 ± 2.4	<b>Hym</b>	n	ns 124	Dry	69.0 ± 4.3
			Con	38.2 ± 1.9				Con	62.6 ± 2.9
			Wet	38.9 ± 1.8				Wet	68.0 ± 4.4
<b>Ana</b>	p Chi <sup>2</sup> n	0.0707 5.298 141	Dry	63.7 ± 2.7	<b>Pla</b>	p Chi <sup>2</sup> n	0.0772 5.123 139	Dry	82.2 ± 4.3
			Con	56.5 ± 2.8				Con	68.6 ± 3.9
			Wet	55.2 ± 2.1				Wet	73.5 ± 4.2
<b>Ono</b>	n	ns 128	Dry	78.8 ± 7.0	<b>Sco</b>	p Chi <sup>2</sup> n	<0.0001 20.781 111	Dry	97.4 ± 4.7
			Con	81.4 ± 3.1				Con	85.1 ± 3.1
			Wet	77.7 ± 3.7				Wet	72.8 ± 3.0
<b>Li str</b>	n	0.0005 15.1 127	Dry	111.6 ± 8.5	<b>Tri</b>	n	ns 89	Dry	101.1 ± 8.9
			Con	180.5 ± 11.5				Con	79.7 ± 7.2
			Wet	158.4 ± 9.0				Wet	88.4 ± 4.8
<b>Aeg</b>	p Chi <sup>2</sup> n	0.0903 4.8 144	Dry	187.3 ± 9.7	<b>Tor</b>	n	ns 116	Dry	95.5 ± 7.7
			Con	210.7 ± 11.4				Con	107.1 ± 9.4
			Wet	191.9 ± 8.1				Wet	113.2 ± 8.2
<b>Atr</b>	p Chi <sup>2</sup> n	0.0779 6.078 111	Dry	75.4 ± 5.2	<b>Av</b>	p Chi <sup>2</sup> n	0.0474 6.099 151	Dry	341.1 ± 20.2
			Con	81.6 ± 5.6				Con	285.7 ± 13.5
			Wet	96.6 ± 5.7				Wet	306.6 ± 14.6
					<b>Uro</b>	p Chi <sup>2</sup> n	0.0192 7.909 87	Dry	159.9 ± 10.4
				Con				203.8 ± 13.7	
				Wet				144.6 ± 10.0	
					<b>Li co</b>	p Chi <sup>2</sup> n	0.0004 15.555 87	Dry	287.3 ± 18.7
				Con				189.9 ± 13.6	
				Wet				214.9 ± 14.8	
					<b>Cru</b>	n	ns 73	Dry	440.6 ± 30.9
				Con				427.5 ± 38.5	
				Wet				366.0 ± 27.6	

Tab. 5c)

## Vegetative biomass

Semi-arid site			Mean	StErr	Mediterranean site			Mean	StErr			
<b>Bis</b>	p	<0.0001	Dry	0.019 ± 0.005	<b>Bis</b>	ns	Dry	0.045 ± 0.008				
	Chi <sup>2</sup>	45.62	Con	0.044 ± 0.004			Con	0.042 ± 0.008				
	n	35	Wet	0.010 ± 0.005			Wet	0.038 ± 0.008				
<b>Hel</b>	ns	39	Dry	0.056 ± 0.029	<b>Hel</b>	p	Dry	0.074 ± 0.015				
			Con	0.084 ± 0.019			Chi <sup>2</sup>	5.23	Con	0.069 ± 0.012		
			Wet	0.074 ± 0.014			n	68	Wet	0.113 ± 0.015		
<b>Hym</b>	ns	82	Dry	0.092 ± 0.014	<b>Hym</b>	ns	Dry	0.157 ± 0.016				
			Con	0.089 ± 0.012			Con	0.143 ± 0.015				
			Wet	0.080 ± 0.013			Wet	0.125 ± 0.016				
<b>Ana</b>	ns	97	Dry	0.107 ± 0.013	<b>Ana</b>	p	Dry	0.111 ± 0.011				
			Con	0.078 ± 0.013			Chi <sup>2</sup>	13.05	Con	0.075 ± 0.009		
			Wet	0.074 ± 0.011			n	112	Wet	0.059 ± 0.009		
<b>Ono</b>	p	0.0102	Dry	0.129 ± 0.028	<b>Pla</b>	ns	Dry	0.054 ± 0.006				
			Chi <sup>2</sup>	9.18			Con	0.219 ± 0.020	Con	0.047 ± 0.006		
			n	111			Wet	0.156 ± 0.023	Wet	0.038 ± 0.006		
<b>Li str</b>	p	0.0008	Dry	0.065 ± 0.011	<b>Sco</b>	p	Dry	0.258 ± 0.027				
			Chi <sup>2</sup>	14.2			Con	0.118 ± 0.010	Chi <sup>2</sup>	7.857	Con	0.167 ± 0.025
			n	109			Wet	0.096 ± 0.010	n	89	Wet	0.162 ± 0.030
<b>Aeg</b>	p	0.0068	Dry	0.350 ± 0.040	<b>Tri</b>	ns	Dry	0.062 ± 0.007				
			Chi <sup>2</sup>	99.93			Con	0.390 ± 0.042	Con	0.050 ± 0.006		
			n	105			Wet	0.233 ± 0.037	Wet	0.047 ± 0.006		
<b>Atr</b>	ns	114	Dry	0.429 ± 0.049	<b>Tor</b>	ns	Dry	0.019 ± 0.003				
			Con	0.518 ± 0.053			Con	0.024 ± 0.003				
			Wet	0.366 ± 0.055			Wet	0.019 ± 0.003				
<b>Av</b>	p	0.0244	Dry	0.489 ± 0.054	<b>Uro</b>	ns	Dry	0.432 ± 0.077				
			Chi <sup>2</sup>	7.43			Con	0.441 ± 0.075				
			n	105			Wet	0.336 ± 0.057	Con	0.441 ± 0.075		
<b>Li co</b>	p	0.0002	Dry	0.146 ± 0.015	<b>Li co</b>	p	Dry	0.146 ± 0.015				
			Chi <sup>2</sup>	16.825			Con	0.064 ± 0.017	Chi <sup>2</sup>	16.825	Con	0.064 ± 0.017
			n	69			Wet	0.093 ± 0.015	n	69	Wet	0.093 ± 0.015
<b>Cru</b>	p	0.0154	Dry	0.442 ± 0.050	<b>Cru</b>	p	Dry	0.442 ± 0.050				
			Chi <sup>2</sup>	8.347			Con	0.279 ± 0.048	Chi <sup>2</sup>	8.347	Con	0.279 ± 0.048
			n	119			Wet	0.259 ± 0.048	n	119	Wet	0.259 ± 0.048



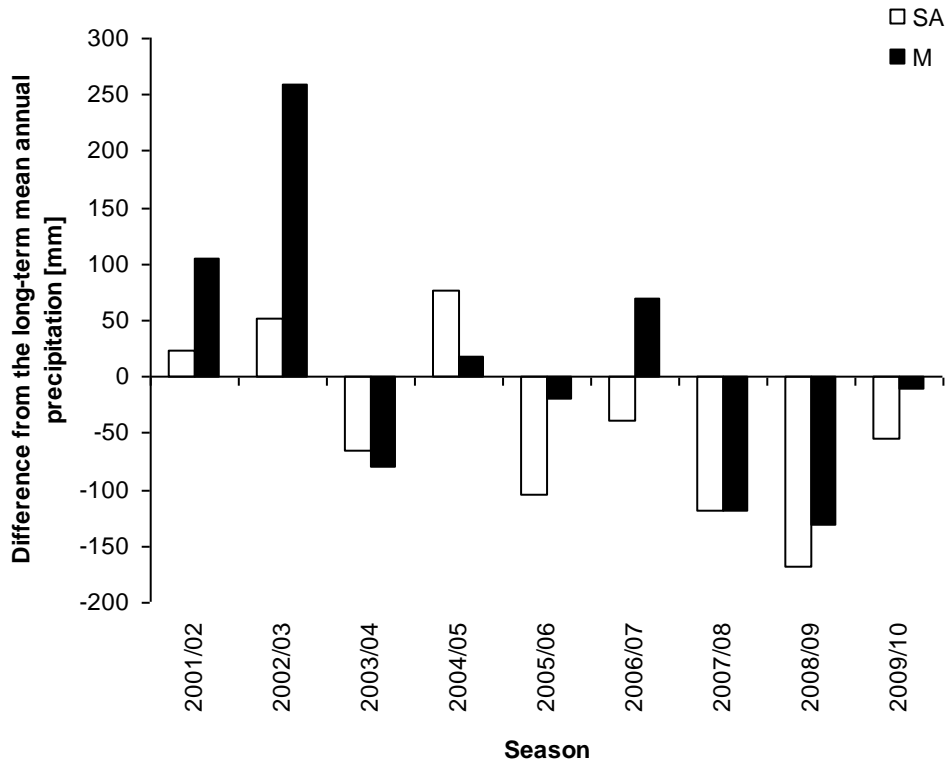


Fig. 7: Annual fluctuation of the amount of rainfall in the Semi-arid (SA) and the Mediterranean (M) site plotted as deviation from the long-term mean (1977-2008).

## Chapter 2: Long-term *in situ* rain manipulation caused adaptive responses in life-history traits in a set of common annual plants

### Abstract

The potential of species to adapt *in situ* to environmental changes plays a key role for the future of biodiversity under global change. Climatic models predict a severe decrease in precipitation in the Mediterranean Basin that might exceed the tolerance range of annual plant species in this region and cause local extinctions. In this chapter, we evaluated the inherited intraspecific variation in response to *in situ* rain manipulation treatments to assess the potential impact of changes in precipitation on life-history traits. Semi-natural plant communities in a semi-arid and a Mediterranean site in Israel were subjected to increased or decreased natural rainfall (30%) for eight years. We collected seeds from each rain manipulation treatment and grew plants under similar conditions in a greenhouse for two generations to equalise maternal effects. The second greenhouse generation was used to test for inherited phenotypic shifts caused by the different rain manipulation treatments in the field. In this chapter we present evidence for contemporary adaptation in five out of eight studied species in response to the field rain manipulation. Corresponding to our prediction, decreasing water availability in the field caused an acceleration in the onset of flowering, a reduction in plant height, each in one species and an increase of reproductive allocation in three species. Our results also showed that the dry-treatment affected trait variation to a greater extent than the wet-treatment in the semi-arid site and vice versa in the Mediterranean site. Many species have the potential to adapt, but the potential for antagonistic developments might increase with the number of selective factors involved.

## Introduction

Plant species are known to have a wide range of plastic response to stress, which enables them to inhabit a great variety of environments (Bradshaw 1965, Aronson et al. 1990). However, the impact of long-term decreasing precipitation, as predicted by climatic models for the Mediterranean Basin (IPCC 2012), might be beyond their capacity of plastic response and cause local extinctions by reducing reproductive success and increasing mortality (Visser 2008, Cahill et al. 2012). Additionally, the projected rate of climate change is likely to outpace the migration rate of many plant species (Davis and Shaw 2001, Corlett and Westcott 2013), and therefore, the potential of species to rapidly adapt *in situ* to the changes plays a key role for the future of biodiversity under global change (Walther et al. 2002, Schiffrers et al. 2013). Assessing the potential to quickly adapt to climate change does not only allow a better understanding of evolutionary processes, but is of high importance for predicting species habitat ranges, improving management strategies in the conservation of species and, ultimately, to confront biodiversity loss.

Despite the wide consent regarding the negative impact that climate change might have on species diversity, evidence of the ability to quickly evolve to tolerate the new conditions has been reported by only a small number of studies and with contradicting findings. The most concrete example of rapid adaptation was the acceleration of the onset of flowering in response to drought after seven generations found in a population of *Brassica rapa* (Franks et al. 2007). In *Fumana thymifolia*, an indirect evidence was found for genotypic shifts due to drought and warming treatments (Jump et al. 2008). On the other hand, in birch (*Betula pubescens*) the rate of change in spring bud burst has been shown to be relatively slow compared to the rate of global warming (Billington and Pelham 1991). Similarly, antagonistic genetic correlations between traits have been shown to slow rate of evolutionary response in *Chamaecrista fasciculata* (Etterson and Shaw 2001). There is an obvious lack of studies that involve more species in various environments.

The inherited shifts in fitness related traits in response to a rain manipulation treatment provide information about the potential of species to quickly adapt to the changes in precipitation. Genotypes that allow a maximisation of fitness under the new conditions

are favoured and can become dominant in the population (Endler 1986). Adaptive evolution resulting from a shift in the genotypic composition of a population is a likely consequence if the environmental changes are severe enough and the genetic variation in the respective traits is high (Fox 1990, Franke et al. 2006, Rathcke and Lacey 2007). The chances to measure evolutionary differentiation already after a short time is high, because evolution has been shown to happen in the first few generations after the environment changed (Wu et al. 1975, Kinnison and Hendry 2001, Bone and Farres 2001). The direction of adaptive shifts in response to changing precipitation can be predicted on the basis of trends in the variation of life-history related traits along rainfall gradients (Etterson and Shaw 2001, Volis et al. 2002, Franke et al. 2006, Petrů et al. 2006, Lampei and Tielbörger 2010, Kigel et al. 2011).

These trait variations along the gradient reveal general strategies of adaptation to the clinal change in precipitation as well as to the change in predictability of rain events. Adaptations to a shortening of the growing season under more arid conditions involve an earlier switch from vegetative to reproductive phase (Cohen 1976). Earlier flowering reduces the potential to greater biomass which could support the production of more seeds (Blum 1996). This obvious disadvantage is a drought escape strategy in more arid environments where resource limitation can cause early mortality (Levitt 1972). An acceleration of the development might also be the result of decreasing precipitation because the decreasing competition for light allows a shorter plant size and a higher reproductive allocation. In arid environments early flowering has been shown to be correlated to smaller plant size (Aronson et al. 1992). Additionally, theory predicts that higher reproductive allocation is favoured in less predictable environments to compensate for low reproductive output in occasionally occurring favourable seasons (Levins 1963). A higher reproductive allocation in arid populations compared to Mediterranean populations was found for a number of annual species (Aronson et al. 1990, Volis et al. 2002, Petrů et al. 2006).

Adaptive evolution depends on the ecological history of the species. Life-history traits can respond differently to an environmental shift when populations from different origins are regarded (Lechowicz 1988, Milla et al. 2009). General trends of the macroclimate can be masked by factors on the microgeographic scale (Neuffer 2011). The relevance of differing trait expressions and their association with habitat heterogeneity is basic

knowledge (Grime 2001). Accordingly, it is likely that the hierarchy of trait relevance for plant fitness shifts as the abiotic factors and the linked biotic factors vary between microhabitats (Thompson 1998, Carroll et al. 2007). In a Mediterranean environment the main environmental constraint is most likely the density of neighbouring plants, while precipitation is the more important factor in the more arid site (Petrů et al. 2006, Liancourt and Tielbörger 2009). These contrasting environmental pressures led to opposing effects of decreasing water availability on survival, growth and phenology in the studied species (first chapter). Based on these paradigms and our observations in the field, we expect that a similar relative reduction in precipitation caused a selection on the life-history traits that differs in magnitude and even in direction within the same species, depending on the selection history experienced in either a semi-arid or a Mediterranean site. Even though the understanding of the role of these ecotypic differentiations in the evolutionary process is poor, it can be argued that affiliation to species within a community and affiliation to ecotype across differing environments are likely to determine adaptive processes and was therefore the second focus of this chapter.

In regions where water is already limited, as in the Mediterranean Basin, the increasing intensity and number of drought events is expected to have a strong negative impact on natural populations (Ackerly et al. 2000, Knapp et al. 2002, Chaves et al. 2003). In the Eastern Mediterranean plant communities winter annuals are the dominating life-form (Zohary 1937, Noy-Meir 1973). Their short generation time and the tendency to autogamy (Aarssen 2000, Snell and Aarssen 2005) increases the probability of a shift in the genotypic composition on an ecological time-scale (Hoffmann and Willi 2008). Here, we subjected a set of annual species for eight years to *in situ* rain manipulation experiments in a semi-arid and a Mediterranean site and tested their descendants for inherited phenotypic shifts as an indication of adaptive evolution in response to the changes in precipitation.

Predictions:

- The rain manipulations treatments resulted in inherited shifts in trait variation according to the trends observed between the two sites along the aridity gradient.
- In the absence of competition in the greenhouse, we expect the reproductive output of dry-adapted genotypes to be increased.

- Plants originating from the semi-arid site are expected to be stronger affected by the dry-treatment and populations originating from the Mediterranean site are expected to show adaptive responses to the wet-, rather than to the dry-treatment.

## Methods

In order to test for an adaptive response, it is necessary to distinguish between the plastic reaction and adaptation. Part of the environmentally caused plasticity can influence the offspring generation even under similar growth conditions via maternal effects due to differing conditions during seed production (Stratton 1989, Rossiter 1996). To equalise these maternal effects we compared plants derived from seeds that were produced under similar greenhouse conditions.

### Plant material

For our experiment we used seeds from eight co-existing annual species (Tab. 1) which were subjected to eight years of rain manipulations in both field sites. They were collected in April-May 2010 and kept in a nethouse in Israel for two months during the summer to break seed dormancy (Petrů et al. 2006). From these seeds a first greenhouse generation (G1) was reared in 2010-11. The seeds/fruits of plants in the G1 were mainly produced by selfing. They were harvested in 2011 and sent to Israel for over-summering as in the previous year. Plants of both generations were grown under greenhouse conditions that excluded competition and resource limitation. Plants descending from G1 individuals in the second greenhouse generation (G2) in 2011-12 represented all of the rain manipulation treatments in the two sites, and were used to measure phenotypic variation in life-history traits.

Tab. 1) Studied species and Initial samples sizes of the second greenhouse generation (G2), collected in the Mediterranean and Semi-arid sites after 8 years of rain manipulations (dry, con=control and wet) and grown for two generations under similar conditions in a greenhouse.

G2	Family	Semi-arid			Mediterranean		
		dry	con	wet	dry	con	wet
<i>Aegilops geniculata</i> ROTH	<i>Graminae</i>	24	30	33			
<i>Biscutella didyma</i> L.	<i>Brassicaceae</i>	19	19	17	28	28	20
<i>Brachypodium distachyon</i> (L.) P.BEAUV.	<i>Graminae</i>	49	45	46	47	49	41
<i>Hymenocarpus circinnatus</i> (L.) SAVI	<i>Fabaceae</i>	23	24	18	24	22	22
<i>Avena sterilis</i> L.	<i>Graminae</i>				31	35	27
<i>Crupina crupinastrum</i> (MORIS) VIS.	<i>Asteraceae</i>				27	30	26
<i>Plantago afra</i> L.	<i>Plantaginaceae</i>				32	27	28
<i>Urospermum picroides</i> (L.) SCOP. EX F.W.SCHMIDT	<i>Asteraceae</i>				16	20	12

### Cultivation of the greenhouse generations

The University of Tuebingen provided the greenhouses for the cultivation of the plants in both seasons (2010/11 and 2011/12). During both growth periods the temperature ranged from 16°C at night to 22°C during the day, and natural lighting was used. Between 5 and 20 mature seeds of the 8 species (depending on seed size and availability) per mother individual were sown in autumn of the respective year into customary pots (90 mm x 90 mm, 100 mm deep) and covered with a thin layer of substrate to prevent desiccation. As substrate a mixture of sand and garden soil with low nutrient content (1:1) and 100 ml Osmocot Scott® fertilizer (15 % N, 9 % P<sub>2</sub>O<sub>5</sub>, 9 % K<sub>2</sub>O) per 15 l of soil/sand mixture was used. In 2010 the day of initial irrigation was the 17<sup>th</sup> of November, and in 2011 the 21<sup>th</sup> of November. Pots were randomised before every other irrigation. After the germination, seedlings were randomly thinned down to one individual per pot to avoid competition.

### Measurement of life-history traits

Onset of flowering was the time of first flower opening (anthesis). In the case of *Graminae* the emergence of the anthers was used as indication of onset of flowering. To keep

the measurement of the onset of flowering comparable to the data collected in the field, measurements were carried out every 2-3 days. Plant height was measured at the onset of flowering. In the case of grasses the size was measured from the soil surface to the tip of the plant, after carefully straightening the plants. For all other species we measured size from the cotyledonary node to the tip to avoid confounding effects due to strong elongation in the hypocotyle in some individuals but not in others, depending on the light situation at the time germination. Seeds or fruits were defined as fully mature, when they were “well developed” (fully grown), appeared brown and dry, and by carefully touching the seeds/fruits to test if they were still firmly attached to the plant or loose and easy to remove.

Plants were individually wrapped in organza bags at the start of seed maturation to avoid the loss of seeds/fruits. After the peak of seed-set, irrigation was stopped and plants were harvested when they were fully dry. Biomass was separated into vegetative biomass and mature seeds/fruits. The vegetative biomass was additionally dried at 70°C for 24 h in a dry oven, and weighted (Kern und Sohn GmbH, d=0.01 g). The seeds were not oven dried since they were needed for sowing in the next season. All seeds/fruits per individual were weighed (Denver Instruments APX-153, d=0.001 g) to assess the total reproductive biomass. Additionally a subsample was weighed and counted to calculate the average weight per seed/fruit, which was used to estimate the total number of seeds/fruits per individual as a measure of fitness. Reproductive allocation was calculated as the proportion of reproductive biomass (total mass of all mature seeds/fruits) of total biomass.

### Statistical analyses

All statistical analyses were computed with the software package JMP 7.0.1 (SAS). The analyses were done for each site separately, using a generalised linear model. For *Biscutella*, *Brachypodium* and *Hymenocarpus*, that occurred in both sites, a full factorial model was used including site as a factor. For onset of flowering, the model was based on a Poisson distribution and a log-link function. For plant size, total biomass, reproductive allocation, and number of fruits/seeds, we used an exponential distribution and reciprocal link-function. For correcting the alpha in post-hoc multiple comparisons, Holm's sequential Bonferroni test (Rice 1989) was applied. The significance levels used in all analyses are 0.05 (\*), 0.01 (\*\*), and 0.001 (\*\*\*).



## Results

### Fitness

In all species from the semi-arid site, the number of seeds/fruits was higher in plants originating from the dry-treatments compared to the control. However, the difference in fitness between rain manipulation treatments, which were calculated as separate model per species, was significant in a only in species (Fig. 1). Neither semi-arid nor Mediterranean populations showed a significant effect of the wet-treatment on fitness.

In the semi-arid population of *Biscutella* the treatment significantly affected seed production ( $p=0.0071$ ,  $\text{Chi}^2=9.899$ ). Plants from the dry-treatment produced 15% more and the wet-treatment 20% less diaspores compared to the control. In populations from the Mediterranean site significant variation in fitness was found in *Brachypodium* ( $p=0.0183$ ,  $\text{Chi}^2=8.001$ ) and *Urospermum* ( $p=0.0086$ ,  $\text{Chi}^2=9.598$ ). *Brachypodium* from the dry-treatment produced 23% more seeds compared to plants from the control-treatment and 27% more than plants from the wet-treatment. *Urospermum* from the dry-treatment increased seed production by 41% compared to the control. No significant differences between treatments were found in the other species. None of the species studied showed significantly differing seed production in the G1.

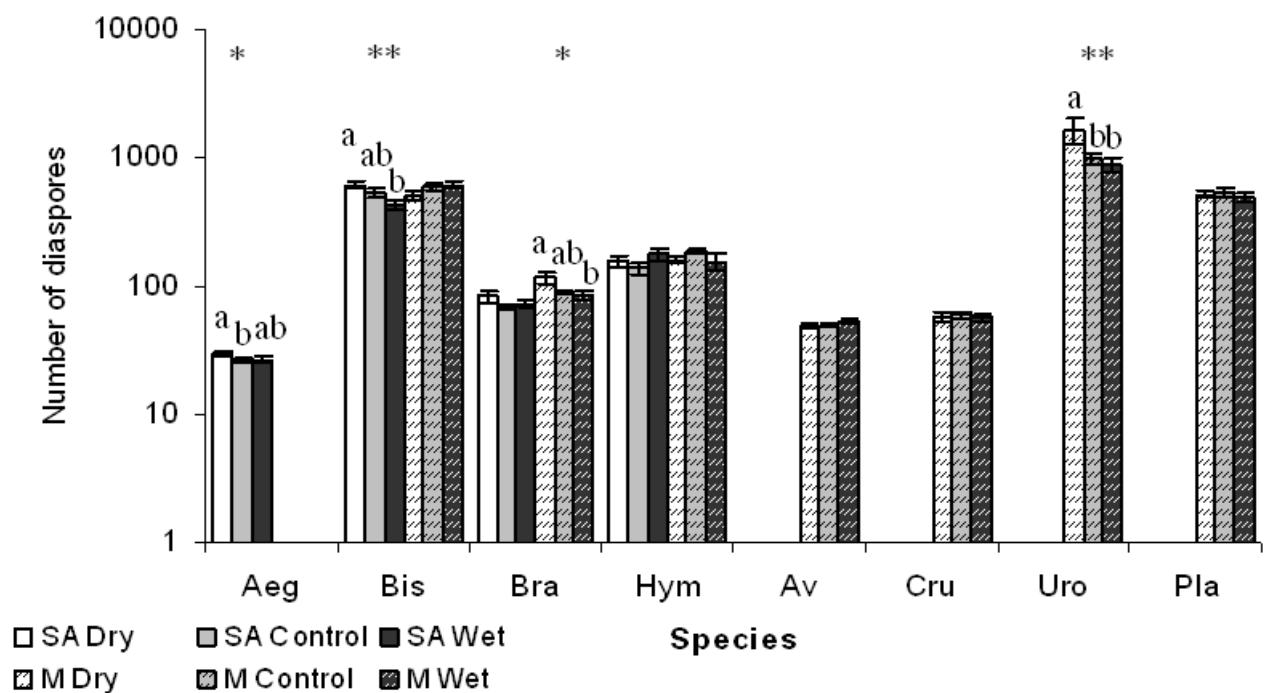


Fig. 1) Number of mature seeds/fruits per plant (+/- Standard Error). The small letters represent significant differences in phenotypic variation due to the rain manipulation treatments.

## Phenotypic variation between the semi-arid and the Mediterranean site

The effect of site on the phenotypic variation of the measured life-history traits was tested in the three species occurring in both sites. A clear trend between the sites was found in all but the reproduction related traits over all tested species (Tab. 2). Semi-arid plants flowered significantly earlier, were shorter and produced less biomass than Mediterranean plants. Only *Biscutella* differed in reproductive allocation between sites: the semi-arid plants had a significantly higher reproductive allocation compared to the Mediterranean plants. The number of diaspores differed between sites only in *Brachypodium*: The Mediterranean population produced more seeds than the semi-arid one.

Tab. 2) Intraspecific comparison between plants originating from the semi-arid and the Mediterranean climate station.

Differences between sites of origin				Direction of trend
Trait	Species	Chi <sup>2</sup>	p	SA - M
Onset of flowering	Bis	41.389	<0.0001	<
	Bra	11.661	<0.0001	<
	Hym	158.782	<0.0001	<
Plant size	Bis	96.147	<0.0001	<
	Bra	3.625	0.0569	<
	Hym	24.305	<0.0001	<
Total biomass	Bis	20.551	<0.0001	<
	Bra	16.925	<0.0001	<
	Hym	9.270	0.0023	<
Reprod. allocation	Bis	7.359	0.0067	>
	Bra	ns		
	Hym	ns		
Number of seeds/fruits (fitness)	Bis	ns		
	Bra	15.885	<0.0001	<
	Hym	ns		

## Phenotypic variation within site origins

## Phenology - Onset of flowering

A significant treatment effect on the onset of flowering was found only in *Aegilops* ( $p=0.0228$ ,  $\text{Chi}^2=7.559$ ). Individuals of *Aegilops* that originated from the dry-treatment in the field had an average acceleration of 6.7 days (5.6%) compared to the control group (Fig. 2). The same trend was also measured in G1 ( $p=0.0009$ ,  $\text{Chi}^2=13.967$ ). The direction corresponded in both generations to the trend which was found between sites for other species (Tab. 2).

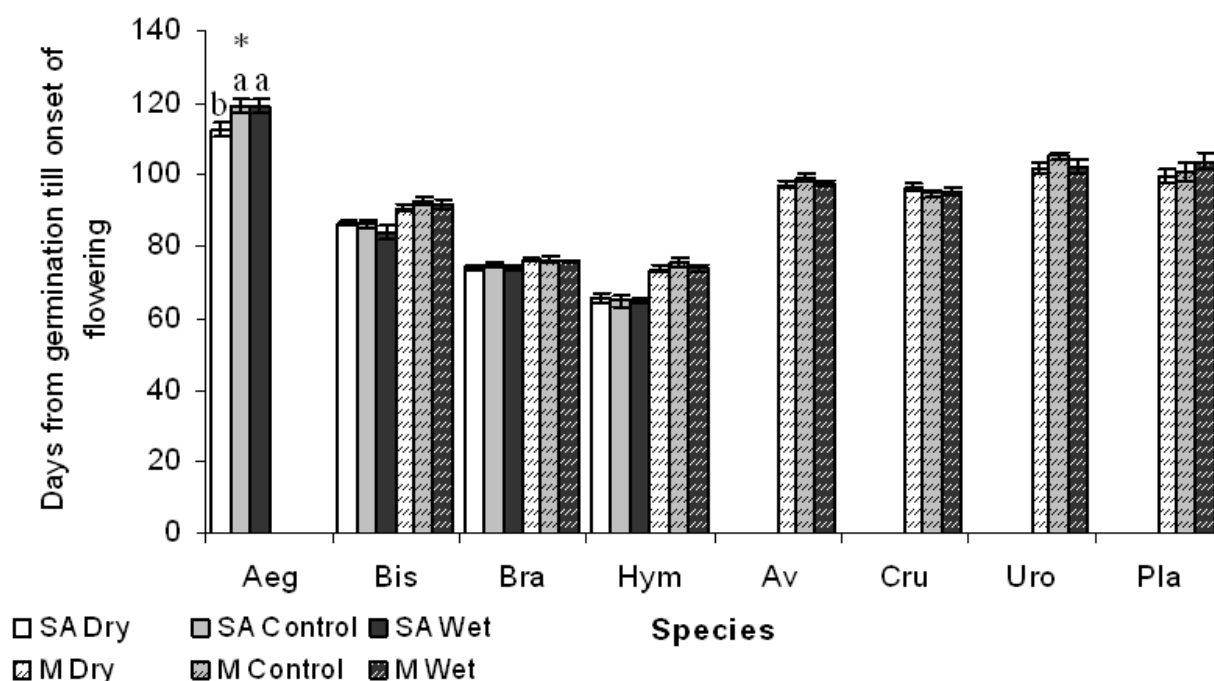


Fig. 2) Time to onset of flowering (+/- Standard Error). Significance level refers to treatment effect within species by site of origin.

## Plant size and total biomass

The plant size at anthesis differed significantly between treatments, though the effect was species-specific (Fig. 3). Significant effects of the rain manipulation treatments on plant size were found in two species originating from the semi-arid site: in *Aegilops* ( $p=0.0186$ ,  $\text{Chi}^2=7.969$ ) individuals of the wet-treatment were 42.5 mm (7.6%) shorter at the onset of flower and in *Hymenocarpus* ( $p=0.0276$ ,  $\text{Chi}^2=7.969$ ) plants originating from the dry-treatment were shorter by 15.9 mm (7.4%) compared to the control.

Among the Mediterranean populations, *Crupina* was the only species showing variation in plant size in the G2 ( $p=0.0219$ ,  $\text{Chi}^2=5.253$ ). Here, plants from the dry-treatment were on average 44.5 mm (9.4%) shorter compared to the control which resembled the trend that was found between sites in the three species occurring in both sites (Tab. 2). Also, in the previous generation this trend was significant ( $p=0.0038$ ,  $\text{Chi}^2=11.127$ ) in *Crupina*.

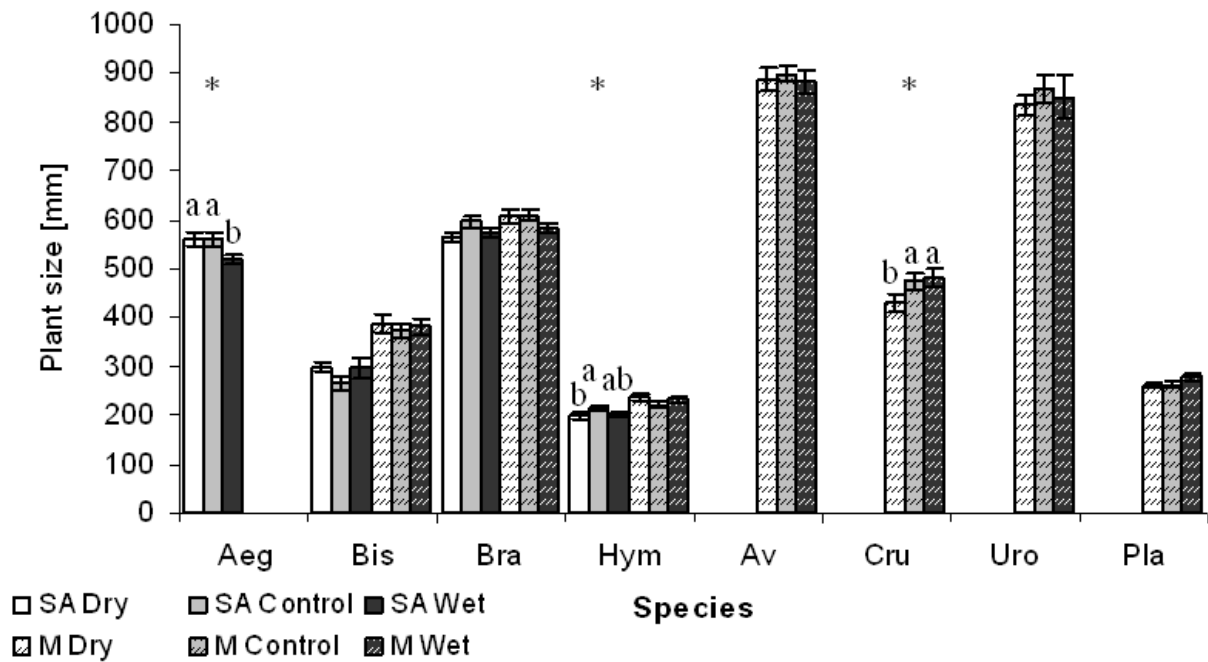


Fig. 3) Plant size at the onset of flowering (+/- Standard Error).

The effect of rain manipulation history on total biomass remained in the G2 in only two species (Fig. 4). In the semi-arid population a significant increase in total biomass ( $p=0.0135$ ,  $\text{Chi}^2=8.606$ ) due to the wet-treatment occurred only in *Hymenocarpus* and corresponded to the increase in plant size. The biomass production reached the levels of the Mediterranean population with an average 1.4 g more biomass (18.3%) compared to semi-arid control group. The semi-arid population of *Brachypodium* plants from the dry-treatment produced 0.26 g more biomass (16.2%) compared to the control. Although the treatment effect was significant ( $p=0.0395$ ,  $\text{Chi}^2=6.466$ ) the trend over all three groups of rain manipulation treatments was inconsistent.

In *Brachypodium* originating from the Mediterranean site total biomass significantly decreased with increasing precipitation in the field treatments ( $p=0.0230$ ,  $\text{Chi}^2=7.548$ ). Plants originating from the dry-treatment produced 14.8 g (8%) more and from the wet-

treatment 0.27 g less biomass (15%) compared to the control. This was in accordance with the variation in the reproductive biomass, which significantly increased with decreasing water availability in the field treatments ( $p=0.001$ ,  $\text{Chi}^2=10.946$ ). None of the studied species varied significantly in biomass production between treatment origins in G1.

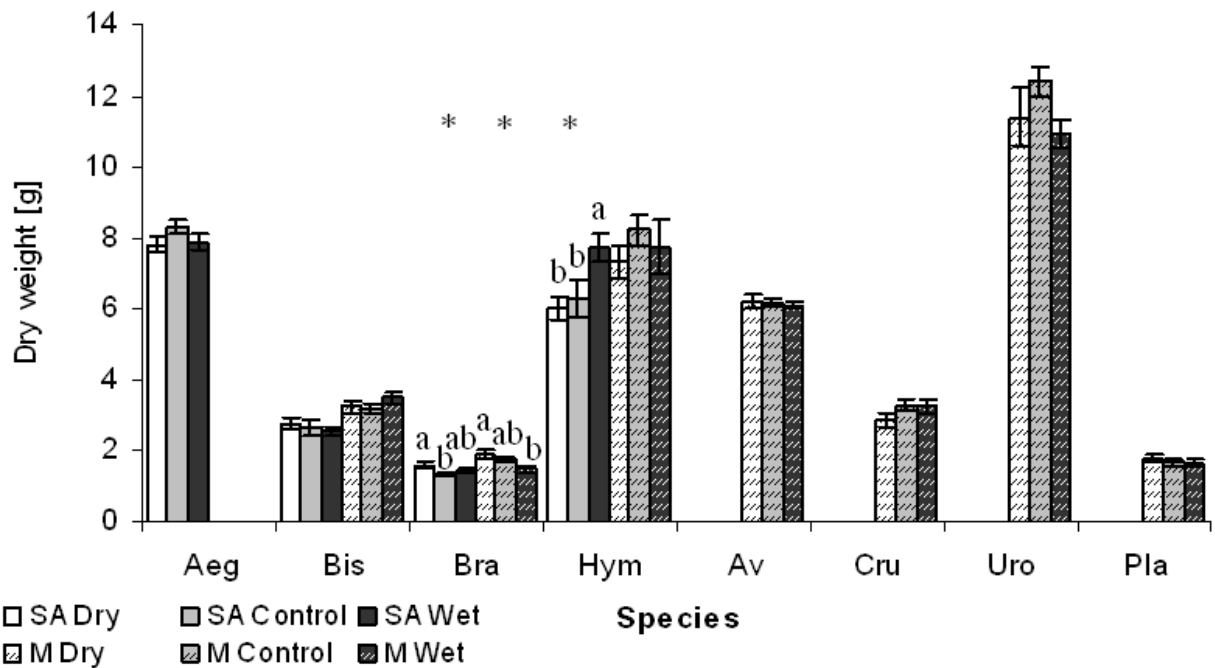


Fig. 4) Total biomass (+/- StErr).

### Reproductive allocation

In the three species that exhibited a significant trait variation, reproductive allocation increased with decreasing water availability in the field treatments (Fig. 5), which resembled the trend between sites in *Biscutella* (Tab. 2). Among the species from the semi-arid site this trend was found in *Aegilops* ( $p=0.0005$ ,  $\text{Chi}^2=15.086$ ) and *Biscutella* ( $p=0.004$ ,  $\text{Chi}^2=11.063$ ). In the Mediterranean population a significantly lower reproductive allocation was found in *Hymenocarpos* originating from the wet-treatment ( $p=0.0153$ ,  $\text{Chi}^2=8.361$ ), whereas all other species were unaffected.

In *Aegilops* the trend resembled the findings from the G1 ( $p<0.0001$ ,  $\text{Chi}^2=18.742$ ), while in *Biscutella* and *Hymenocarpos* the differences the previous generation (G1) were nonsignificant.

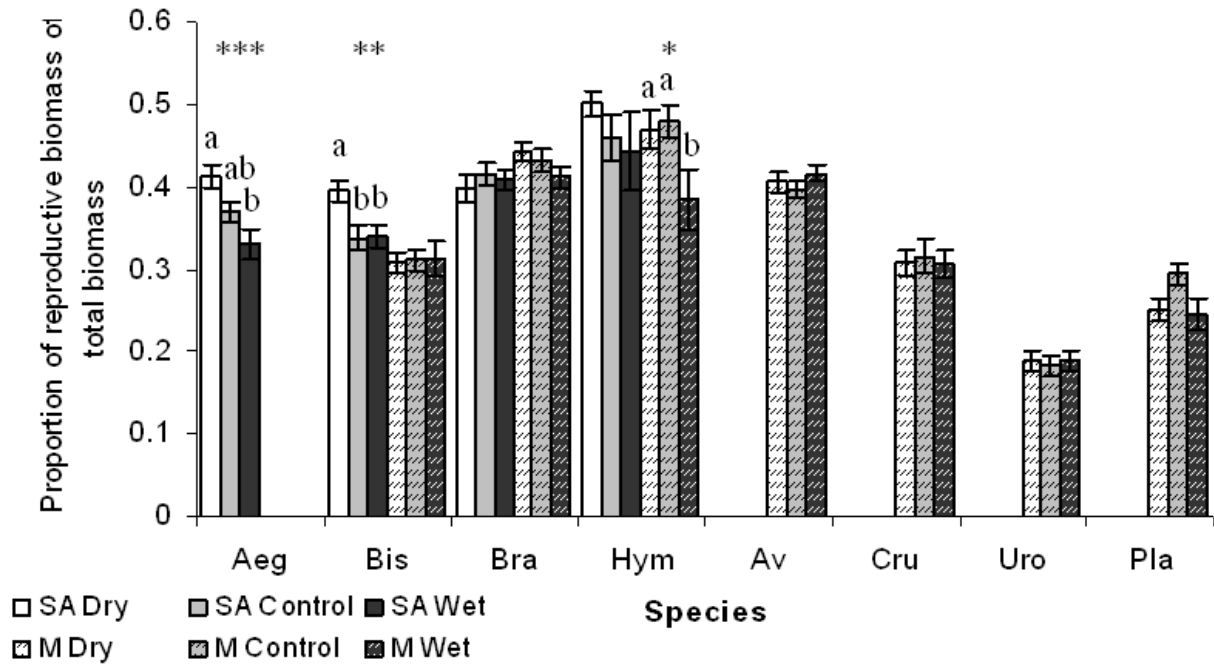


Fig. 5) Reproductive allocation (+/- Standard Error).

Tab. 3) Summary of all affected species per trait and site regarding the rain manipulation treatment, which caused a significant shift compared to the control. The dry-treatment affected more traits in more species in the semi-arid site (SA, 4 studied species) in relation to the Mediterranean site (M, 8 studied species).

Site	SA (4)		M (8)	
	Dry	Wet	Dry	Wet
Onset of flowering	Aeg			
Plant size	Hym	Aeg	Cru	
Total biomass	Bra	Hym	Bra	Bra
Reproductive allocation	Bis, Aeg	Aeg		Hym
Sum	<b>5</b>	<b>3</b>	<b>2</b>	<b>2</b>

## Discussion

We found evidence for inherited trait shifts in response to eight years of rain manipulation in more than half of the studied species. Phenology was affected only in one species from the semi-arid site. A significant effect on growth related traits was found in five species from both sites with contrasting trends. Reproductive allocation consistently increased with decreasing water availability in two semi-arid and one Mediterranean species. These findings confirm assumptions that contemporary evolution occurs frequently (Bradshaw and McNeilly 1991, Reznick and Ghalambor 2001, Carroll et al. 2007) and show that the effect of rain manipulation on the measured life-history traits was species-specific, and the site of origin was an important factor influencing the adaptive response to the changes in the precipitation regime.

### Adaptive responses of the measured life-history traits

We observed an accelerated onset of flowering by 6.7 days in response to decreasing precipitation in *Aegilops*. Phenology shifts are an often observed response to global warming (Parmesan and Yohe 2003), and as a drought escape strategy, earlier flowering is associated with a shortening of the growing season or a decreasing predictability of rain events rather than the decrease of the amount of rain (Levitt 1972, Franks et al. 2007). The magnitude of this shift in *Aegilops* is comparable to the advancement of 8.5 days which was observed in *Brassica rapa* after seven generations that experienced a series of dry years and shortened growing season (Franks et al. 2007). Since the length of the growing season was not manipulated, we assumed that the acceleration of flowering time was an indirect result of accelerated growth due to lower plant densities, which might have resulted in a higher number of diaspores. In a more productive region as the Mediterranean site, competition for light is a limiting factor to an earlier switch from vegetative to reproductive phase (Weiner 1988, Petru et al. 2006, Holzappel et al. 2006). In the Mediterranean site, competition might have selected against shorter plant size and decreased biomass production, even under dry-treatment conditions. In this light an acceleration of flowering time in more productive environments appears unlikely as long as competition is a stronger selective factor.

Assuming the possibility of continued vegetative growth after the onset of flowering, earlier reproduction is advantageous as it maximises the reproductive output in preferable years, and increases the chances to reproduce in years with little rain. The question arises, why this trait shift was observed only in *Aegilops*. Among the studied species *Aegilops* flowered latest (Fig. 2). The earlier a species flowers the more likely a further acceleration of development is limited by the necessity to reach a minimum vegetative size rather than competition (Weiner 1988, Blum 1996, Kigel et al. 2011). Other species in the semi-arid site might be stronger limited by their vegetative size.

The optimal strategy of vegetative growth is a pivotal element in the life-history of an annual plant as it determines competitive abilities and influences phenological and reproductive traits. An effect of the changed precipitation on biomass production and plant size was found in several species originating from both sites, though the trends varied between species. The predicted increase of trait values with increasing water availability in the field as found in two of the studied species (*Crupina*, *Hymenocarpos* semi-arid origin) and corresponded to previous findings obtained along the rainfall gradient in the region (Aronson et al. 1990, 1993, Petru et al. 2006). The phenotypic variation in plant size in *Aegilops* was opposite to our predictions and showed a reduction in response to the addition of water, whereas biomass production was not different between treatments. Plants from the wet-treatment grew wider instead of taller, as a possible adaptive response to increased competition, which remains to be tested. Similar changes in architecture were reported previously for *Biscutella* (Petru et al. 2006). The increase in total biomass production with decreasing water availability in the field treatments in *Brachypodium* from the Mediterranean site was related to an increasing reproductive biomass, though reproductive allocation did not vary significantly.

The relative allocation of resources to reproductive biomass increased with decreasing water availability in the field, confirming our prediction. This reproductive allocation was the most consistently affected trait, however, the comparison between sites of origin showed that for this trait a distinct trend between sites remained only in *Biscutella*. Whether responses in reproductive allocation are plastic or adaptive appears to be species-specific. Our findings are in line with the controversial nature of previous findings (Levins 1963, Hickman 1975, Aronson et al. 1993, Petru et al. 2006). For *Biscutella* we can state that shifts in reproductive allocation are adaptive, and that the rain manipula-



tion in the Mediterranean site did not cause an inherited shift in this trait. Also, *Hymenocarpus* plants originating from both sites, exhibited a trend according to the prediction. In open habitats, a high number of seeds is an advantage, which compromises the competitive ability of a plant to persist under high plant density (Harper 1967). The inheritance of the trait shift in the Mediterranean population is an indication for an adaptive response, but it is possible that the phenotypic variation in this trait was not fully expressed under the nonstressful conditions in the greenhouse. We suggest that in *Hymenocarpus* reproductive allocation decreases as a plastic response to competition for light.

#### The effect of site of origin on the adaptive responses

Our results also confirmed the second prediction: the impact of the dry-treatment was greater than that of the wet-treatment in the semi-arid site and vice versa in the Mediterranean site (Tab. 3). Some species showed adaptive responses in several traits while others did not vary in any trait in the G2. Nonresponding species were exclusively of Mediterranean origin (*Avena*, *Plantago*, *Urospermum*). Previous studies showed that competition is higher in the Mediterranean site (Petrů et al. 2006, Holzapfel et al. 2006) and that it is likely that in the Mediterranean site drought is a less severe selective factor compared to competition (Liancourt and Tielbörger 2009). The finding of an increased mortality in few species in the wet-treatment of the Mediterranean site (Chapter 1), supports this conclusion. It is possible that differences due to the rain manipulation history are weak under common greenhouse conditions and might be detectable only under drought or competition, especially in the case of effects on the ability to survive. Since both factors were excluded in the greenhouse, the response to competition and drought was not tested. These results are in line with the hypothesis that the impact of decreasing precipitation is higher on populations in regions where water is already limited (Knapp et al. 2002).

Selection pressure can differ in its magnitude depending on the history of selection of a population, because of differences in the correlation between life-history traits and their effects on survival and fitness (Milla et al. 2009). The local environment affects adaptation and in turn, intraspecific trait variation influences abiotic and biotic factors (Hendry

et al. 2011, Bolnick et al. 2011). Whether adaptation is promoted or slowed down depends on a number of environmental and biotic factors and their interactions. There is consent about the influence of some of these factors like population size, genetic covariance between and genetic variation in the traits under selection (Bradshaw and McNeilly 1991, Willi et al. 2006, Jump et al. 2009). Others, like phenotypic plasticity, maternal effects, dispersal and community dynamics, leave us with many hypotheses and questions (Antonovics 1976, Bone and Farres 2001, Davis et al. 2005, Carroll et al. 2007, Matesanz et al. 2010). The adaptive potential of a population might be higher if less selective factors are involved, because the occurrence of dynamics and interactions between abiotic and biotic factors that slow contemporary evolution might be less frequent.

### Conclusion

Our findings support the view that rapid adaptation is not an exception, and instead was found in more than half of the studied species. Adaptation can happen on ecological timescales, but not all species might respond to the environmental changes with genetic differentiation. Climate change is not just decreasing precipitation but also alters community dynamics in an unpredictable way, which emphasises the need for studying the whole community instead of single species. Our study contributes findings for eight common species, still it does not allow to draw conclusions how the changes in precipitation affect rare species. The implication for rare species is that the bar for adaptation is much higher, because their fitness is already limited by more factors than just changing precipitation. The variation in magnitude and direction of selection on life-history traits was species-specific and depended on the ecological history and the complex interactions of biotic and abiotic factors which are unique in each of the studied sites. This shows that results derived from a single habitat are not a reliable basis for predictions outside this habitat. We can conclude that many species have the potential to adapt, but correlations between traits can restrict adaptation and the potential for antagonistic developments might increase with the number of selective factors. *In situ* adaptation might be the strategy of choice to ensure the local persistence of a population as long as it involves less changing environmental factors than migration.

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## Appendix and supplemental material

Tab. 4) Results of full factorial models (GLM) testing for the effects of species, sites of origin and rain manipulation treatment on phenotypic variation in the studied species originating from both study sites. Degrees of freedom (d.f.) were the same for all models.

Tab. 4a) Source of variation	Onset of flower		Size at flower		Total biomass		
	Chi <sup>2</sup>	p	Chi <sup>2</sup>	p	Chi <sup>2</sup>	p	d.f.
Species	1104.774	<b>&lt;.0001</b>	3493.965	<b>&lt;.0001</b>	2025.915	<b>&lt;.0001</b>	2
Station	190.242	<b>&lt;.0001</b>	94.983	<b>&lt;.0001</b>	41.117	<b>&lt;.0001</b>	1
Treatment (Field)	2.942	0.2297	0.573	0.751	5.439	0.0659	2
Species*Station	75.705	<b>&lt;.0001</b>	84.151	<b>&lt;.0001</b>	16.764	<b>0.0002</b>	2
Species*Treatment	2.246	0.6907	8.719	0.0685	14.023	<b>0.0072</b>	4
Station*Treatment	2.757	0.2519	2.984	0.2249	2.865	0.2387	2
Spec.*Stat.*Treatm.	5.740	0.2194	9.052	0.0598	7.025	0.1346	4

Tab. 4b) Source of variation	Reprod. allocation		Number of diasp.		
	Chi <sup>2</sup>	p	Chi <sup>2</sup>	p	d.f.
Species	118.113	<b>&lt;.0001</b>	1668.335	<b>&lt;.0001</b>	2
Station	3.613	0.0573	15.894	<b>&lt;.0001</b>	1
Treatment (Field)	8.408	<b>0.0149</b>	5.001	0.0821	2
Species*Station	9.211	<b>0.01</b>	13.985	<b>0.0009</b>	2
Species*Treatment	8.860	0.0647	7.564	0.1089	4
Station*Treatment	0.719	0.6982	2.917	0.2326	2
Spec.*Stat.*Treatm.	8.030	0.0905	8.945	0.0625	4

Tab. 5) Results of generalised linear models testing the effects of species and rain manipulation treatment on phenotypic variation including all species per site.

Trait	Source of variation	Semi-arid			Mediterranean		
		Chi <sup>2</sup>	p	d.f.	Chi <sup>2</sup>	p	d.f.
Time to 1 <sup>st</sup> flower	Species	791.330	<b>&lt;.0001</b>	3	840.336	<b>&lt;.0001</b>	6
	Treatment	1.805	0.4055	2	3.696	0.1575	2
	Treatm.*Spec.	12.155	0.0586	6	10.783	0.5476	12
Plant size at 1 <sup>st</sup> flower	Species	2604.573	<b>&lt;.0001</b>	3	4681.635	<b>&lt;.0001</b>	6
	Treatment	0.762	0.6832	2	1.315	0.5182	2
	Treatm.*Spec.	18.637	<b>0.0048</b>	6	13.480	0.3351	12
Total biomass	Species	2366.831	<b>&lt;.0001</b>	3	3232.975	<b>&lt;.0001</b>	6
	Treatment	4.882	0.0871	2	2.920	0.2322	2
	Treatm.*Spec.	19.469	<b>0.0034</b>	6	17.579	0.1291	12
Reprod. Allocation	Species	52.686	<b>&lt;.0001</b>	3	567.328	<b>&lt;.0001</b>	6
	Treatment	13.241	<b>0.0013</b>	2	0.100	0.9512	2
	Treatm.*Spec.	12.793	<b>0.0464</b>	6	22.981	<b>0.0279</b>	12
Number of diaspores	Species	557.371	<b>&lt;.0001</b>	3	4290.816	<b>&lt;.0001</b>	6
	Treatment	12.519	<b>0.0019</b>	2	0.759	0.6844	2
	Treatm.*Spec.	10.602	0.1015	6	24.041	<b>0.0201</b>	12

Tab. 6) Effects of site of origin and rainmanipulation treatment on phenotypic variation. Given are the number of observations (n), the mean trait values per rain manipulation treatment origin for each species in each site (SA=semi-arid, M= Mediterranean).

Tab. 6a)

			Number of diaspores						
			Mean ± Standard Error			Site effect		Treatment effect	
Spec.	Site	n	Dry	Control	Wet	Chi <sup>2</sup>	p	Chi <sup>2</sup>	p
Bis	SA	40	616 ± 35.4	538 ± 50.4	431 ± 39.1	ns		10.611	0.005
	M	44	509 ± 39.6	576 ± 33.4	583 ± 49.6			ns	
Bra	SA	59	81 ± 8.1	69 ± 2.9	73 ± 4.2	19.845	<.0001	ns	
	M	55	106 ± 7.7	96 ± 4.7	81 ± 5.3			10.523	0.0052
Hym	SA	39	157 ± 19.0	138 ± 14.9	168 ± 18.7	ns		ns	
	M	38	162 ± 11.6	186 ± 14.1	154 ± 22.3			ns	
Aeg	SA	74	30 ± 1.3	26 ± 1.3	27 ± 1.6	-		6.01	0.0495
Av	M	83	48 ± 1.9	49 ± 1.4	53 ± 1.9	-		ns	
Cru	M	66	59 ± 4.3	60 ± 3.9	56 ± 3.5	-		ns	
Pla	M	72	504 ± 34.9	500 ± 32.0	458 ± 39.5	-		ns	
Uro	M	37	1687 ± 390.1	994 ± 104.1	876 ± 112.5	-		ns	

Tab. 6b)

			Onset of flowering [number of days after germination]						
			Mean $\pm$ Standard Error			Site effect		Treatment effect	
Spec.	Site	n	Dry	Control	Wet	Chi <sup>2</sup>	p	Chi <sup>2</sup>	p
Bis	SA	58	84.9 $\pm$ 1.0	86.5 $\pm$ 1.1	84.1 $\pm$ 2.0	36.522	<0.0001	ns	
	M	76	90.8 $\pm$ 1.1	92.7 $\pm$ 1.0	91.8 $\pm$ 1.0			ns	
Bra	SA	140	74.2 $\pm$ 0.6	74.8 $\pm$ 0.5	74.0 $\pm$ 0.5	15.612	<0.0001	ns	
	M	140	76.5 $\pm$ 0.6	76.3 $\pm$ 0.7	75.4 $\pm$ 0.6			ns	
Hym	SA	69	65.8 $\pm$ 1.4	64.9 $\pm$ 1.4	65.3 $\pm$ 1.2	69.513	<0.0001	ns	
	M	71	73.8 $\pm$ 1.2	75.0 $\pm$ 1.4	75.3 $\pm$ 1.2			ns	
Aeg	SA	87	112.6 $\pm$ 2.0	119.3 $\pm$ 1.7	119.0 $\pm$ 1.8	-	-	7.372	0.0251
Av	M	95	98.7 $\pm$ 1.5	99.2 $\pm$ 0.9	97.6 $\pm$ 0.8	-	-	ns	
Cru	M	86	96.6 $\pm$ 1.0	94.9 $\pm$ 1.1	97.4 $\pm$ 1.4	-	-	ns	
Pla	M	87	99.6 $\pm$ 2.1	101.0 $\pm$ 2.5	103.7 $\pm$ 2.4	-	-	ns	
Uro	M	48	101.7 $\pm$ 1.7	105.4 $\pm$ 1.0	102.1 $\pm$ 1.8	-	-	ns	

Tab. 6c)

			Plant size [mm]						
			Mean $\pm$ Standard Error			Site effect		Treatment effect	
Spec.	Site	n	Dry	Control	Wet	Chi <sup>2</sup>	p	Chi <sup>2</sup>	p
Bis	SA	59	299 $\pm$ 11.3	267 $\pm$ 13.4	297 $\pm$ 22.2	43.284	<0.0001	ns	
	M	75	389 $\pm$ 20.0	373 $\pm$ 15.3	381 $\pm$ 17.2			ns	
Bra	SA	139	564 $\pm$ 10.1	586 $\pm$ 10.3	577 $\pm$ 9.5	4.609	0.0318	ns	
	M	142	607 $\pm$ 12.8	609 $\pm$ 11.7	584 $\pm$ 11.4			ns	
Hym	SA	69	199 $\pm$ 8.5	215 $\pm$ 5.7	202 $\pm$ 5.0	19.83	<0.0001	ns	
	M	71	236 $\pm$ 7.4	221 $\pm$ 7.4	232 $\pm$ 6.1			ns	
Aeg	SA	87	562 $\pm$ 14.4	562 $\pm$ 13.9	519 $\pm$ 10.6	-	-	7.133	0.0282
Av	M	95	888 $\pm$ 24.6	899 $\pm$ 15.5	882 $\pm$ 22.6	-	-	ns	
Cru	M	86	430 $\pm$ 17.6	474 $\pm$ 15.4	496 $\pm$ 22.4	-	-	7.286	0.0262
Pla	M	85	262 $\pm$ 6.5	264 $\pm$ 7.9	278 $\pm$ 8.2	-	-	ns	
Uro	M	48	834 $\pm$ 21.1	869 $\pm$ 27.6	853 $\pm$ 46.3	-	-	ns	

Tab. 6d)

			Total biomass [mg]						
			Mean $\pm$ Standard Error			Site effect		Treatment effect	
Spec.	Site	n	Dry	Control	Wet	Chi <sup>2</sup>	p	Chi <sup>2</sup>	p
Bis	SA	42	2.77 $\pm$ 0.14	2.65 $\pm$ 0.24	2.53 $\pm$ 0.14	24.908	<.0001	ns	
	M	43	3.26 $\pm$ 0.17	3.22 $\pm$ 0.12	3.50 $\pm$ 0.18			ns	
Bra	SA	59	1.52 $\pm$ 0.11	1.30 $\pm$ 0.04	1.44 $\pm$ 0.06	13.666	0.0002	ns	
	M	58	1.85 $\pm$ 0.10	1.76 $\pm$ 0.09	1.49 $\pm$ 0.09			7.635	0.022
Hym	SA	39	6.01 $\pm$ 0.34	6.30 $\pm$ 0.52	7.70 $\pm$ 0.38	5.750	0.0165	8.993	
	M	38	7.33 $\pm$ 0.44	7.88 $\pm$ 0.53	7.74 $\pm$ 0.76			0.0111	
Aeg	SA	72	7.81 $\pm$ 0.21	8.28 $\pm$ 0.20	7.88 $\pm$ 0.22	-	-	ns	
Av	M	83	6.19 $\pm$ 0.20	6.18 $\pm$ 0.11	6.03 $\pm$ 0.12	-	-	ns	
Cru	M	65	2.87 $\pm$ 0.21	3.27 $\pm$ 0.16	3.16 $\pm$ 0.16	-	-	ns	
Pla	M	74	1.79 $\pm$ 0.07	1.61 $\pm$ 0.10	1.65 $\pm$ 0.09	-	-	ns	
Uro	M	37	11.40 $\pm$ 0.80	12.26 $\pm$ 0.85	10.91 $\pm$ 0.39	-	-	ns	



Tab. 6e)

			Reproductive allocation						
			Mean $\pm$ Standard Error			Site effect		Treatment effect	
Species	Site	n	Dry	Control	Wet	Chi <sup>2</sup>	p	Chi <sup>2</sup>	p
Bis	SA	40	0.40 $\pm$ 0.01	0.34 $\pm$ 0.01	0.34 $\pm$ 0.01	14.79	0.0001	10.977	0.0041
	M	44	0.31 $\pm$ 0.01	0.31 $\pm$ 0.01	0.31 $\pm$ 0.02			ns	
Bra	SA	60	0.40 $\pm$ 0.02	0.42 $\pm$ 0.02	0.41 $\pm$ 0.01	ns		ns	
	M	58	0.44 $\pm$ 0.01	0.43 $\pm$ 0.02	0.41 $\pm$ 0.01			ns	
Hym	SA	39	0.50 $\pm$ 0.02	0.46 $\pm$ 0.03	0.44 $\pm$ 0.05	ns		ns	
	M	38	0.47 $\pm$ 0.02	0.48 $\pm$ 0.02	0.38 $\pm$ 0.04			7.166	0.0278
Aeg	SA	73	0.41 $\pm$ 0.01	0.37 $\pm$ 0.01	0.33 $\pm$ 0.02	-		14.976	0.0006
Av	M	83	0.41 $\pm$ 0.01	0.40 $\pm$ 0.01	0.42 $\pm$ 0.01	-		ns	
Cru	M	66	0.31 $\pm$ 0.02	0.32 $\pm$ 0.02	0.31 $\pm$ 0.02	-		ns	
Pla	M	73	0.25 $\pm$ 0.01	0.30 $\pm$ 0.01	0.25 $\pm$ 0.02	-		ns	
Uro	M	37	0.19 $\pm$ 0.01	0.18 $\pm$ 0.01	0.20 $\pm$ 0.01	-		ns	

### Chapter 3: Rain manipulation history affects phenotypic variation and fitness under different irrigation levels in annual plants

#### Abstract

In the context of global change, the ability of species to adapt to high rates of environmental changes is a key factor, determining whether populations face local extinction or can persist. Here we asked: a) does *in situ* adaptation occur after few generations under altered precipitation? And b) does it lead to improved survival and fitness under drought? We subjected species to rainfall manipulations *in situ* within their communities, thus providing a realistic scenario for selection processes. We raised plants of two winter annuals (*Biscutella didyma*, *Hymenocarpus circinnatus*) under an irrigation gradient in the greenhouse from seeds produced by mother plants growing under different rain manipulation treatments in a Mediterranean and a semi-arid site. We measured phenology, growth and reproduction related traits and compared survival and fitness under drought. We predicted higher survival and higher fitness under drought due to adaptive shifts in life-history traits. The trends in these shifts were expected to resemble the trends between the Mediterranean and the semi-arid population. In *Biscutella didyma*, eight years of selection under altered water availability in the field caused higher seed number under drought in plants originating from the dry-treatments of the semi-arid site compared to plants from the control and the wet-treatment. According to our predictions, selection under lower water availability favoured smaller diaspores and a higher reproductive allocation. Our results provide a reliable indication that some species might be able to cope with rapidly changing environments, which confirms that *in situ* adaptation is an important alternative to migration.

## Introduction

Under the predicted changes in environmental conditions such as decreasing precipitation (IPCC 2007, 2012), species can either migrate in order to track their favourable climatic conditions or change their physiology to tolerate the reduced water availability. *In situ* adaptation is widely acknowledged as a key factor for species to persist under climate change (Lynch and Lande 1993, Burger and Lynch 1995, Davis and Shaw 2001, Thuiller et al. 2005, Jump and Penuelas 2005), but the likelihood of plant species to quickly adapt to environmental changes is highly debated (Kinnison and Hendry 2001, Reznick and Ghalambor 2001, Etterson and Shaw 2001, Ackerly 2003, Franks et al. 2007, Gienapp et al. 2008). Decreasing precipitation as a consequence of climate change is likely to increase mortality and reduce the rate of reproductive success, and thus, driving evolutionary change (Boyer 1982, Lee and Bazzaz 1986, Ackerly et al. 2000, Chaves et al. 2003, Cahill et al. 2012).

Widely distributed species are locally adapted to their immediate environments, which increases fitness as costs of plasticity are reduced (DeWitt 1998). Locally adapted genotypes have a fitness advantage over transplanted genotypes (Joshi et al. 2001). Accordingly, we assume that inherited phenotypic shifts in response to selection under drought result in a fitness advantage of the subpopulation that experienced this selection over a subpopulation which did not. So far, only two studies tested if such phenotypic shifts resulted in improved survival and reproductive success in the altered environment and showed that such adaptive processes are possible within few generations (Franks et al. 2007), although they might be too slow compared to the rates of environmental change (Etterson and Shaw 2001).

Life-history traits are under two opposing selective forces along aridity gradients (Petrů et al. 2006, Schiffrers and Tielbörger 2006, Liancourt and Tielbörger 2009). At the arid end, water limitation causes high mortality and is a major constraint to plant growth and reproduction (Boyer 1982, Lee and Bazzaz 1986, Chaves et al. 2003). At the productive end, plant density is high and biotic interactions, e.g. competitive exclusion, are the major selective force. Biotic interactions play an important role in the partitioning of ecological niches and, in addition to adaptation, determine the distribution of species and community composition in a given habitat (Herrera 1992, Weiher et al. 1995). The

complex dynamics of biotic interactions might affect *in situ* adaptation (Ackerly 2003, Wiens and Graham 2005). Climate change alters these biotic interactions by affecting growth and competition (Ayres 1993, Lynch and Lande 1993). Hence, selection can occur also due to altered biotic interactions. The direct effect of changed precipitation on selective processes might be weakened by biotic interactions at the productive end of the gradient.

Fitness related traits such as vegetative growth, timing of the reproductive phase and allocation of resources to reproductive organs and diaspores are key traits in the study of adaptation to long-term environmental stress (Antonovics 1976, Grime 1977, Schlichting 1986, Stearns 1992, Ackerly et al. 2000). Phenotypic variation in these traits, that corresponds to the variation in precipitation, has been observed along rainfall gradients within a number of species (Aronson et al. 1990, Petrů et al. 2006, Volis 2006, Liancourt and Tielbörger 2009, Kigel et al. 2011).

A compensation for higher mortality and fitness loss, either under drought or increased competition, can be achieved by a number of physiological and morphological adjustments. At the mesic end of the gradient light is a limiting factor due to high plant density. Hence, selection favours increased plant size and biomass in order to outcompete neighbours and maximise fitness (Cohen 1976, Weiner 1988). Although a prolonged phase of vegetative growth is associated with a delay in reproduction, it promotes the acquisition resources that are available for reproduction (Antonovics 1980, Bazzaz et al. 1987). Toward the arid end, decreasing precipitation reduces the above-ground competition, and biomass production is limited by water availability. Adaptations that minimise water loss include reduced plant height and a slow vegetative growth (Fox 1990). A higher relative allocation of biomass to diaspore production instead of vegetative biomass can maximise the reproductive output (Marshall 1986, Aronson et al. 1993, Petrů et al. 2006, Holzapfel et al. 2006, Liancourt and Tielbörger 2009). The reduction of vegetative growth has limits since plants have to reach a certain size before they are able to reproduce (Holdsworth 1956, Harper and White 1974, Waller 1988, Schmid et al. 1995).

The timing of the switch from vegetative to reproductive phase is a crucial element in plant life-history as it determines fitness to a great extent (Cohen 1976). Under high

competition, an early switch to reproductive phase might be a disadvantage since reproduction requires resources that are no longer available for further vegetative growth and result in suppression by neighbour plants (Antonovics 1980, Bazzaz et al. 1987). In arid environments, where fluctuations of the growth season length is high, early flowering individuals might be able to reproduce while late flowering can lead to reproductive failure and exclusion from the population (Rathcke and Lacey 2007). Earlier flowering is a drought escape strategy under terminal drought at the end of the season, that has been observed in multiple species along aridity gradients (Aronson et al. 1992, Heschel and Riginos 2005, Petru et al. 2006, Volis 2006, Sherrard and Maherali 2006, Lampei 2011, Kigel et al. 2011).

Seed size is a trait that influences initial seedling size and, thus, can enhance seedling establishment and survival (Marshall 1986, Westoby et al. 1992, 2002, Leishman and Westoby 1994). Selection for a high seed number is considered antagonistic to larger seed size. Larger seeds can be advantageous under both high competition and unpredictable environments, therefore, hypotheses on adaptive response of seed mass are ambiguous. At the community level seed mass was found to decrease with increasing aridity (Harel et al. 2011). This finding may indicate the direction of selection under drought and rain unpredictability.

We studied the effect of selection under different rain manipulation treatments in the field, on the survival, reproductive success and life-history traits in the greenhouse, under an irrigation gradient. The purpose of the rain manipulations in the field was to simulate the predicted change in precipitation in the eastern Mediterranean Basin in the wake of global warming (Holzapfel et al. 2006, Sternberg et al. 2011). The experimental treatments consisted of reduced natural precipitation by approximately 30% (dry-treatments), an unaltered control (control-treatment) and, additional irrigation (wet-treatments) which increased the amount of water by 30% as a contrasting treatment. The treatments did not change the frequency of rain events. Plants of two annual species were raised from seeds produced by mother plants growing under the rain manipulation treatments in both, the semi-arid and the Mediterranean site. Their offspring were grown under an irrigation gradient in a greenhouse that simulated and exceeded the water availability conditions in the field. This long-term experiment

allowed us to show whether inherited phenotypic shifts due to eight years of rain manipulation in the field can contribute to fitness under drought.

#### Predictions:

##### - Survival and fitness

We predicted that plants originating from the semi-arid site have a higher survival and produce more seeds under drought, compared to plants from the Mediterranean site. The same trend was expected within each site: Lower water availability *in situ* selects for better adapted genotypes which surpass plants from the control- and wet-treatments in survival and fitness under drought. We expected that the rain manipulation treatments in the field had a stronger effect on survival and fitness in the semi-arid populations compared to the Mediterranean populations.

##### - Selection on life-history traits

We predicted that lower water availability *in situ* selects for earlier flowering, reduced plant size, smaller seeds, and higher reproductive allocation. We expected to find that the effect of rain manipulation history differs between the sites of origin. Since our irrigation gradient experiment excluded biotic interactions, we expect phenotypic shifts benefitting fitness under drought to be weaker in the Mediterranean population compared to the semi-arid population, because in the Mediterranean environment biotic interactions are a stronger selective force.

## Methods

Study species:

*Biscutella didyma* (*Brassicaceae*) and *Hymenocarpus circinnatus* (*Fabaceae*) are common winter annuals with a Mediterranean and Irano-Turanian distribution (Zohary 1937). Diaspores of *Biscutella* are one seeded, while in *Hymenocarpus* the dispersal unit is a dry legume with two seeds. Both species are abundant in the semi-arid and the Mediterranean sites and could easily be cultivated in the greenhouse. To equalise maternal effects, the seeds for the experiment were produced under same standard conditions in the greenhouse in the previous season (G1), and kept in a nethouse in the Faculty of agriculture of the Hebrew University of Jerusalem in Rehovot, Israel for two months to break dormancy (Petrů et al. 2006). Full-sibs of each rain manipulation site x treatment combination were raised under different irrigation levels in a greenhouse. Since the studied species are selfers, we assume that the siblings are genetically similar.

Irrigation gradient:

In order to compare the effect of the field rain manipulations under optimum conditions and different levels of water availability, an irrigation gradient was set up in the greenhouse of the University of Tuebingen, Germany. The irrigation system was built according to the line source irrigation presented by Johnson et al. (1982) and modified by Lampei (2011, Thesis unpubl.) to meet the requirements of this experiment. The system consisted of seven irrigation levels, which were replicated for each species 8 times (irrigation gradient units). The amount of irrigation was adjusted using the mean annual precipitation measured (+/- 30% for the treatments) and compared to the mean annual soil humidity, both of which were recorded over the manipulation period from 2002 until 2010 (GLOWA Project Database). The range of water supply as well as soil humidity exceeded the range of precipitation and soil humidity in the field manipulations (Fig. 1). The lowest irrigation level (IL1) caused a high mortality, thus showing that the physiological limit of the plants was reached.

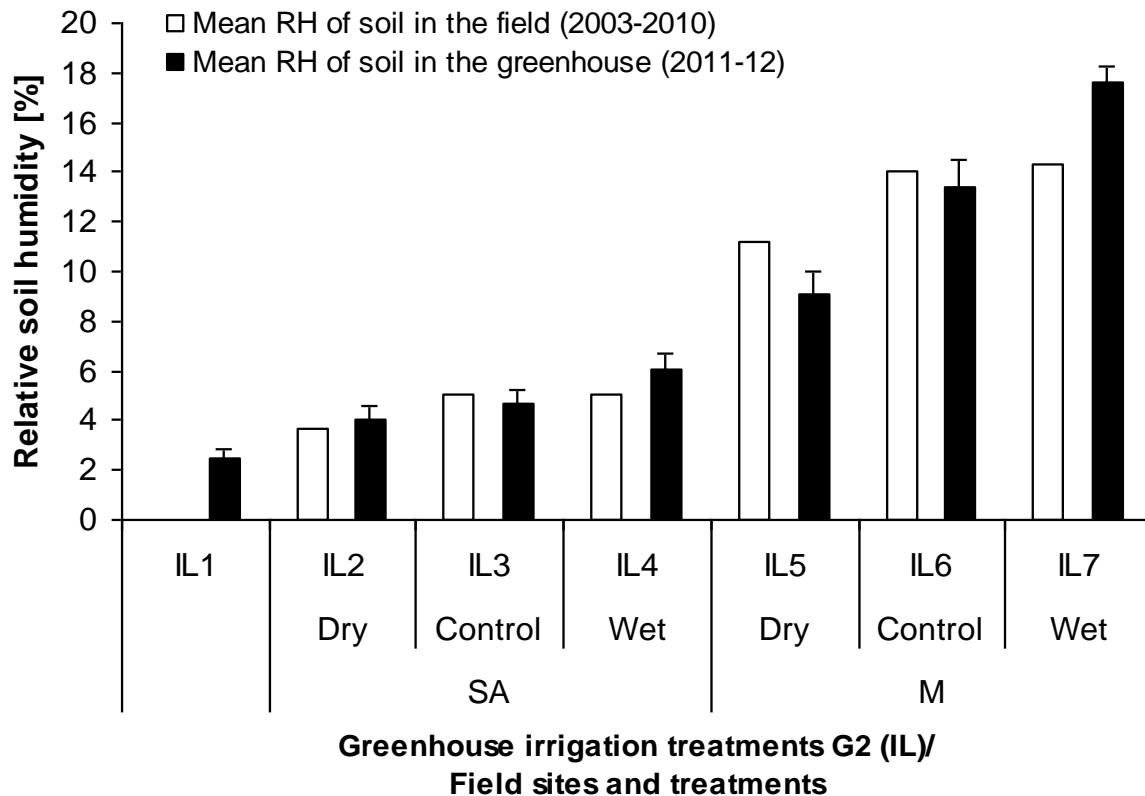


Fig. 1) Comparison of the mean relative soil humidity in the field sites for the last 7 years of rain manipulations, and for the irrigation gradient in the greenhouse ( $\pm$  Standard error). (For precipitation/irrigation comparison see Tab. 1b in the Appendix.)

#### Cultivation:

As substrate we used a mixture of sand and garden soil with low nutrient content (1:1) and 100 ml Osmocot Scott® fertilizer (15 % N, 9 % P<sub>2</sub>O<sub>5</sub>, 9 % K<sub>2</sub>O) per 15 l of soil/sand mixture. Ten (*Hymenocarpus*) or 12 (*Biscutella*) seeds per mother plant were sown into pots (90 mm x 90 mm, 100 mm deep) and covered with a thin layer of substrate to prevent desiccation. Seeds of *Hymenocarpus* had to be manually scarified to ensure germination. The day of initial irrigation was the 21<sup>th</sup> of November 2011. The temperature in the greenhouse ranged from 16°C at night to 22°C during the day, and natural lighting was used. Pots were randomised before each irrigation within each irrigation level and once per month over all eight irrigation gradient units in the greenhouse. The relative substrate humidity was measured before every second irrigation with a TRIME®-FM2 (IMKO Mikromodul-technik GmbH) in 1 out of 9 pots (11%). Whenever the soil humidity in the drought stress level IL2 and 3 was below 5% all plants were irrigated. Irrigation time increased with the development of the plants,



first from 2 min 30 s every 3-4 days at seedling stage to 3 min 45 s every other day when plants were fully grown.

#### Measurements:

Fitness was evaluated by the number of mature diaspores produced per plant, as calculated from the total diaspore weight per plant and the weight per diaspore. The latter was calculated from the weight of 20 randomly chosen diaspores per individual. The day of germination was recorded for each plant to obtain a precise time for the onset of flowering. At the day of first open flower the plant size (i.e. height/length) was measured from the cotyledonary node to the tip of the plant. Since germination spanned 4 weeks, the hypocotyl length was not included in the assessment of plant size, since it varied according to the light conditions during germination and seedling emergence. The hypocotyl length could have confounded results, especially in the more stressing irrigation levels because of the strong size reduction of these plants. At the end of the growth cycle and ensuing plant senescence, plants were harvested. Above-ground vegetative biomass was dried at 70°C for 24 h and weighed. Dry diaspores/fruits were collected separately, stored under room conditions and weighed. Reproductive allocation was calculated as the ratio of the total mass of mature diaspores/fruits from total above-ground plant biomass.

#### Statistics:

All statistical analyses were computed with the software package JMP 7.1 (SAS). A survival analysis was applied to the plants in the irrigation gradient to analyse whether the rain manipulation history of the respective groups had an effect on mortality under drought conditions. The number of surviving individuals at the time of seed set was counted for both species to compare survival. A Poisson distribution and a log link-function were used in a full-factorial generalised linear model. The model included irrigation level, site (semi-arid or Mediterranean origin of the populations), treatment (individuals originating from the rain manipulation treatments Dry, Control and Wet) as fixed factors, and the parameter "initial number of individuals" as a covariate.

The significance of the effects on the measured traits was tested with a multiple regression model. Trait values were averaged for each site x treatment combination in each irrigation level. The independent variables in the model were soil humidity (as a continuous variable instead of irrigation levels), site and rain manipulation treatment.

For the data of *Hymenocarpus* we used a log-transformation of relative soil humidity to achieve linearity for all variables except total biomass and number of fruits. In the case of *Biscutella* this transformation was used only for the data of plant size. Tukey-tests were used for post-hoc multiple comparisons of the means. The significance levels used in all analyses are 0.05 (\*), 0.01 (\*\*), and 0.001 (\*\*\*).

## Results

### Survival analysis

The effect of the irrigation levels on survival was similar in both species. Survival of the semi-arid population of *Biscutella* was significantly higher in the most stressed irrigation level (IL1 with 3.18% soil humidity) compared to the Mediterranean population. Differences in survival due to rain manipulation history were not significant. The survival of *Hymenocarpus* differed only between irrigation levels.

Tab. 1) Results of the survival analysis, testing the effects of irrigation level in the greenhouse gradient, and site and treatment origin on survival.

Source of variation	d.f.	Biscutella		Hymenocarpus	
		Chi <sup>2</sup>	p	Chi <sup>2</sup>	p
Irrigation level (IL)	6	298.246	<b>&lt;.0001</b>	181.992	<b>&lt;.0001</b>
Site	1	13.249	<b>0.0003</b>	1.626	0.2022
Treatment (Field)	2	3.592	0.1660	3.015	0.2215
IL*Site	6	41.175	<b>&lt;.0001</b>	7.180	0.3046
IL*Treatment	12	5.711	0.9299	14.210	0.2875
Site*Treatment	2	0.141	0.9138	2.173	0.3373
IL*Site*Treatment	12	8.039	0.7821	13.536	0.3313

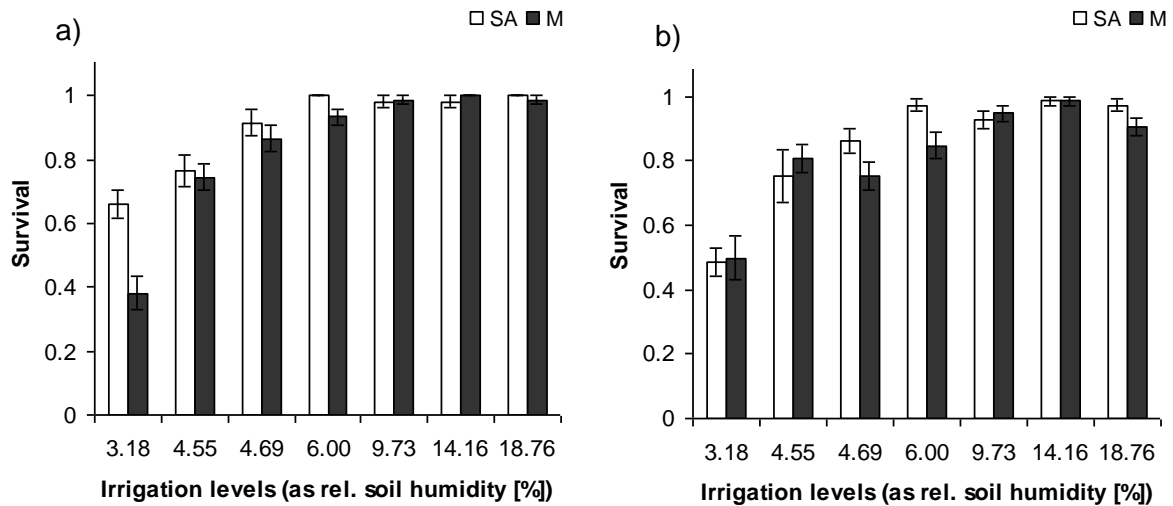


Fig. 2) Survival until the time of seed set in *Biscutella* (a) and *Hymenocarpus* (b) under seven levels of irrigation (from IL1 as the driest level to IL7 with the highest amount of water), displayed over mean relative soil humidity.

### Fitness

The number of diaspores per plant was positively correlated to water availability in both species (Fig. 3). In *Biscutella*, no difference in fitness was found between the two sites of origin (Tab. 2a), but the increase in diaspore number with soil humidity was significantly higher in the Mediterranean population ( $p=0.0231$ ,  $F=5.729$ ). No variation in fitness was found in relation to the rain manipulation treatments in the Mediterranean site. In the semi-arid population, on the other hand, plants originating from the wet-treatments produced significantly less diaspores (Tab. 2a) compared to plants from the control- and dry-treatments. Under drought, they reached 50% and under the highest irrigation level 70% of the number of diaspores that was produced by plants from the dry-treatment, which had the highest reproductive output. The increase in diaspore number with higher water availability was smaller in plants of wet-treatment origin compared to plants from the control- and the dry-treatment. In *Hymenocarpus*, the number of fruits changed significantly only in response to water availability in the irrigation gradient, but not between sites nor between treatments within sites (Tab. 2b).

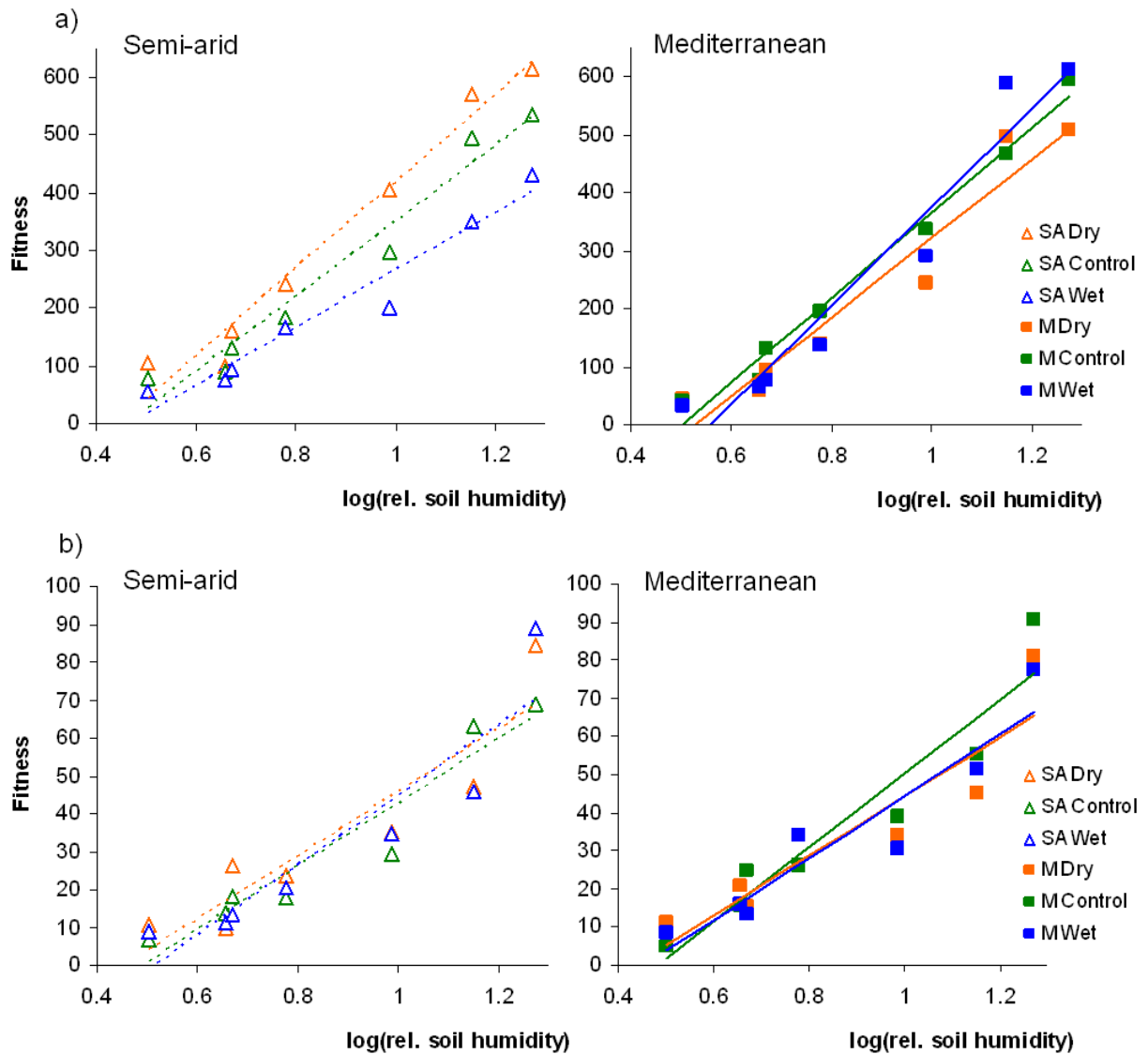


Fig. 3) The variation in mean fitness, measured as number of mature diaspores in *Biscutella* (a) and fruits in *Hymenocarpus* (b) across all irrigation levels in the greenhouse, displayed as mean relative soil humidity.

### Phenotypic responses to the irrigation gradient

Almost all measured traits in both studied species were affected by differences in water availability across the greenhouse irrigation gradient (Tab 2a+b, for the results of the full model see tables 3a and b in the Appendix). Under highest irrigation, the onset of flowering in *Biscutella* was earlier by 4-7 days in the semi-arid population and 11-13 days in the Mediterranean population compared to the level of lowest irrigation (Fig. 4a). In *Hymenocarpus*, the increase of irrigation had the opposite effect on the flowering time in plants from the two site origins. The onset of flowering was accelerated by 3-9 days

with increasing irrigation in Mediterranean plants, while in semi-arid plants a delay by 1-3 days was observed (Fig. 4b). In both species the plant size at the onset of flowering, biomass production and reproductive allocation increased with increasing irrigation in the greenhouse gradient (Figs. 5 and 6, a and b respectively). It is notable, that the plastic responses of diaspore weight to variation in water availability differed between the two studied species. The response of diaspore weight with increasing irrigation was a reduction in *Biscutella* (Fig. 7a) and an increase in *Hymenocarpus* (Fig. 7b).

#### Differences between sites of origin

In *Biscutella* and *Hymenocarpus*, site origin had a significant effect on all measured traits, except for diaspore weight (Tab. 2a+b). In both species, the Mediterranean populations flowered later than the semi-arid ones with a difference of approximately 8 days in *Biscutella* and 12 days in *Hymenocarpus* (Figs. 4a+b). The time until the onset of flowering in *Biscutella*, increased with decreasing water availability significantly more in the Mediterranean population compared to the semi-arid population ( $p=0.0329$ ,  $F=5.003$ ). Also in *Hymenocarpus*, the onset of flowering was significantly more delayed in plants from the wet-treatment of the Mediterranean population compared to the other two treatments ( $p=0.0478$ ,  $F=4.259$ ). In both species, Mediterranean plants were taller, produced more biomass and had a higher reproductive allocation compared to those from the semi-arid site (Figs. 5,6). The Mediterranean population of *Hymenocarpus* had a significantly greater biomass increase with increasing irrigation compared to the semi-arid population ( $p=0.0347$ ,  $F=4.896$ ). The variation of diaspore weight within the semi-arid population of *Biscutella* exceeded the variation of the Mediterranean population. Although the Mediterranean population had a significantly higher diaspore weight compared to the semi-arid population, the highest weights were measured in plants from the semi-arid wet-treatment (Fig 7). In *Hymenocarpus*, no significant differences in mean weight per diaspore were found between sites.

## Effect of selection history

The effect of the *in situ* rain manipulation treatments on the measured life-history traits was species- and site-specific. The effect of the selection history on flowering time was significant only for *Biscutella* (Tab. 2a). Plants from the control-treatments flowered approximately 3-4 days (Mediterranean population) and 2-3 days (semi-arid population) later compared to plants from the respective dry- and wet-treatments. The rain manipulation treatments had a significant effect on plant size in both species, though only on the respective semi-arid population.

In *Biscutella*, plants from control-treatment were shorter, whereas in *Hymenocarpus*, they were taller at the onset of flowering compared to plants from the dry- and wet-treatment (Fig. 5, Tab. 2). In contrast to plant size, total biomass was not affected by selection history in *Biscutella* nor in *Hymenocarpus*. The rain manipulation treatments had no effect on reproductive allocation in *Hymenocarpus* and in the Mediterranean population of *Biscutella*. *Biscutella* plants from semi-arid dry-treatment origin had a significantly higher reproductive allocation compared to those from the control- and the wet-treatment (Tab. 2a, Fig. 6). In the semi-arid population of *Hymenocarpus* the weight per seed was highest in plants from the control-treatment (Tab. 2b). Here, plants from the wet-treatment had a notable, though nonsignificant seed weight increase with increasing water availability in the irrigation gradient (Fig. 7b). A significantly higher weight per diaspore was found in plants from the wet-treatment in the semi-arid site of *Biscutella*, which was opposite to the trend in plants from the Mediterranean site where the dry-treatment had the highest weight per diaspore (Fig. 7a).

Tab. 2) Differences in phenotypic variation in response to the greenhouse irrigation gradient, depending on site and rain manipulation treatment origin in a) *Biscutella*, b) *Hymenocarpus*.

Tab. 2a) <i>Biscutella</i>	Response to irrigation gradient	Differences between sites	Differences between treatments within sites	
			Semi-arid	Mediterranean
Number of diaspores	p < 0.0001, F=734.92	ns	p=0.0003, F=14.551	ns
Onset of flowering	p < 0.0001, F=47.348	p < 0.0001, F=128.343	p=0.0058, F=7.405	p=0.0468, F=3.782
Height at onset of flowering	p < 0.0001, F=231.671	p < 0.0001, F=100.128	p=0.0155, F=5.577	ns
Total biomass	p < 0.0001, F=65.665	p=0.0037, F=9.935	ns	ns
Reprod. allocation	p < 0.0001, F=346.178	p < 0.0001, F=28.838	p=0.0006, F=12.581	ns
Weight per diaspore	p < 0.0001, F=98.057	p=0.0427, F=4.481	p=0.0004, F=14.040	p=0.0163, F=5.484

Tab. 2b) <i>Hymenocarpus</i>	Response to irrigation levels	Differences between sites	Differences between treatments within sites	
			Semi-arid	Mediterranean
Number of diaspores	p < 0.0001, F=526.784	ns	ns	ns
Onset of flowering	ns	p < 0.0001, F=260.465	ns	ns
Height at onset of flowering	p < 0.0001, F=533.028	p < 0.0001, F=70.242	p=0.0044, F=7.946	ns
Total biomass	p < 0.0001, F=983.807	p=0.0003, F=17.145	ns	ns
Reprod. allocation	p < 0.0001, F=26.511	p < 0.0001, F=20.526	ns	ns
Weight per diaspore	p < 0.0001, F=33.077	ns	p=0.0141, F=5.740	ns

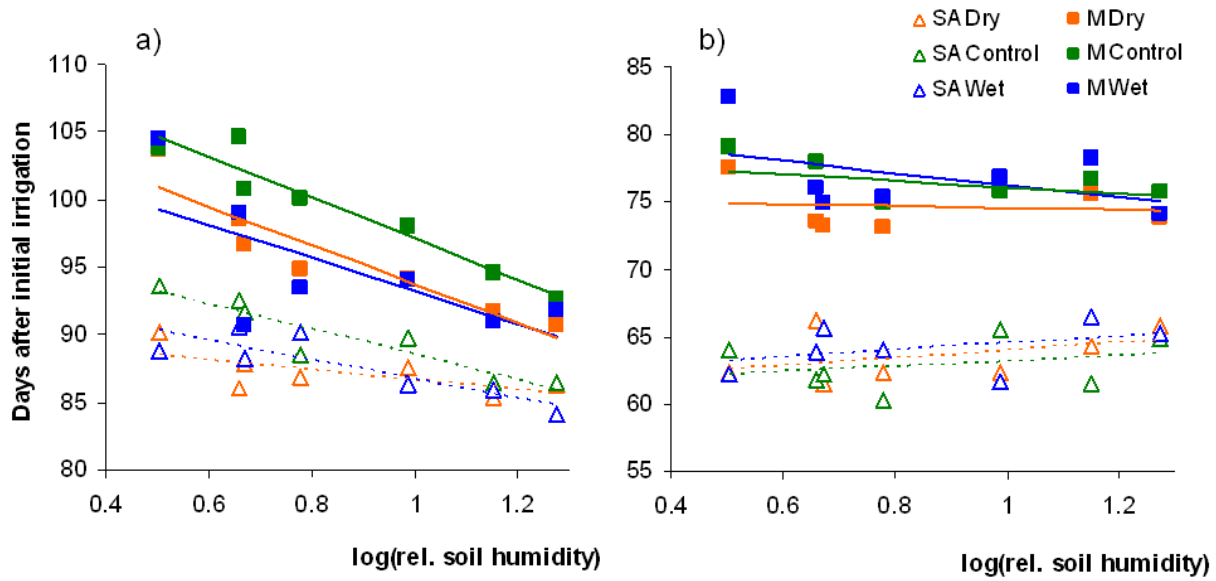


Fig. 4) Onset of flowering under increasing irrigation levels in *Biscutella* (a) and *Hymenocarpus* (b), plotted over increasing relative soil humidity.

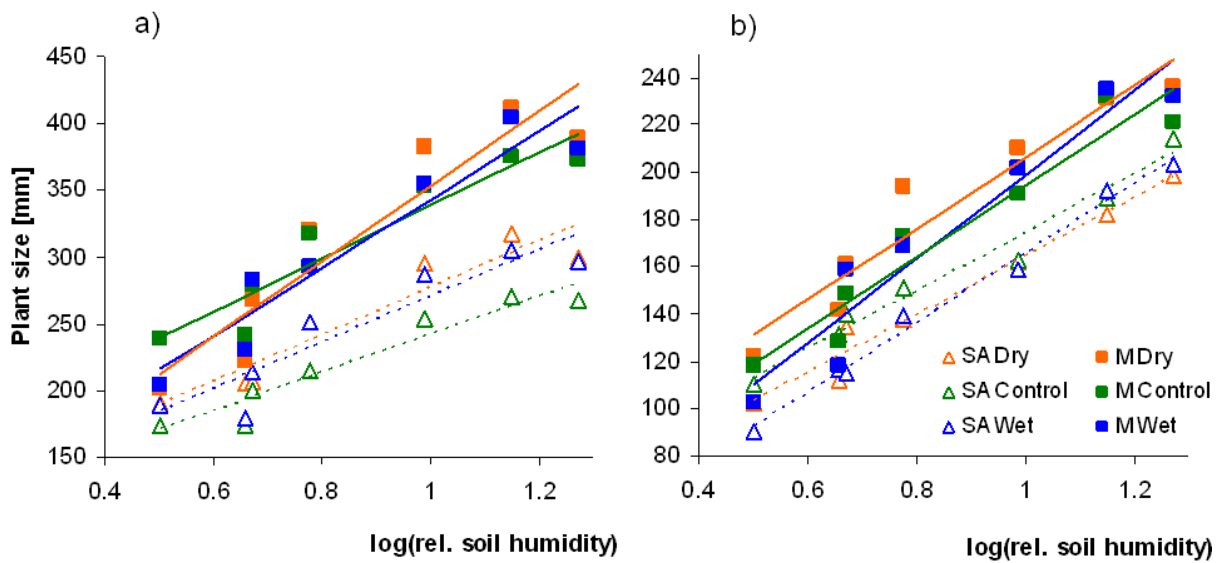


Fig. 5) Plant height at the onset of flowering in *Biscutella* (a) and *Hymenocarpus* (b).



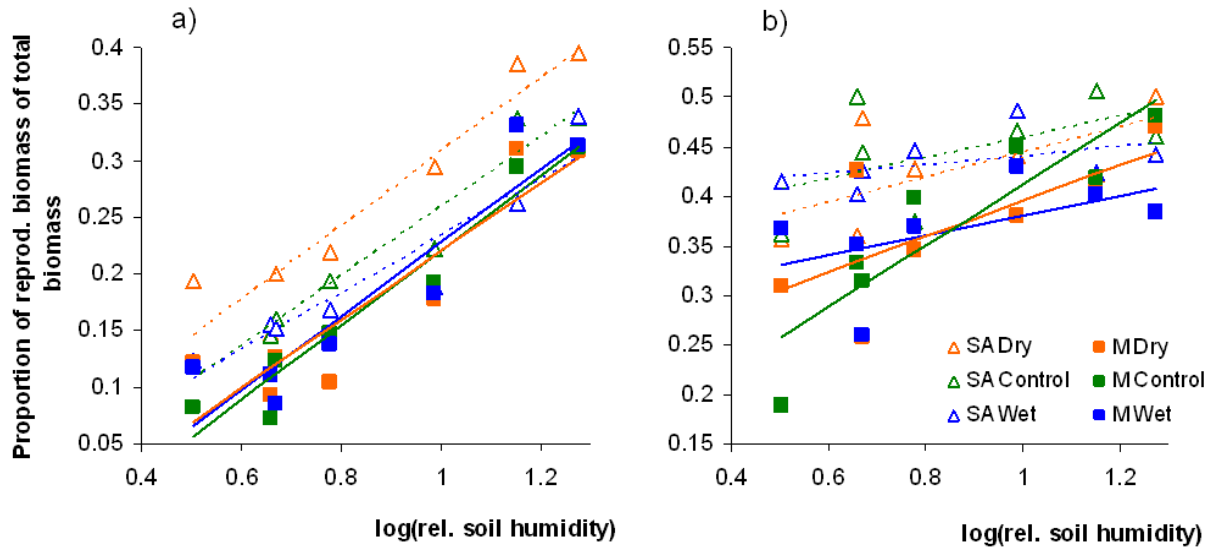


Fig. 6) Reproductive allocation in *Biscutella* (a) and in *Hymenocarpos* (b).

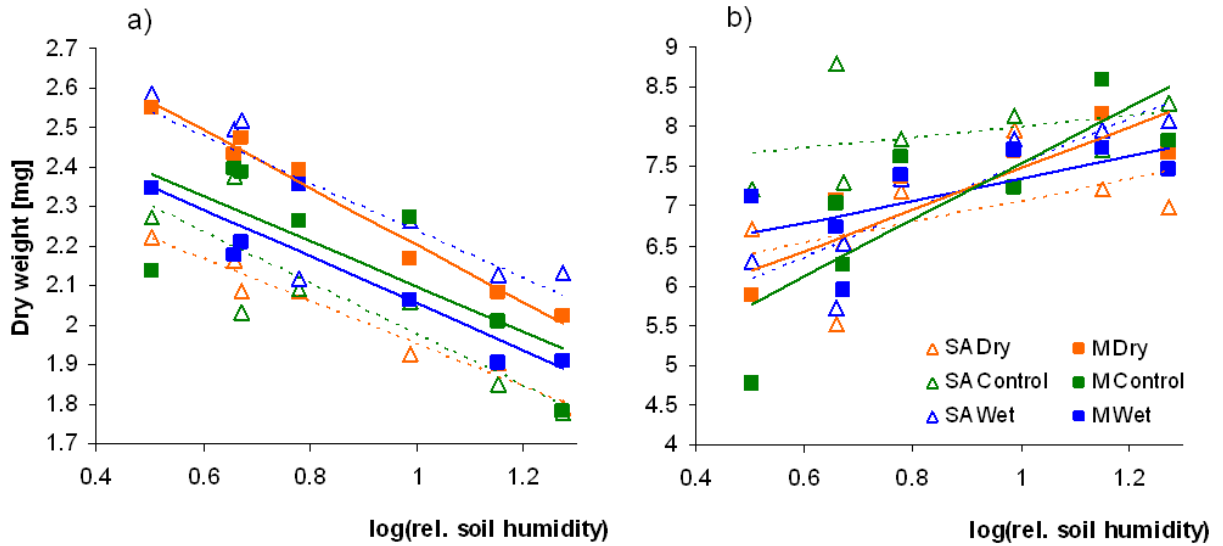


Fig. 7) Weight per diaspore in *Biscutella* (a) and per seed in *Hymenocarpos* (b).

## Discussion

Our results provide evidence for *in situ* adaptation after eight years of rain manipulation. Changes in the precipitation regime in the field led to adaptive shifts in life-history traits, altering fitness under drought in the greenhouse. A higher fitness in *Biscutella* plants originating from the semi-arid dry-treatments in relation to plants from the respective control-and wet-treatments was observed under all greenhouse irrigation levels. This improved fitness under drought was associated to adaptive shifts in the reproduction related traits.

Firstly, the increase in resource allocation to reproductive biomass allowed a higher number of diaspores. The observed shifts in reproductive allocation in the semi-arid population of *Biscutella* resembled the trend between sites as predicted, and corresponded to previous findings (Petrů et al. 2006). So far, previous studies could not decisively show whether responses in reproductive allocation are plastic under low water availability and low above-ground competition, as found by Aronson et al. (1993) and Hickman (1975), or whether this trait has indeed the potential of a fast adaptive response to increasing aridity as suggested by Levins (1963) and Petrů et al. (2006). For *Biscutella* we can show that reproductive allocation is an adaptive trait, quickly responding to changes in the amount of precipitation and contributing to improved fitness under drought. Secondly, *Biscutella* of semi-arid wet-treatment origin had an adaptive shift in seed weight that negatively influenced fitness in these plants, regardless of the water availability in the greenhouse. Increasing water availability selected for an increased diaspore weight in the semi-arid population, which corresponds to our prediction and is supported by the findings of Harel et al (2011).

Interestingly, short-term selection on diaspore weight under the rain manipulation was profoundly affected in *Biscutella* by the long-term selection history of the two field sites. Within the Mediterranean population of *Biscutella* the trend in response to the rain manipulation treatments was opposite to that in the semi-arid population. The diaspores produced by plants originating at the dry-treatment were the largest, while the lowest weight per diaspore was found in plants originating from the wet-treatment. A decrease in seed weight in response to water shortage can maximise the number of seeds (Fox 1990). An increase in weight, on the other hand, is an investment of limited resources in

well-equipped rather than numerous seeds and can improve the survival of the seedlings under drought, but also under competition (Marshall 1986, Westoby et al. 1992, 2002, Leishman and Westoby 1994). Our data from the greenhouse experiment gave no indication how larger diaspores influenced growth and phenology and improved survival under drought. But maybe the key for understanding the trends in seed weight response are seed predation and dispersal, or other biotic factors, which were not part of this greenhouse experiment (Thompson 1987, Westoby et al. 1992).

The second characteristic that was measured to assess the occurrence of adaptation was survival under drought in the greenhouse. Long-term selection in the semi-arid environment resulted in higher survival rates under drought in the greenhouse gradient. In concordance to our prediction, the semi-arid population of *Biscutella*, had a higher survival rate under drought and therefore, are better adapted to arid conditions in relation to the Mediterranean population. In neither of the two studied species survival was affected by the rain manipulation treatments. It is likely that a change in rain predictability or the shortening of the season has a severe effect on survival (Franks et al. 2007).

We found a lack of adaptive shifts in phenology and growth related traits in *Biscutella* and the absence of any differences in survival or fitness in *Hymenocarpus*. In both species the later flowering of the Mediterranean populations was associated with a larger size at this time. Within site of origin, however, neither plant growth nor total biomass did vary, or the trends were not consistent. Therefore, and contrary to our expectations, no indirect selection on flowering time resulted from shifts in growth related traits.

Our findings in the first chapter showed that decreasing precipitation had no effect on the survival in the field, which is a sign of adjustment to the environment. Then, it was unclear whether this adjustment was caused by a shift in genetic composition or by plasticity. Now, the lack of differences in these traits in *Biscutella* and the absence of any effect of drought in the greenhouse irrigation gradient on survival and fitness in *Hymenocarpus* verifies that the phenotypic trait variation in the field was a plastic response. The range of physiological plasticity allowed to buffer directional selection caused by the rain manipulation treatments on phenology and growth related traits. The

strong between year fluctuations of resource limitation and competition in environments like in our study sites demand a high plasticity to maximises fitness (Cohen 1971, 1976, Bazzaz et al. 1987, Schmid et al. 1995). We suggest that the environmental change and consequently the selection pressure was not strong enough to cause an adaptive response to the rain manipulation treatments in *Hymenocarpos*. This interpretation can be supported by the lower phenotypic variation between sites of origin in most measured traits compared to *Biscutella*. An additional factor to delay adaptive processes in the case of *Hymenocarpos* is probably the high seed dormancy. This might be a bias in our results due to plants that germinated from seeds which were produced prior to the rain manipulation period.

Our findings for fitness and survival under drought in the greenhouse imply that the studied species can persist under conditions that correspond to the current projection of climate change in the region. In relation to the maximum number of fruits/diaspores, plants of both species had a fitness loss of up to 95% under drought. Despite 60% mortality rate, the Mediterranean population of *Biscutella* might be able to persist even under water availability conditions similar to those in the lowest irrigation level of the greenhouse gradient. Regarding the rather stable survival rates we can assume for *Hymenocarpos* that this species has the ability to persist under climate change.

As much as it is important to assess the occurrence and magnitude of adaptation, it is also highly relevant to learn about possible reasons why species do not respond to altered precipitation. Such species might be either the most or the least sensitive to a change in water availability. For example, high seed dormancy can lead to a relative lower number of generations under selection compared to non-dormant species, and thus constrain evolutionary processes. In this context, a comparative study on adaptive response under stress of a large number of species with differing dormancy could provide an answer. In species like *Biscutella*, phenotypic shifts that improve fitness under drought might cause a disadvantage due to a possibly reduced competitive ability (Grime 1974, Liancourt and Tielbörger 2009). Therefore, future research needs to evaluate in experiments that include the complex interaction in the community, whether the potential for adaptation matches the rate of environmental change. According to our results, adaptation occurs not only under drought conditions, and we might also find adaptive trait shifts which contribute to survival and fitness that were caused by

selection under the wet-treatments. After all, adaptation to biotic factors may also be important in coping with environmental changes and their impact on plant community composition.

### Conclusion

We show evidence for *in situ* adaptation in response to changed precipitation, which can enable species to persist under climate change. Therefore, adaptation is an important alternative to the too low migration rates. The adaptations reported here, may provide a way for plants to avoid extinction. Therefore it is highly important to understand how frequent this capability is and to assess whether it can allow to withstand the future climate change.

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## Appendix

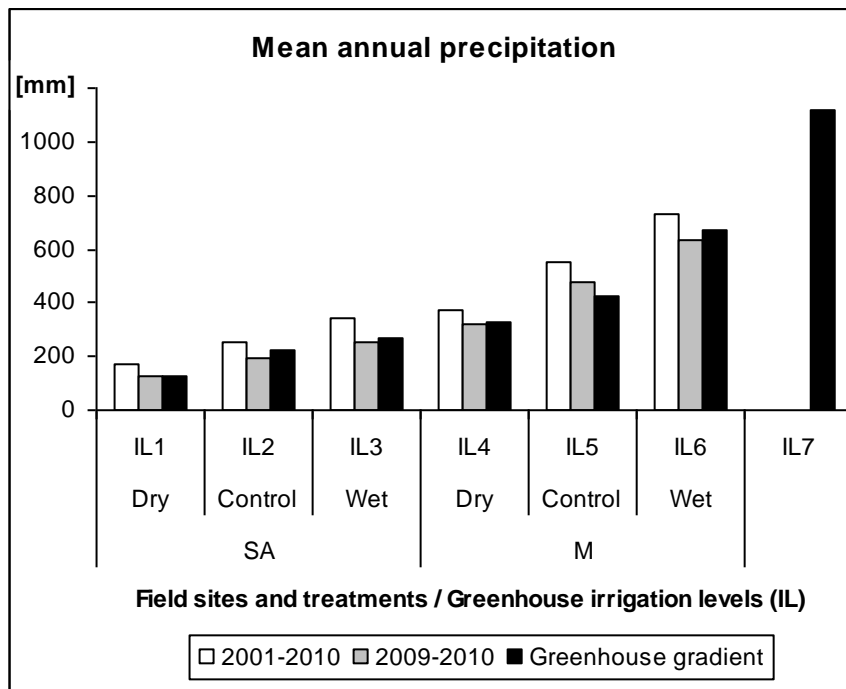


Fig. 1b) Mean annual amount of water in the field sites for the entire time of rain manipulation and the study year (estimated for the dry and wet treatments), and for the irrigation gradient in the greenhouse.

Tab. 3a)	Source of variation	d.f.	Onset of flowering		Plant size		Total biomass	
			F	p	F	p	F	p
Biscutella	Soil humidity (SH)	1	47.348	<b>&lt;.0001</b>	231.671	<b>&lt;.0001</b>	65.665	<b>&lt;.0001</b>
	Site	1	128.343	<b>&lt;.0001</b>	100.128	<b>&lt;.0001</b>	9.935	<b>0.0037</b>
	Treatment	2	8.012	<b>0.0016</b>	1.850	0.1747	0.513	0.6041
	SH*Site	1	5.003	<b>0.0329</b>	9.301	<b>0.0048</b>	2.448	0.1282
	SH*Treatment	2	0.683	0.5127	1.648	0.2093	0.217	0.8065
	Site*Treatment	2	0.736	0.4876	1.764	0.1886	0.412	0.6659
	SH*Site*Treatment	2	0.519	0.6003	0.305	0.7391	0.222	0.8024
Hymenocarpus	Soil humidity (SH)	1	0.001	0.9796	533.028	<b>&lt;.0001</b>	983.807	<b>&lt;.0001</b>
	Site	1	391.133	<b>&lt;.0001</b>	70.242	<b>&lt;.0001</b>	17.145	<b>0.0003</b>
	Treatment	2	1.639	0.2112	1.566	0.2254	0.528	0.5952
	SH*Site	1	4.259	<b>0.0478</b>	5.146	<b>0.0307</b>	4.896	<b>0.0347</b>
	SH*Treatment	2	0.234	0.7928	1.784	0.1854	2.786	0.0777
	Site*Treatment	2	1.307	0.2857	3.739	<b>0.0355</b>	0.491	0.6171
	SH*Site*Treatment	2	0.210	0.8119	0.007	0.993	1.635	0.2119

Tab.3) The results of the full factorial model testing the effects of relative soil humidity, site and treatment on life history traits and number of diaspores as a measure for fitness. The degrees of freedom (d.f.) were equal for all analysed traits.

Tab. 3b)	Source of variation	d.f.	Reprod. Allocation		Weight per diasp.		Number of diaspores	
			F	p	F	p	F	p
Biscutella	Soil humidity (SH)	1	346.178	<b>&lt;.0001</b>	98.057	<b>&lt;.0001</b>	734.923	<b>&lt;.0001</b>
	Site	1	28.838	<b>&lt;.0001</b>	4.481	<b>0.0427</b>	0.308	0.5833
	Treatment	2	4.240	<b>0.0239</b>	3.658	<b>0.0379</b>	3.847	<b>0.0326</b>
	SH*Site	1	0.636	0.4313	0.413	0.5253	5.729	<b>0.0231</b>
	SH*Treatment	2	0.167	0.8467	0.166	0.8479	0.158	0.8544
	Site*Treatment	2	5.100	<b>0.0124</b>	17.056	<b>&lt;.0001</b>	10.234	<b>0.0004</b>
	SH*Site*Treatment	2	0.524	0.5973	0.174	0.841	5.677	<b>0.0081</b>
Hymenocarpus	Soil humidity (SH)	1	26.511	<b>&lt;.0001</b>	33.077	<b>&lt;.0001</b>	526.784	<b>&lt;.0001</b>
	Site	1	20.526	<b>&lt;.0001</b>	1.030	0.3184	0.972	0.3321
	Treatment	2	0.123	0.885	2.236	0.1244	0.190	0.8282
	SH*Site	1	3.541	0.0696	1.473	0.2343	0.079	0.7811
	SH*Treatment	2	1.997	0.1534	0.021	0.9797	0.382	0.6857
	Site*Treatment	2	0.159	0.8536	3.173	0.0562	0.816	0.4518
	SH*Site*Treatment	2	0.789	0.4636	3.135	0.058	1.177	0.3221



Curriculum Vitae

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