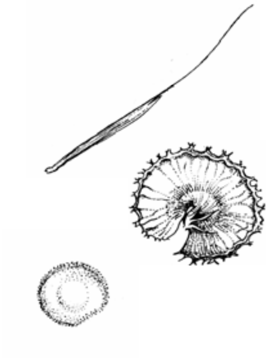
Life-history expressions of annual plants in unpredictable environments: From theoretical models to empirical tests



Ph.D. Thesis

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Life-history expressions of annual plants in unpredictable environments: From theoretical models to empirical tests

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When a seed breaks dormancy, it is extremely vulnerable.

A man who works with his hands is a labourer; a man who works with his hands and his brain is a craftsman; a man who works with his hands and his brain AND his heart is an artist… (or scientist?) **…** [Louis Nizer]

To the many good and cheerful labourers, craftsmen and artists of my life

Acknowledgments

I could much easily (and probably much faster) write a booklet of an extent of this thesis expressing my most sincere thanks to all of YOU, who have facilitated the progress of this mission. And not very long ago this mission still seemed impossible.

I would acknowledge you in full extent in the future, and title it 'In-between the Worlds', 'Right in the Middle' or else… Once I would do so. And it would also be four chapters like in this thesis, with the first 'original study' being written in 'the original' (Czech). It would be a chapter written in capitals, and it would be The First One, because there it all started, although I can hear the Hebrew voices arguing that it was 'Over there' where 'It ALL started'. Well, in fact it all started in Germany, and so let's make the German chapter the first one, followed by a Hebrew chapter (because those guys when they argue they argue loud). The next would be an American chapter, and the Honorary last would be the one in Czech. There also would be few Appendixes, in Arabic and Spanish and French and… Norwegian.
Let me provide you here with the 'Abstract'.

Since our living environments are highly variable and unpredictable and prone to changes, one needs to optimize while discovering the intrigues of life, e.g. the joy of learning. This may be possible through spatial and temporal variation of approaches, or through a specific approach, adapted to local conditions. For this social study, I implemented an original international multi-cultural approach based on diverse interactions and the phenomenon of migration. It has been a 'population level' study.

Commonly throughout my research, I have encountered many good and cheerful labourers, craftsmen and artists in many habitats, starting in 'a social desert'. Coming to this new habitat, I made plentiful 'a priory' observations and over the almost four years of my study, I recorded highly favourable 'environmental conditions' making the social desert bloom. I greatly enjoyed the densely blooming wadis for the community spirit. Consequently, flourishing social deserts have been my favourite and frequently sampled habitat. I sampled a lot of data points and attempted to analyse the data carefully, however the design had severe problems as most ecological (and perhaps also social) field studies have - pseudoreplications. However…

I established several experiments with the first study site being Potsdam, and my first 'data point' was Prof. Dr. Katja Tielbörger, my supervisor, who am I thankful first and foremost for the introduction to my thesis subject and for the professional manoeuvring of this voyage. I have learned a lot and greatly appreciate the time Katja found for me even during the highly stressful periods of her own career. I am especially thankful for the much-needed support during the thesis finalizing.

Prof. Florian Jeltsch supported me with ‘sampling plots’ in his working group and generously provided with ‘sampling equipment’. During this time, ‘the best secretary of my German Life’ was ultimately Birgit Seifert, also the ultimate flat-mate and wonderful friend. My many thanks go to the other ‘Potsdamians’ (ordered randomly): Katjas, Monika, Evas, Niels, Matthias, Jörgs, Bernd, Florence, Katrin, Nina, Michael, Michel, Jutta, Annette, Doreen, Uli, Jule, and Julia. Finally, I would like to thank to Heike Küchmeister for the inspiring friendship and kindness.

After concluding my field studies in Potsdam, I went to analyze and summarize ‘my data’ to Tübingen. My analyses have been challenging, but considerably eased by Monika Schwager, who has been generously sharing her home with me, and Clara and Katja and Bara. Cornelia and Richard, Merav and Tal and Achim (my second-favorite ‘German secretary’) and Christian and Johannes and others ‘up on the hill’ made my social time here more pleasant. My special thank belongs to ‘Micha’ Koltzenburg, who has shared his ‘nosy’ (and noisy) office. Thanks for the constant cheer that has been loosing some degrees of stress in favor of the future ‘degrees of freedom’. Thank you also for the tips on how to play the Photoshop game even better and successfully waste even more time.

I would like to include in this ‘German Analyses’ a ‘geographical outlier’ from Hannover, Rüdiger Prasse, and thank for keeping me from the deepest depths of … (also the Red and Med seas) up (also to the skies) throughout this Odyssey, and supplying me a constant flow of amusement and enthusiasm shared over exploration of the natural world. **Danke Schön**.

My main study in Israel started from a ‘pilot’, however expanded exponentially within the least (im)possible time to an impressive network of interactive studies (very likely confounded). It is extremely hard to summarize in few sentences the three-times-nine months of my recent life without forgetting anybody who has been part of it in a certain aspect. There are many of you, really. And I will devote you the full version of these Acknowledgments. I cannot possibly valuate the sincere friendships, and the hospitality and the many adoptive homes that made my life happier and easier and more cheerful, when I was getting lost or desperate in the ‘dangerous’ Holy Land.

Starting chronologically, I owe a lot to Claus Holzapfel and Hadas Parag for most of the shared fun on our ‘outing’ and birding. Thank you also for all the logistical and organizational help. I appreciate various support of Marcelo Sternberg and Jaime Kigel, and their lab members: Netta, Ofri, Yaelim, Azaria, Efrat, Sharon, Matangi, and Ilana and Maya from the adjacent labs. I would like to thank to several professionals who have willingly spared some of their precisious time to discuss my research; being that inspiring and encouraging Dany Cohen and Deborah Goldberg, Jaime Kigel, Avi Shmida and Dini Eisikowitch.

I highly value the friendships and technical help of Clara Ariza, Henri, Yaely, Yoni, Aliza, Ruthie, Yiftach and Hila, Renana, Tamar, and Maital, and others. I also would like to thank to Dan Malkinson for hosting me in the Golans, and further to all, who with all the enthusiasm in this world have shared the truly beautiful and interesting outdoors of Israel. My sincere thanks go to Hagar Leshner, Avi Shmida, Mimi Ron, Ori Fragman, Yuval, Yossi, Dvorale, Ely, Jankele and others. All of this, beyond the science, has been a rewarding life experience…it has been 'emotional' **תודה רבה**

To continue breaking the cultural borders, a geographically distant study took place at the Biological Station of the Michigan University. Here, not only the level of bureaucracy has been significantly (<0.001) lower than in Israel (although non-significantly different from the German bureaucracy), but the entire 'Biostation' life has been an unforgettable experience. I am grateful for the unique friendships that emerged there and have been maintain over the long-distance till now. My most thanks belong to Bob Pillsbury and Samara Hamzé, Melanie Gunn and Chuck Davis and to Anthony Arnold, especially. And Sharon, Noel, Brad, Tereza, Mark, Stephanie, Adam, Maribeth, and Liza among the others. **Thank you**.

My local adaptation study with the Middle-European perspective has proved a very low level of my local adaptation, although I have felt homesick at times. However, my 'old' friends remained my friends even I have not been good in keeping in touch. Thank you for the patience Kláro, Hanys, Šuspo, Mileno, Járo, Evo and Radky. I am mostly grateful to Tereza Kumstátová and Adam Petrusek for the 'always ready temporary home', and David Lacina, who has made sure that I made it to this point. I clearly needed a second Czech around before submitting my thesis in Germany. Thank you Dejf for the careful double-čeking of the thesis text, and for your friendship that is so much appreciated.

My family in its full extend suffered from my absence in many happy family occasions that I did not manage to prioritize over my important mission in Israel… Truly, without the support of my parents, who have not completely understood until now what made me returning to that 'Paradise', I would have not been able to carry over the family disconnection as cheerfully and as relaxed as I did. I would like to reward them once with a bit more readable book. **Díky**.

Finally: **Gracias** Marcelo, Jaime, and Clara. **Merci** Florence and Pierre, and **Takk** Vigdis.

In conclusion, this work has greatly benefited from the diverse social interactions with all of YOU, and I also hope this work can contribute to understanding of this complicated world, as it is not simple, and it is not simple to understand it. Although, what won't be simple, simply won't be…

Abstract

Survival of annual plants in unpredictably varying environments may be possible through life-history adaptations, i.e. seed dormancy, or through phenotypic variation in life-history traits. In this thesis we studied these evolutionary and environmental life-history expressions along a steep rainfall gradient in Israel, spanning from arid habitats with highly variable and unpredictable rainfall to less variable and more predictable mesic Mediterranean habitats. We studied three winter annual species abundant along the gradient, and differing in dormancy from low to high: *Bromus fasciculatus*, *Biscutella didyma* and *Hymenocarpos circinnatus*.

The major objective of this thesis was to perform original empirical tests for optimal germination strategies that were frequently theoretically modeled, but rarely empirically proved. Namely, we tested the pioneering model that predicts the germination fractions are constant over time and proportional to increasing amounts and predictability of rainfall (Cohen's 1966). We studied the effects of average annual rainfall separately from maternal effects, which has not been done before. Our results show germination fractions of *Biscutella* and *Bromus* varying in a clinal fashion along the gradient, supporting the Cohen's (1966) model. The trend for *Biscutella* was consistent during three consecutive years indicating a strong genetic determinant of germination. This genetic basis still needs to be established, and we advocate careful addressing of maternal effects too.

Maternal effects have been considered in optimal germination strategy only recently, when predictive germination was modeled with respect to competition and environmental variability (Tielbörger & Valleriani 2005). For the first time, we tested this model in real field populations, namely the prediction that maternal fecundity is positively related to rainfall amounts and offspring germination is negatively related to maternal fecundity. We document maternal effects in more humid and more competitive environment (the Mediterranean), and suggest their short-term advantage in controlling sibling competition. With this pioneering approach to test maternal effects on predictive germination, we encourage more rigorous empirical studies to find out whether the observed patterns are universal.

In a large-scale field experiment we tested the predicted optimal germination strategies (Cohen 1966, 1967) under naturally varying climatic conditions. Simultaneously, we evaluated the importance of separating climatic from other environmental variables. We reciprocally transplanted conspecific seeds of the species from and to the four sites along the gradient and expected higher germination fractions of seeds from wetter origins and at wetter sites. By sowing seeds on both local and standard soil, we separated climatic from edaphic and neighbor effects. We found strong substrate effects but relatively weak climate effects. This indicates that local environmental conditions may override effects of climate and should be critically addressed in future studies testing for local adaptation to current climate and for the potential of species to adapt to changing climate.

Finally, adaptations to local climate and pre-adaptations to changing climate may reflect in life-history traits beyond germination. While exploring phenotypic variation in several morphological and reproductive traits of *Biscutella didyma*, we found clinal trends in the trait expression along the gradient, indicating two strongly opposing selective forces at the two extremes of the aridity gradient, resulting in contrasting strategies within the species. These clinal trends in the studied traits were consistent with germination trends of *Biscutella* in our parallel studies and indicate ecotypic differentiation/local adaptation of the climatic populations.

Regarding survival under variable climate and potential climate changes, our observed trends in seed dormancy and other life history traits encourage future research on within-population genetic variation related to adaptive traits.

Contents

Synopsis

Introduction

Spatial and temporal variation is inherent to natural environments worldwide. For instance, in the Eastern-Mediterranean region, highly variable climate and weather patterns generate unpredictable and inconsistent environmental conditions, under which survival may be possible through phenotypic plasticity in life-history traits (e.g. Evenari et al. 1982, Schlichting 1986), or through specific life-history adaptations (e.g. Noy-Meir 1973, 1983). Seed dormancy of annual plants has been a classical example to demonstrate how selection had favored a mechanism that allows survival under unpredictable and unfavorable conditions.

Analogous to dispersal in space, seed dormancy has been regarded as an adaptive strategy enabling seeds to germinate during appropriate environmental conditions. Since seed longevity and delayed germination reduce the risk of low success in unfavorable years (e.g. after a single catastrophic season) and allow temporal spreading of the extinction risk, seed dormancy has been regarded as an adaptive risk-spreading (bet-hedging) strategy, buffering against the detrimental effects of temporal environmental variation. Consequently, many theoretical models formulated the optimal germination behavior under these conditions (Cohen 1966, 1967, Mac-Arthur 1972, Slatkin 1974, Stearns 1976, Venable & Lawlor 1980, Mac Donald & Watkinson 1981, Bulmer 1984, Cohen & Levin 1985, Ellner 1985a,b, 1986, 1987, Léon 1985, Brown & Venable 1986, Klinkhamer et al. 1987, Venable & Brown 1988, Venable 1989).

In the pioneering model (Cohen 1966), optimal germination fractions were proportional to the favorability and predictability of environments (i.e. rainfall conditions), and constant over time. Subsequent models extended this original form to so-called ‘predictive germination’ and regarded bet-hedging germination as phenotypic plasticity to highly reliable cues, which indicated the favorability of the upcoming season, e.g. the first rainfall of the season (Cohen 1967, Venable & Lawlor 1980, Bulmer 1984, Brown & Venable 1986). In the following studies, predictive germination was extended by an alternative view of seed dormancy, i.e. a mechanism of escaping unfavorable biotic conditions. High neighbor density was a reliable cue indicating favorability of the future environment. Optimal germination fractions were modeled negatively density-dependent and constant under generalized or sibling competition (Westoby 1981, Bulmer 1984, Ellner 1985a,b, 1986, 1987, Léon 1985, Nilsson et al. 1994, Kobayashi & Yamamura 2000). The most recent study (Tielbörger & Valleriani 2005) innovated the previous models by integrating intraspecific sibling competition with interspecific generalized competition, and by assuming that seeds do not have constant, but different germination probabilities. This novel approach coupled predictive germination generated by variable environmental conditions with dormancy strategies evolving via sibling competition.

Compared to the myriad of models on the germination theory, *rigorous empirical* tests for their predictions are very rare. As a result, verification of the various models from natural populations is not complete. For example, the relevance of the Cohen's (1966) original model is still being discussed (Maffei et al. 2005). This clearly indicates an urgent need of more rigorous experimental tests. The currently existing empirical evidence for the various models is limited to a handful of studies (Philippi 1993a,b, Pake & Venable 1996, Hyatt & Evans 1998, Clauss & Venable 2000), and they have major methodological constraints. These studies were done under laboratory conditions, in no real populations, for one species and/or one site only, or were confounded by diverse environmental effects. For example, the empirical support of the initial prediction (Cohen 1966) is limited to four rigorous studies (Philippi 1993a,b, Clauss & Venable 2000, Volis et al. 2002) that were confounded by environmental maternal effects. While no previous empirical study attempted to separate maternal effects from effects of climate/environment, the increasing evidence of maternal effects from natural populations (Philippi 1993b, Hyatt & Evans 1998, Tielbörger & Valleriani 2005) highlights the need of their methodological consideration.

Apparently, empirical testing of ecological theories has not been unproblematic. Yet, validating theoretical models from natural population is one of the underlying tasks of modern ecological research and it has also been the major effort of this thesis.

We attempted original rigorous tests of the above-mentioned theories. Namely, we tested for the Cohen's (1966) and Tielbörger & Valleriani's (2005) models, aiming to overcome limitations of the previous empirical studies.

In our empirical approach, we utilized a steep aridity gradient in Israel, and four long-term ecological research stations established along this gradient in arid, semi-arid, Mediterranean and mesic Mediterranean zones (Sternberg et al., unpublished). We also used field rainfall manipulations located in a transition zone from arid to mesic climatic region, reducing rainfall amounts with rainout shelters and supplementing rainfall with artificial irrigation. We selected three common winter annual species differing in their dormancy: a grass *Bromus fasciculatus* with low dormancy, a crucifer *Biscutella didyma* with medium dormancy and a legume *Hymenocarpos circinnatus* with high dormancy, and used their seeds from the natural populations, and from the rainfall manipulations. Subsequently, we tested for predictions of the two above-mentioned models by germinating the seeds in an array of controlled and field conditions, including large-scaled reciprocal seed transplants between the stations along the gradient.

In this field experiment, we tested if the optimal germination strategies may be adaptations or pre-adaptations to varying climatic conditions. Simultaneously we evaluated the importance of separating climatic from other environmental variables. Considering the predicted climate change effects (e.g. Palutikof et al. 1992 for the Eastern-Mediterranean region), empirical studies on climatic changes have been greatly increasing in importance, as they may determine the vulnerability of species to climate change (Rehfeldt et al. 2002). The ability of a species to persist under climate change largely depends on the degree of its local adaptation to current climate and potential pre-adaptation to future climatic conditions (Jump & Peñuelas 2005).

Adaptations and pre-adaptations to local and changing climate may reflect also to other life-history traits, e.g. traits maximizing species fitness, such as phenology, growth, sexual reproduction or reproductive allocation. Consequently, climatic gradients have provided a useful framework for evaluating relative importance of differential selection for adaptive shifts in these traits (Callahan and Waller 2000, Hauser and Weidema 2000, Olsson and Ågren 2002, Sugiyama 2003, Stinson 2004). For example, the highly contrasting selection pressure on the extreme gradient ends suggest that life history at the arid end of the gradient may be adaptated to drought and unpredictable conditions, while adaptations to the highly competitive environment may prevail at the mesic Mediterranean end. Within single annual plant species persisting along the entire gradient, this may results in contrasting strategies in life-history traits beyond germination.

Thesis objectives and organization

Summarized in four chapters, the thesis objectives were three-fold:

(i) To rigorously empirically test (using controlled-condition experiments) for theoretical predictions of seed dormancy under variable and unpredictable environmental conditions, namely for the effects of average annual rainfall and maternal effects (Chapters 1 and 2).

(ii) To test for the germination strategies under varying climatic conditions in a field experiment, and to evaluate the importance of separating the climatic from other environmental variables (Chapter 3).

(iii) To study phenotypic variation in several morphological and reproductive traits along a steep aridity gradient, testing whether the trait expression indicates different selective forces at the extreme gradient ends that may results in contrasting life-history strategies (Chapter 4).

The four chapters are written as independent manuscripts aiming to convey a specific message to the international audience. This approach results, regrettably, to a certain repetition of some parts of the manuscripts, namely the introduction and methods.

Chapter four is currently in press in *Ecography*, chapter three in review in *New Phytologist*, and the remaining two chapters are previewed for submission to international scientific journals in co-operation with the co-author(s). That is the reason for the first person plural throughout the thesis.

Key Results

'Cohen's (1966) prediction is not wrong': Optimal germination strategy along an aridity gradient. (Chapter 1)

The classic, but rarely tested Cohen's (1966) original model on optimal germination strategy predicts that germination fractions are proportional to increasing mean annual rainfall and its predictability. We tested this prediction along the rainfall gradient, with an original approach to separate the effects of average annual rainfall from confounding maternal effects. In our results, germination fractions of a crucifer *Biscutella didyma* and a grass *Bromus fasciculatus* increased in a clinal fashion between the two extreme ends of the gradient, supporting the Cohen's (1966) prediction. In *Biscutella*, this trend was also prominent and consistent for seeds from natural conditions during three consecutive years, indicating that germination has a strong genetic determinant. Maternal effects did not vary under very similar rainfall conditions in the natural populations among the three years. We address maternal effects on germination in the following study.

Maternal effects reduce sibling competition in competitive environments. (Chapter 2)

We introduced a rigorous test for a novel model predicting maternal fecundity positively related to the favorability of environment and offspring germination negatively related to the maternal fecundity. For the first time, we related predictive germination to competition. We found strong maternal effects for two annual plant species (*Biscutella didyma* and *Bromus fasciculatus*) from the Mediterranean site (540 mm average annual rainfall), where seed production was lower in dry and higher in wet maternal environment and *vice versa*. The offspring germination was strongly negatively correlated with the maternal fecundity (seeds from the wet maternal environment had lower germination fractions than seeds from the dry environment). We found no such relationship in drier, less productive and less competitive semi-arid site (300 mm average annual rainfall). Apparently, maternal effects are stronger in more competitive environments, as a short-term advantage in controlling sibling competition.

Local environmental conditions can override effects of climate in field experiments. (Chapter 3)

We improved standard methods for studying local adaptation of plants to climate in the field, by separating climatic from other environmental effects (i.e. edaphic and neighbor effects) and by focusing on germination, a trait that is largely determined by climate alone. We tested for climate effects on optimal germination strategies by reciprocal transplanting of conspecific seeds from and to four sites along the aridity gradient on local and standard substrate. The substrate effects were strong, i.e. the germination fractions were consistently lower on local soil, indicating that local environmental conditions can override climatic effects that were, to the contrary relatively weak.

Opposing selective forces at the extreme ends of an aridity gradients create contrasting strategies in life-history traits. (Chapter 4)

While comparing four populations of a winter annual crucifer *Biscutella didyma* growing along the aridity gradient, we found distinct directional trends in growth morphology, architecture, and fitness-related traits. Plants from arid environments were faster in phenological development, more branched in architecture and tended to maximize reproduction, while the Mediterranean plants invested mainly in vertical vegetative growth. When we cultivated plants and compared the diaspore production between the cultivation and the field, diaspore production was much larger for arid populations as opposed to Mediterranean ones, indicating a larger potential to increase reproduction under favorable conditions. The overall findings indicate two strongly opposing selective forces at the two extremes of the aridity gradients, which result in contrasting strategies within the studied annual plant.

Discussion

The main objective of this thesis was to perform original empirical tests for theoretical models on optimal germination behavior in variable and unpredictable environments. Namely, we tested for the effects of average annual rainfall (Cohen 1966) separately from maternal effects on seed dormancy (Tielbörger & Valleriani's (2005) in two separate studies.

While testing for the Cohen's (1966) model (Chapter 1), we controlled for confounding by maternal effects, which has not been considered in the previous studies (Philippi 1993a,b, Clauss & Venable 2000). Overall, our results of germination fractions of seeds cultivated under standard maternal environment, of a crucifer *Biscutella didyma* and *Bromus fasciculatus* support the Cohen's prediction. We further found germination fractions of *Biscutella* from natural populations following a clinal trend along the aridity gradient, consistently during three consecutive years. This particular result supports one of the key hypotheses of Cohen's (1966) model, i.e. constant germination fraction across the years, that has not been empirically documented thus far (comp. Philippi 1993a, Clauss & Venable 2000). The germination fractions varied greatly among populations, indicating the adaptive value of dormancy in relation to aridity clines, e.g. genetic differentiation and local adaptation. With this observation we advocate proper methodological approaches for establishing the genetic basis for our germination patterns with clearly addressed maternal effects.

We studied maternal effects under field-manipulated rainfall conditions, and present the first experimental evidence of predictive germination regarding competition in real field populations (Chapter 2). Interestingly the effects differed between sites and years. Our results for *Biscutella* and *Bromus* from Mediterranean region in Israel indicate that the maternal seed production and offspring germination fractions are strongly negatively correlated in more humid/productive environments, where competition is the major constraint. On the contrary, in more arid/less productive environment, where competition is not as important, no such obvious relationship can be found. Maternal effects reducing sibling competition in competitive environments seem to be a short-term advantageous mechanism for predicting future success. While in the long-term, germination should be positively, not negatively correlated with annual rainfall and its predictability (Cohen 1966, 1967), with this pioneering study we encourage more field tests to prove the generality of the patterns observed.

Under field conditions, the germination patterns were less straightforward (Chapter 3). Our reciprocal transplant experiment indicated that environmental effects may virtually override the tested climatic effects. This points out some major methodological constraints of field experiments studying local adaptation within the context of climate change research. Future experiments should focus on separating climatic forces from other selective forces along climate gradients.

Throughout the three above-mentioned germination studies, we found species-specific germination patterns reflecting the various degrees of species dormancy. The medium-dormant crucifers (*Biscutella didyma*) apparently followed the theoretical predictions the closest. In contrast, grasses (*Bromus fasciculatus*) with low dormancy, usually germinate within the first season following seed production and do not build up a permanent seed bank. Legumes (*Hymenocarpos circinnatus*), on the other hand, have high dormancy and ability to spread germination over multiple years up to a decade (Kigel, unpublished). Therefore both the studied grasses and legumes may not have been the best model species for testing the theories on seed dormancy evolution. These large differences among species of the same life form call for a community-wide approach in future studies of seed dormancy, particularly with respect to climate changes.

While exploring life-history traits other than germination in *Biscutella didyma* (Chapter 4), we found distinct directional trends in the expression of traits. Our comparison of field and common-garden conditions suggests that these trends may be genetically based (Turesson 1922, Clements et al. 1950, Langlet 1971, Rehfeldt et al. 1999, 2002, Hauser and Weidema 2000). Namely, aboveground competition intensity at the wet end favored vegetative growth, and low resource availability and highly pulsed resources at the arid end select for high reproductive allocation. Interestingly, these largely opposing strategies predicted for between-species differences in habitat-trait relationships (Grime 1973a,b, 1977, Harper 1977) can be found even within a single species.

The consistent clinal trends of germination and other life history traits (morphological and reproductive life history traits of *Biscutella didyma*) suggest (at least for this species) genetically differentiated and locally adapted populations. With these results we advocate proper methodological approaches to establish the genetic basis for our germination patterns in future studies. Since maternal effects may mask the real expression of the genetic differences among the populations (Chapter 1), we highlight the need of their careful consideration (Chapter 3). Further tests should separate the different germination components, such as the different maternal effects (i.e. environmental vs. genetic maternal effects) in order to define also the genetic basis for the susceptibility to maternal effects. Finally, other life history traits should be studied to quantify the genetic differences among the climatic populations. Also the level of local adaptation should be established as a measure of vulnerability to potentially changing climatic conditions, as predicted for the region (Palutikof et al. 1992, Ben-Gai et al.1998).

Overall Conclusions

(i) Germination fractions, positively correlated with annual rainfall and its predictability (Cohen 1966), indicate long-term advantageous strategy.

(ii) Maternal effects reducing sibling competition in competitive environments seem to be a short-term advantageous mechanism for predicting future germination success.

(iii) Maternal effects need to be clearly addressed in future studies on weather- and climate effects on germination.

(iv) Other local environment effects (i.e. soil and neighbor density) can override effects of weather- and climate on germination.

(v) Local environmental conditions should be critically considered in future field studies, e.g. empirical tests for the potential of species to adapt to changing climate.

(vi) Species- specific germination responses indicate different levels of local adaptation to climate and advocate community-wide approach to climate change studies.

(vii) Life-history traits (i.e. germination and morphological and reproductive traits) vary in clinal fashion along aridity gradient under strong selective forces operating in opposite directions and this results in contrasting strategies within the studied species.

(viii) The consistent clinal trends in diverse life history traits along the aridity gradient indicate ecotypic differentiation and local adaptation.

Declaration of my own contribution to the presented manuscripts

In the first three studies I designed all the experiments, collected the field data, performed the statistical analysis of the data and wrote the major part of the manuscripts by myself using relevant literature. While conducting the research, I was advised by Prof. Dr. Katja Tielbörger, who discussed the results and contributed to advanced drafts and final stages of the manuscripts as a co-author.

The last study was initiated as an independent student project designed by my advisor and carried out by Ruthie Belkin under supervision of Dr. Marcelo Sternberg. Ruthie Belkin collected the major portion of the data and contributed to the first draft of the manuscript. I analyzed the data and worked on the manuscript drafts that were reviewed and finalized by my advisor. Prof. Dr. Florian Jeltsch has been a partner in the discussions of the results together with the other mentioned co-authors.

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Chapter 1

Optimal germination strategy of annual plants along an aridity gradient

Optimal germination strategy of annual plants along an aridity gradient

Abstract

Seed dormancy of annual plants has been viewed as a form of bet hedging, optimizing survival and fitness in variable and unpredictable environments. Many theoretical models have formulated the optimal germination strategy, however their empirical validation, including the original Cohen's (1966) model, is not complete. The Cohen's model predicts germination fractions constant over time and proportional to increasing annual rainfall predictability. Here we tested this prediction along a steep rainfall gradient in Israel, and for the first time we separated the effects of average annual rainfall from maternal effects. Under standard maternal environment we propagated F-1 seeds of three common annual plants from four climatic populations, and in a growth chamber we examined the predicted germination differences. A parallel experiment in a growth chamber evaluated germination of seeds collected from the natural populations for three years. By comparing the germination patterns among the years varying in rainfall, we expected the predicted pattern confounded by maternal effects. Two of our study species (*Biscutella didyma* and *Bromus fasciculatus),* in which germination fraction of the F-1 seeds varied in a clinal fashion along the gradient, supported the Cohen's model. Under very similar rainfall conditions in the natural populations among the years, maternal effects were not detectable. The germination patterns in the natural populations were very consistent over the three years, indicating that germination has a strong genetic determinant.

Introduction

Seed dormancy (i.e. delayed germination) of annual plants has been regarded as an adaptive bet-hedging behavior, reducing variation in fitness and buffering against the risk of extinction. Consequently many theoretical models formulated the optimal germination strategy in spatially and temporally varying and unpredictable environments (Cohen 1966, 1967, Mac-Arthur 1972, Slatkin 1974, Stearns 1976, Venable & Lawlor 1980, Mac Donald & Watkinson 1981, Bulmer 1984, Cohen & Levin 1985, Ellner 1985a,b, 1986, 1987, Léon 1985, Brown & Venable 1986, Klinkhamer et al. 1987, Venable & Brown 1988, Venable 1989). In contrast to this impressive theoretical base, very little advances have been made in finding adequate *empirical* support for the theoretical predictions from natural populations of annual plants.

Classical models were developed in the late 1960's, followed by a myriad of models in the 1980's. In the original study (Cohen 1966), germination fractions were proportional to increasing favorability and predictability of environments (i.e. rainfall conditions), and constant over time. Preceding any empirical verification, this original form was extended in subsequent models to 'predictive germination', i.e. phenotypic plasticity in response to an external cue that indicated the favorability of the upcoming season, e.g. the first rainfall of the season (Cohen 1967, Venable & Lawlor 1980, Bulmer 1984, Brown & Venable 1986). While several empirical studies suggested the occurrence of predictive germination (Rice 1985, Philippi 1993b, Evans & Cabin 1995, Pake & Venable 1996, Clauss & Venable 2000), there are hardly any well-designed rigorous tests for the original Cohen (1966) model.

The fact that the relevance of the Cohen's original model, namely the constant germination fraction across years, has been discussed until now (Maffei et al. 2005), and the general lack of supporting empirical evidence, clearly indicate the urgent need of more rigorous tests. For example, annual plants surviving along a gradient of gradually changing environmental conditions (e.g. along a steep gradient varying in amount and predictability of rainfall) represent an ideal system, where Cohen's (1966) model should hold. Therefore, it is surprising that not many studies have utilized environmental gradients for testing the Cohen's (1966) predictions. The current existing support for the Cohen's (1966) model from observational and experimental studies is inconsistent (Jain 1982, Freas & Kemp 1983, Gutterman & Agami 1987, Gutterman & Edine 1988, Platencamp 1991, Kigel 1995, Clauss & Venable 2000, Cavieres & Arroyo 2001). These studies compared mainly xeric vs. mesic conditions, and they did not relate their results to environmental predictability along gradients. Also Philippi (1993a,b) found positive correlation of the germination fraction with the mean winter rainfall among several populations of six winter annual plants from the southwest US. However, these two parallel studies were correlative and did not explicitly associate their results to a rainfall gradient. Only two studies utilized environmental (rainfall) gradients to rigorously test for bet-hedging germination (Clauss & Venable 2000, Volis et al. 2002). First, Clauss & Venable (2000) performed a four-year field germination test in several populations of a desert annual *Plantago insularis*. In the second study, Volis et al. (2002) compared germination fractions among four ecotypes of an annual grass *Hordeum spontaneum* along an environmental gradient in Israel. Both studies documented bet-hedging germination, however neither of the studies avoided confounding. Namely, the inter-annual germination differences in both studies indicate confounding by uncontrolled site-specific physical and climatic variation.

Until the recent study of Tielbörger & Valleriani (2005), none of the former theoretical models recognized the role of maternal effects in germination. Consequently, no previous empirical studies distinguished for maternal effects, and were likely confounded. In their model, Tielbörger & Valleriani (2005) regarded high neighbor density as a reliable cue indicating favorability of the future environments (Westoby 1981, Bulmer 1984, Ellner 1985a, 1985b, Léon 1985, Nilsson et al. 1994, Kobayashi & Yamamura 2000). They accounted for the quality of maternal environment, and coupled predictive germination generated by variable environmental conditions with dormancy strategies evolving via sibling competition.

Considering the growing evidence of maternal effects from natural populations (Philippi 1993b, Hyatt & Evans 1998, Tielbörger & Valleriani 2005, Petrů & Tielbörger, unpublished), their role in confounding germination experiments needs to be clearly addressed. Maternal effects on germination can be particularly strong if seeds matured under extremely wet or drought conditions (Tielbörger & Valleriani 2005). In most previous studies that collected seeds *in situ*, environmental maternal effects were frequently neglected. This was unfortunate, since environmental maternal effects can mask genetically based germination differences (Roach & Wulff 1987). Therefore, in order to eliminate maternal effects across the different populations, F-1 seed generation should be propagated under uniform maternal environment, such as in studies of Hacker (1984) and Hacker & Ratcliff (1989). Here, maternal effects were successfully eliminated. Namely, dormancy was found higher in ecotypes from drier regions compared to humid-region ecotypes, and these interpopulation differences were maintained in the F-1 seed generation. While these results document adaptive bet-hedging germination and suggest its genetic base, neither of the two studies considered the Cohen's (1966) theory in their objectives, or when interpreting the results.

To summarize, there is a major shortage of rigorous empirical studies of the Cohen's (1966) model, and these studies have major shortcomings. Namely, previous studies along rainfall gradients did not account for maternal effects and/or confounded climate with other factors. Furthermore, F-1 seed generations devoid of maternal effects are needed. In studies where this was done, Cohen's model was not tested and no association to gradient has been made. Therefore, our study is the first one to separate long-term evolutionary from environmental effects on germination.

Specifically, we tested the following hypotheses:

(i) Germination fractions of seeds from different populations increase proportionally to more predictable rainfall conditions, i.e. from arid to mesic Mediterranean (Cohen 1966).

(ii) Inter-annual differences in germination patterns vary according to the variation in rainfall (i.e. maternal environments) with seeds produced in wetter years having lower germination fraction and *vice versa*.

Methods

Field sites

Four stations (referred also to as ‘sites’) along a S-N oriented gradient in Israel were our original natural sources of seeds. The amounts, distribution patterns and predictability of rainfall increase along the gradient from an arid station (90 mm mean annual rainfall), to a semi-arid (300 mm), a Mediterranean (540 mm) and to a mesic Mediterranean (780 mm) station, while the rainfall variation increases in the opposite direction (N-S). The arid site is located 3 km N of Sde Boqer in central Negev, the semi-arid site is 20 km north of Beer Sheva in the northern Negev, the Mediterranean site is 15 km south-west of Jerusalem in the Jerusalem Mountains and the mesic Mediterranean site is located 10 km east of Nahariya in north-eastern Galilea. The sites are situated on south-facing slopes on the same calcareous bedrock. Mean annual temperatures are similar, ranging between 17 ºC and 19 ºC (Sternberg et al. unpublished). The vegetation at the stations varies from open vegetation to closed shrubland, where shrub and annual cover increase from the arid to the mesic Mediterranean site (Holzapfel et al. in press, Tielbörger et al. unpublished). The length of the growing season at the stations corresponds to the rainfall distribution; the season is shorter at the arid site (December-March), prolonging towards the mesic Mediterranean site (October-May). Seeds of annual plants mature during heat spells, first and more quickly in the arid site, from approx. March, and later and more slowly in the mesic Mediterranean site. Over the three seasons of this study, the total rainfall amounts at the sites varied: 2001-2 was an average to above-average season, 2002-3 season was above the average (except for the arid site) and the 2003-4 season was below the average (except for the mesic Mediterranean site, Figure 1). However this inter-annual variation was relatively small (Figure 1).

Focus species

We studied three common winter annual species, differing in seed dormancy (Petrů & Tielbörger, unpublished). The species have wide distribution including both the arid and the mesic Mediterranean habitats, and were abundant at least at three stations along the gradient. *Biscutella didyma* (further referred to as '*Biscutella*') with Mediterranean and Irano-Turanian distribution (Zohary 1966) grows along the entire gradient. *Biscutella* is a crucifer germinating from fragmented double-silicles with intermediate dormancy. *Bromus fasciculatus* (further '*Bromus*') with Mediterranean, Saharo-Arabian and Irano-Turanian distribution (Feinbrun-Dothan 1986) grows in the three southern stations. *Bromus* is a grass with low dormancy caryopses. *Hymenocarpos circinnatus* (further '*Hymenocarpos*') distributed in the Mediterranean region (Zohary 1987) and in the three northern stations along the gradient, is a legume with pods containing two highly dormant seeds.

Experiment I

To evaluate the interpopulation germination differences without maternal influence, we propagated F-1 seed generations of the different populations under standard maternal environments. We collected diaspores from the natural populations in spring (March-May) 2002. To assure natural breaking of summer dormancy, diaspores were bagged in organza (permeable transparent synthetic fabric), and the bags were attached to the ground surface in their original sites over the summer. In September 2002, we collected the diaspores from the field, and at the end of November planted them in a cultivation located in a screen-house at the Botanical Gardens of the Tel Aviv University, Israel (for more details see Petrů et al. in press). Under standard conditions of the cultivation, the established plants grew prevented form cross-pollination between the origins, until May 2003 when all the F-1 diaspores matured. We harvested and bagged the diaspores from each species and site in separate paper bags and stored the bags loosely placed in an open cardboard box in the screen-house over the summer. In September 2003, we transferred the bags to a laboratory for diaspore counting into equal portions and subsequent sowing in a controlled germination experiment.

The F-1 seeds germinated from mid November 2003 in a growth chamber (further 'Phytotron') at the Hebrew University in Rehovot, Israel under temperature set up to 16/10 ºC day/night and excessive watering regime twice a day. We added diaspores: 20 *Biscutella*, 30 *Bromus* or 20 fruits of *Hymenocarpos* (each enclosing 2 seeds) to pots of 12 cm in diameter and 12 cm deep, filled with vermiculite up to 10 cm pot height. To prevent the diaspores from drying out, we covered them with about 1 cm-layer of vermiculite, moistened with tap water. The species-site combination (*Biscutella* from 4, *Bromus* from 3 and *Hymenocarpos* from 2 sites (missing at the semi-arid site) had 10 replicates, resulting into a total of 90 pots. We recorded the first emergence one week after the experimental set-up, and afterwards we recorded and removed the emerging seedlings every second day. No additional emergence occurred after mid December 2003, when we finished the experiment. Results from previous experiments and viability tests indicated that seeds, which did not germinate in the first year, were mostly dormant and not dead.

Experiment II

Parallel germination trials in the Phytotron compared germination fractions among the natural populations during 2002, 2003 and 2004. Every spring we collected diaspores from the natural populations and stored them in their original sites over the summer for natural breaking summer dormancy. In September each year, we collected the diaspores from the field and counted them into equal portions for sowing in a germination experiment. Since diaspores stored outdoors over the summer may loose their viability, every year we first examined the seed viability by laboratory germination of one hundred seeds per species and site. For the viability trials, we placed 20 seeds on moisturized filter paper in each of 5 petri dishes (9 cm diameter) and germinated seeds under 23 ºC for ten days. Subsequently, we poked the ungerminated seeds with a needle examining whether the embryos were fleshy and viable ('poking method' adopted from Pake & Venable 1996). The final percentage of viable seeds was the sum of the germinated seeds and those with fleshy embryos. All three species had high viability, ranging among the sites and years: *Biscutella* 81-98%, *Bromus* 62-96% and *Hymenocarpos* 92-100%.

From mid November to mid December each year we run an experimental trial in the Phytotron. Diaspores germinated in pots filled with vermiculite (pots of the same size with the same added seeds numbers as above). The species-station combination (*Biscutella* from 4 stations and *Bromus* and *Hymenocarpos* from 3 stations) had 20 replicates, resulting into a total of 200 pots every year. The course of the experiment and germination monitoring was the same as in the first experiment.

Data analyses

We estimated the germination percentage as a fraction of seeds germinating from supplemented seeds (experiment I) and from supplemented viable seeds (experiment II). We employed analysis of variance to evaluate the two experiments, where percent germination was the dependent variable. For analyzing the interpopulation differences in the F-1 seeds, we constructed separate one-way ANOVAs for each species with a single factor 'site'. To analyze the second experiment, we used two-way ANOVAs for each species, with 3 years x 4 resp. 3 sites (dependent on species). Tukey's multiple range post-hoc tests ($p<0.05$) compared pair-wise differences among seed origins within the species and sites in both experiments. Data from both experiments satisfied assumptions of ANOVA without transformation.

Results

Germination of F-1 seeds from standard maternal environment

Germination fractions of the F-1 seeds of *Biscutella* were significantly lower for the arid sites compared to the Mediterranean sites (Figure 2, $F_{df} = 117.2_3$, $p < 0.001$). The fraction of germinating arid F-1 seeds of *Bromus* was significantly lower than the fractions of the semi-arid and the Mediterranean seeds (Figure 2, $F_{df} = 91.9_2$, $p < 0.001$). Germination fractions of F-1 seeds of *Hymenocarpos* were low overall (Figure 2); the Mediterranean F-1 seeds had higher germination fraction than the mesic Mediterranean seeds ($F_{df} = 12.6_1$, $p = 0.002$, Figure 2).

Three-year germination patterns of seeds from natural populations

Germination fractions of *Biscutella* increased gradually with increasing rainfall from the arid to the mesic Mediterranean site, and this pattern was consistent over the three years (significant site and non-significant year effect, Table 1, Figure 3). In contrast, the arid and the semi-arid seeds of *Bromus* had high germination fractions that declined significantly in the Mediterranean seeds (significant site effect, Table 1, Figure 3). While the germination fractions of arid and semi-arid seeds of *Bromus* were relatively consistent among the years, there was a large inter-annual variation in germination fraction of the Mediterranean seeds (significant site x year interaction, Table 1, Figure 3). Germination fractions of *Hymenocarpos* were low in all years (Figure 3) and varied inconsistently (all effects significant, Table 1, Figure 3).

Discussion

In this study, we document germination fractions of a crucifer *Biscutella didyma* and a grass *Bromus fasciculatus* varying in a clinal fashion between two extreme ends of a steep aridity gradient in Israel. This germination pattern was clearly manifested in F-1 seeds that were cultivated under standard maternal environment. In *Biscutella*, the trend was also prominent and consistent for seeds from natural conditions during three consecutive years. Here, the germination fractions gradually increased from arid to mesic Mediterranean environment with increasing annual average and predictability of rainfall. Overall, these findings support the classic, though rarely rigorously tested model of optimal germination strategy (Cohen 1966). Specifically, our results support one of the key hypotheses of the model, i.e. the constant germination fraction across the years that has not been shown before (comp. Philippi 1993a, Clauss & Venable 2000).

The germination trends clearly presented in F-1 seeds were species-specific. Most interestingly, the germination fractions of *Biscutella* and *Bromus* were distinctly separated between the arid and Mediterranean populations, suggesting that this germination pattern is genetically determined. To support this, the germination pattern of F-1 seeds of *Biscutella* was consistent with the germination patterns of seeds from natural populations in all three years. In contrast, the germination patterns of F-1 and natural seeds of *Bromus* differed. For example, the fraction of germinating mesic Mediterranean seeds from natural populations was lower in the drier populations, as oppose to the pattern of the F-1 seeds. Moreover, the inter-annual variation in germination fractions of *Bromus* was large. This variation, however, did hot reflect maternal effects in the natural populations. While maternal effects predict the trend in germination fractions and the trend in rainfall amounts reversed, the trends had the same direction over the three years. In fact, the inter-annual germination patters of *Biscutella* and *Bromus* were very consistent, reflecting low rainfall variation among the years. Apparently, inter-annual rainfall differences must be much larger to allow prominent expression of maternal effects on germination (Tielbörger & Valleriani 2005). Therefore, if there were maternal effects within the natural populations, they did not differ among the years since the annual rainfall amounts were very similar. However, we cannot exclude the possibility of confounding by other uncontrolled effects of the field conditions, e.g. by various factors of the physical environment. Therefore, a *real* test with all conditions being equal was our experiments with F-1 seeds.

There were quantitative differences between the two experiments. Namely, the germination fraction of arid F-1 seeds of *Biscutella* and *Bromus* and semi-arid F-1 seeds of *Biscutella* were lower compared to the same seeds from the natural populations germinating within the same year. While this may suggests confounding by maternal or other environmental effects, there may also be another interpretation. Namely, although we standardized the maternal environment when propagating the F-1 seeds, the conditions under which seeds matured were not resembling ‘the same’ year for the arid as for the Mediterranean seeds. The Mediterranean seeds were experiencing ‘average’ conditions similar to the natural conditions, but the arid seeds grew in ‘very wet’ conditions. Therefore, the arid seeds were exposed to excessive maternal effects that were unwanted. Since these ‘unwanted’ maternal effects may mask the real expression of the genetic differences among the populations, we encourage careful addressing of maternal effects in future experiments. Namely, further tests should separate the different germination components, such as the different maternal effects (i.e. environmental vs. genetic effects) in order to define the genetic basis for the susceptibility to maternal effects.

While modeling of optimal germination strategy has been progressing fast, there are almost no advances in relating the existing empirical results to the theory. An exception is a recent study by Maffei et al. (2005) finding encouraging fitting between empirical data (for crustacean *Anostraca*) and the theoretical predictions, indicating validity of the Cohen's theory. Not only that such a link has not been made for annual plants so far, but also results of the very few existing rigorous empirical tests have often been confounded (Philippi 1993a,b, Clauss & Venable 2000, Volis et al. 2002), and no empirical studies have separated maternal effects thus far. In this study we found germination patterns of two species supporting the theory (*Biscutella* and *Bromus*), while another species (*Hymenocarpos*) showed very low and non-clinal germination. Similarly, previous studies documented results that were inconsistent and varying among species (e.g. Jain 1982, Hacker & Ratcliff 1989, Platencamp 1991, Philippi 1993b, Kigel 1995, Clauss & Venable 2000). Such findings (including our own data) indicate that the applicability of the Cohen's (1966) model is species-specific.

In conclusion, our results support the former observation, that germination patterns are maintained within a species and over time in response to environmental variation (Jain 1982, Hacker 1984, Hacker & Ratcliff 1989, Kigel 1995). On the contrary, the populations varied greatly in dormancy. This indicates adaptive value of dormancy in relation to aridity clines. Consequently, it suggests genetic differentiation and local adaptation of the populations, as supported by the consistent germination patterns among the three years of our study and the recently documented identical clines in morphological and reproductive traits of *Biscutella didyma* (Petrů et al. in press, Nachreiner 2005). However, we advocate proper methodological approaches to establish the genetic basis for our observed germination patterns with clearly addressed maternal effects.

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Table 1. Results of ANOVAs for controlled-condition seed germination from the four field sites (arid, semi-arid, Mediterranean and mesic Mediterranean) in three years (2002, 2003 and 2004); separate analyses for each of the three species due to differing species distribution (*Biscutella* at all sites, *Bromus* not in mesic Mediterranean and *Hymenocarpos* not in arid site).

Factor	**Species**					
	Biscutella		*Bromus*		*Hymenocarpos*	
	F_{df}	**p**	F_{df}	**p**	F_{df}	**p**
Site	71.10_3	**< 0.001**	402.10_2	**< 0.001**	25.73_2	**< 0.001**
Year	0.90_2	0.410	171.08_2	**< 0.001**	103.74_2	**< 0.001**
Site x Year	3.29_6	**0.004**	34.48_4	**< 0.001**	63.60_4	**< 0.001**

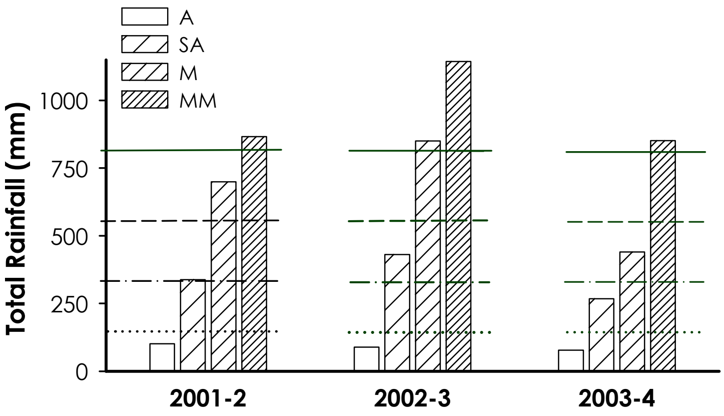

Figure 1. Total annual rainfall at the field sites: arid (=A), semi-arid (=SA), Mediterranean (=M) and mesic Mediterranean (=MM) in 2001-2, 2002-3 and 2003-4. Lines indicate long-term averages per site (dotted line for arid site (90 mm), dash-dotted for semi-arid site (300 mm), dashed for Mediterranean site (540 mm) and solid line for mesic Mediterranean site (780 mm).

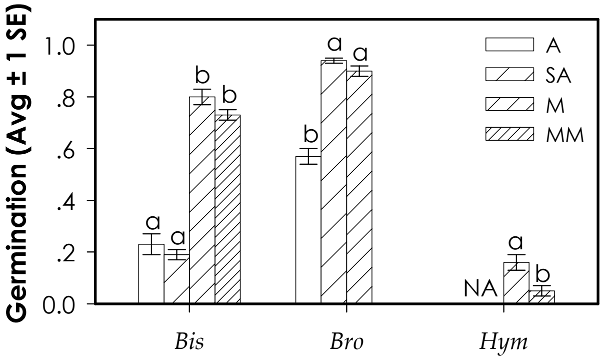

Figure 2. Controlled germination of F-1 seeds (Avg ± 1 SE) of *Biscutella* (=*Bis*), *Bromus* (=*Bro*) and *Hymenocarpos* (=*Hym*) from arid (=A), semi-arid (=SA), Mediterranean (=M) and mesic Mediterranean (=MM) sites. F-1 seeds were propagated in standard nethouse conditions in winter and spring 2002-3 and germinated in a growth chamber in fall 2003. Different letters above error bars indicate significant pair-wise differences among the sites within the species (Tukey's multiple range tests, $p<0.05$). Note that *Bromus* was not present at the mesic Mediterranean site and *Hymenocarpos* was not at the arid site and that data from the semi-arid site were not available (=NA).

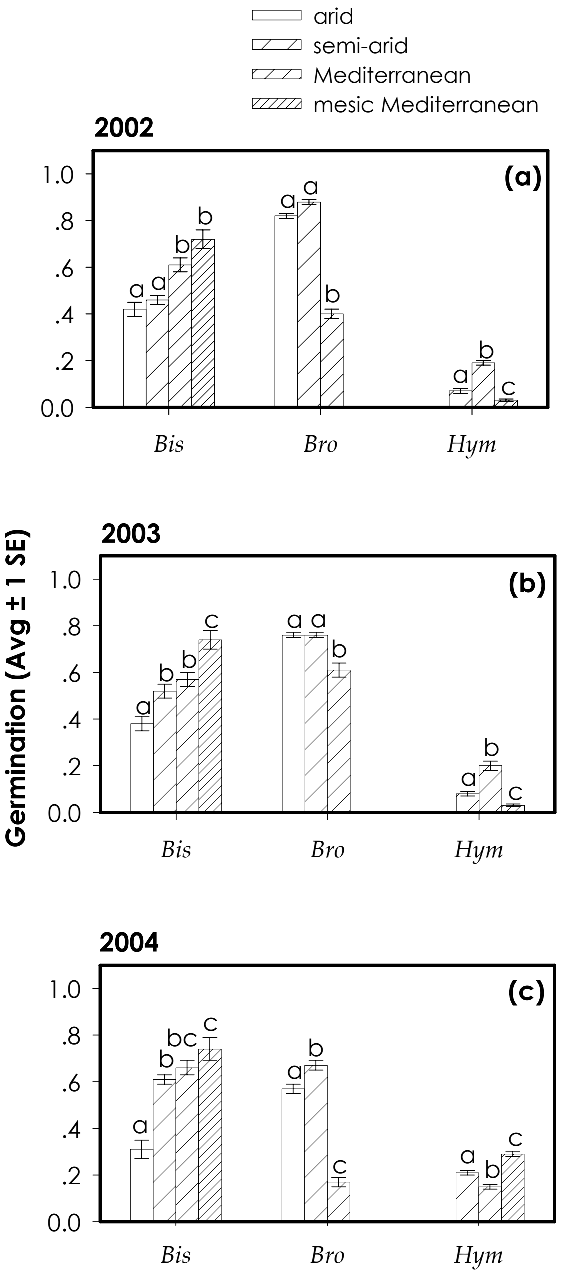

Figure 3. Controlled germination (Avg ± 1 SE) of *Biscutella* (=*Bis*), *Bromus* (=*Bro*) and *Hymenocarpos* (=*Hym*) seeds from natural populations over three years **(a)** 2002, **(b)** 2003 and **(c)** 2004. Different letters above error bars indicate significant pair-wise differences among the sites within the species (Tukey's multiple range tests, $p<0.05$). Note that *Bromus* was not present at the mesic Mediterranean site and *Hymenocarpos* was not at the arid site.

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Chapter 2

An empirical test for maternal effects on seed dormancy under field-manipulated rainfall conditions

An empirical test for maternal effects on seed dormancy under field-manipulated rainfall conditions

Abstract

Maternal effects can have a considerable role in seed dormancy evolution of annual plants. Maternally influenced germination fractions allow predicting (and avoiding) sibling over-crowding. Theoretical studies modeled maternal fecundity positively related to the favorability of environment, and offspring germination negatively related to maternal fecundity. We performed an empirical test for these predictions, and for the first time we related predictive germination to competition in real field populations. For two years we subjected the natural populations of three annual plants in semi-arid and Mediterranean regions in Israel to different maternal environments, where rainfall was either reduced with rainout shelters or enhanced with artificial irrigation. We evaluated seed production among the maternal environments, and subsequently tested the offspring germination under controlled conditions. The rainfall manipulations successfully decreased and increased annual rainfall. Seed production often increased with increasing rainfall in the manipulations at both sites and in both years. Germination in the first offspring generation was negatively related to the maternal fecundity for two of our species from the Mediterranean site. We found no such relationship at the semi-arid site. This suggests that in the more humid and more productive Mediterranean site, greater plant density and greater competition intensity induce stronger maternal selective pressure. Maternal effects were reduced in the second year, indicating their short-term advantage in controlling sibling competition. While in the long-term, germination fractions should be positively, not negatively, correlated with mean annual rainfall and rainfall predictability, more studies of maternal effects are needed to generalize.

Introduction

Seed dormancy has been theoretically modeled as a strategy of escaping from temporally unfavorable conditions in variable and unpredictable environments. By spreading their germination over several years, plants reduce extinction risk, and on the average exploit more favorable conditions. Numerous models attempted to evaluate the optimal germination strategy under certain statistical properties of a given environment. In the pioneering model (Cohen 1966), optimal germination fraction was constant and proportional to the favorability and predictability of conditions between environments. Most of the following models were developed in the 1980's extending the original Cohen's (1966) form to so-called 'predictive germination'. Germination was modeled as a phenotypic plasticity to highly reliable cues, which indicated the favorability of the upcoming season, e.g. the first rainfall of the season (Cohen 1967, Venable & Lawlor 1980). In the more recent studies, predictive germination was extended by an alternative view of seed dormancy, i.e. a mechanism of escaping unfavorable biotic conditions. Namely, high density of neighbors was the cue that indicated favorability of the future environment. Subsequently, optimal germination fractions were modeled as negatively density-dependent and constant under generalized or sibling competition (Westoby 1981, Bulmer 1984, Ellner 1985a,b, 1986, 1987, Léon 1985, Nilsson et al. 1994, Kobayashi & Yamamura 2000).

The most recent model (Tielbörger & Valleriani 2005) innovated the previous approaches by (i) integrating individual-based sibling competition with population-based generalized intraspecific competition and by (ii) assuming that seeds do not have constant, but different germination probabilities (i.e. heterogeneous seed bank). This novel approach coupled predictive germination generated by variable environmental conditions with dormancy strategies evolving via sibling competition. Seed dormancy was predicted higher (or germination lower) if mother plants produced many seeds (large seed families) in favorable (wet) seasons and *vice versa*.

Compared to the myriad of models on germination theory, the amount of rigorous empirical tests verifying the theoretical predictions from natural populations is limited to a handful of studies (Philippi 1993a,b, Pake & Venable 1996, Hyatt & Evans 1998, Clauss & Venable 2000). It is not surprising that the model of Tielbörger & Valleriani (2005) has not been yet rigorously empirically tested. Though there are two studies that had been tested for sibling competition even before heterogeneous seed banks have been modeled (Tielbörger & Valleriani 2005). First Hyatt & Evans (1998) found that seeds of large seed families of a short-lived perennial *Lesquerella fendleri* have lower germination fractions than seeds of small seed families. This study focused solely on the sibling competition hypothesis and found marginal support. However, the authors admitted potential confounding by heterogeneous field conditions, from which maternal plants were collected. Second, Philippi (1993b) showed that the experimentally manipulated plant size of *Lepidium lasiocarpum*, a desert winter annual from the southwest US, was negatively correlated with offspring germination fractions, and he ultimately attributed these results to maternal effects.

Apparently, the two above-mentioned studies focused on specific aspects of the sibling competition theory, and their approaches differed from what would be the proper test for the Tielbörger & Valleriani's (2005) model. The Tielbörger & Valleriani's (2005) model was actually inspired by authors' own field observations of consistent negative relationship between seed production and subsequent germination fractions in three consecutive years. Here, in contrast to the previous two studies, four desert annuals were compared, and whole generations (population averages) of all species expressed the same pattern. However, these results were correlative and not experimental, and thus may have been confounded by inter-annual rainfall variation. Aware of that fact, Tielbörger & Valleriani (2005) highlighted the need of a rigorous test to establish the existence of maternal effects on seed dormancy under 'real-life' conditions in variable environments. They suggested controlling for the spatial and temporal variation of the environment, i.e. to manipulate rainfall within the same site and season, and subsequently test germination of seeds produced under the altered maternal environments.

With this study we aimed to perform such a test, and to study predictive germination under field conditions with respect to competition for the first time. Namely, we tested whether seeds may predict their future competitive environment via negative correlation between maternal seed production and offspring germination. In addition, we tested whether this effect would be detectable in a second year of rainfall manipulation.

Based on the novel theory of predictive germination (Tielbörger & Valleriani 2005), we tested if

(i) maternal seed production is positively correlated with annual rainfall,
(ii) offspring germination fraction is negatively correlated with maternal fecundity, and
(iii) this effect is more pronounced in environments where competition is a major environmental constraint.

Methods

Field rainfall manipulations

We manipulated rainfall during the growing season (approx. November-April) at two sites in a transition zone between arid and mesic climatic regions in Israel. The two sites are referred to as 'semi-arid' (300 mm mean annual rainfall) and 'Mediterranean' (540 mm mean annual rainfall). The semi-arid site is located 20 km north of Beer Sheva in the northern Negev, and the Mediterranean site is 15 km southwest of Jerusalem in the Jerusalem Mountains. Both sites are situated on south-facing slopes and have the same calcareous bedrock. The vegetation can be described as an open shrubland dominated by *Sarcopoterium spinosum* at both sites, and *Thymelaea hirsuta* and *Calicotome villosa* in the semi-arid and the Mediterranean sites, respectively. At the semi-arid site, annuals may reach a cover of 15%, and often grow under the canopy of shrubs. At the Mediterranean site, annuals reach a cover up to 60% in open patches between shrubs (Holzapfel et al. in press).

Plots with unmanipulated rainfall conditions were referred to as 'control' maternal environment. Naturally occurring rainfall was reduced by manually covering roofs of standard rainout shelters during targeted rain showers. Following the same rainfall event, drizzle sprinklers irrigated the wet plots (Sternberg et al., unpublished). At each site, five plots of 10 m x 25 m simulated either scenario of winters with drought (referred to as ‘dry’ maternal environment) or winters with increased rainfall (referred to as ‘wet’ maternal environment).

The rainfall manipulations operated first in the season 2002-3, and reduced and increased rainfall amounts to 83% and 113% of the current year rainfall, respectively, at the semi-arid site (Figure 1a), and to 74% and 119%, respectively, at the Mediterranean site (Figure 1b). Total rainfall exceeded the long-term average at both sites, to 144% at the semi-arid and to 156% at the Mediterranean site.

In the following season 2003-4, the rainfall manipulations reduced and increased the current rainfall amounts to 58% and 134%, respectively, at the semi-arid, and 68% and 141%, respectively, at the Mediterranean site (Figure 1).

The following season 2003-4 was drier with 89% of the long-term average at the semi-arid and 81% at the Mediterranean site (Figure 1).

Study species

We studied three common winter annuals with wide distribution across Israel (Zohary 1966). The species represented different dormancy types. *Bromus fasciculatus* (further ‘*Bromus*’) is a grass with low dormancy caryopses. *Bromus* grows in the Mediterranean, Saharo-Arabian and Irano-Turanian regions (Feinbrun-Dothan 1986). *Biscutella didyma* (further referred to as ‘*Biscutella*’) is a crucifer germinating from fragmented double-silicles with intermediate dormancy. *Biscutella* has Mediterranean and Irano-Turanian distribution. *Hymenocarpos circinnatus* (further ‘*Hymenocarpos*’) is a legume with pods containing two highly dormant seeds. This species has a Mediterranean distribution (Zohary 1987).

Diaspore production under rainfall manipulations

We estimated diaspore production of plants established under the different maternal environments in each of the two growing seasons. We counted matured diaspores of *Biscutella* and *Hymenocarpos* when they were still attached to plants in the field. For counting seeds of *Bromus*, the whole plants were brought to a laboratory. In the first season, the plant abundance under the different maternal environments was low, and we sampled eight randomly chosen plants per maternal environment and site. In the second season, the plant abundance was higher and allowed sampling fifty plants per species and maternal environment. Data for *Hymenocarpos* was missing due to rapid fruit dispersal in this season.

Diaspore collection and storage

We collected diaspores from the maternal environments in both years. Diaspores from the first year are referred to as 'first seed generation'. In the second year, the collection included diaspores of plants established from the previous-year seeds that have naturally dispersed within the plots, and therefore this 'second seed generation' experienced two years of the same quality of maternal environment. For natural breaking summer dormancy, we stored the diaspores collected in each year in their original sites over the summer in bags made of organza (permeable transparent synthetic fabric), attached to the ground surface. In September each year, we collected the diaspore bags from the field and transferred them to a laboratory for seed counting in equal portions and subsequent sowing into a controlled-condition germination experiment.

Seed viability test

Seeds that are stored in the field over summer for dormancy breaking may loose their viability. Therefore, each year we first examined the viability of one hundred seeds per species, site and treatment, germinating them in laboratory conditions (23 ºC) for ten days. For this trial, we placed 20 seeds on moisturized filter paper in each of 5 petri dishes (9cm diameter). Subsequently, we poked the ungerminated seeds with a needle to examine whether the embryos were fleshy and viable ('poking method' adopted from Pake & Venable 1996). The total of viable seeds was the sum of the germinating seeds and those with fleshy embryos. The three species had high viability ranging between the sites and years with no significant variation among the maternal environments (*Biscutella* 0.81–0.91, *Bromus* 0.62–0.96 and *Hymenocarpos* 0.90–1.00).

Controlled germination trials

We initiated germination trials in a growth chamber (further 'Phytotron') at the Hebrew University in Rehovot, Israel in mid November each year, for each species separately. We added 20 *Biscutella* seeds, 40 and 30 *Bromus* (in the first and second year, respectively) or 40 seeds of *Hymenocarpos* (20 fruits; each enclosing 2 seeds) to pots 12 cm in diameter and 12 cm deep. We filled the pots with vermiculite up to 10 cm pot height and soaked the vermiculite with tap water with no added nutrients. To prevent the seeds from drying out, we covered them with a 1 cm-layer of well-moistened vermiculite. We exposed the seeds to a temperature range of 16/10 ºC day/night and watered them excessively twice a day. The experimental design (2 sites x 3 species x 3 maternal environments) had 20 replicates resulting into a total of 360 pots each year. The first seedlings emerged one week after the beginning of the experiment. Afterwards we counted the seedlings every other day and removed them from the pots. The experiment was completed in four weeks, when no additional seedlings emerged. Results from parallel experiments and viability tests indicated that seeds, which did not germinate in the first year, were mostly dormant and not dead.

Data analyses

We used analysis of variance to evaluate seed production per mother plant and offspring germination percentage (the proportion of viable seeds that germinated) for the maternal environments, species and sites in a full factorial design (2 sites x 3 species x 3 maternal environments). We tested the maternal effects on the second seed generation in separate ANOVAs, where the model for seed production included only 2 species, *Biscutella* and *Bromus*. The model for germination was the same as for the first generation. We square-root transformed the seed numbers in both years to meet assumptions of ANOVA. Germination data from both years satisfied assumptions of ANOVA without transformation. Tukey’s multiple range post-hoc tests ($p<0.05$) evaluated pairwise differences among the maternal environments within the species and sites.

Results

Maternal seed production

The seed production in the different maternal environments was positively correlated with rainfall in all species and years (Figure 2a,b, significant differences among maternal environments, Table 1). The pair-wise comparisons of the maternal environments within species and sites were significant with the exception of *Biscutella* and *Hymenocarpos* at the Mediterranean site (Figure 2b).

Offspring germination

The offspring germination trends across the maternal environments differed between the two sites (significant site effect, Table 1). The differences among the maternal environments were significant for the Mediterranean site and non-significant for the semi-arid site (significant interaction site x maternal environment Table 1, Figure 2c,d). The offspring germination was negatively related to maternal fecundity in *Biscutella* and *Bromus* for the Mediterranean site (Figure 2d comp. to Figure 2b), where the germination fractions were significantly greater for the dry maternal environment than for the control and the wet maternal environments (Figure 2d). Germination of *Bromus* at the semi-arid site weakly supported the trend observed at the Mediterranean site (Figure 2c comp. to Figure 2d).

Second-generation maternal seed production and offspring germination

Second-generation maternal seed production increased under higher rainfall amounts in the maternal environments, consistent with the first generation, but the differences were more significant (Figures 3a,b comp. to Figure 2a,b, significant differences among maternal environments, Table 2).

The negative relationship between maternal fecundity and offspring germination, as found in the first generation, did not hold in the second generation. The overall germination differences among the maternal environments were not significant (Table 2). However, at both sites, seeds of *Biscutella* from the wet maternal environment had reduced germination (Figures 3c,d) compared to the drier maternal environments. The sites and species and all the interactions were significant (Table 2), but there was no directional trend across the maternal environments (Figures 3c,d).

Discussion

This study presents the first experimental evidence of predictive germination under field conditions with respect to competition. For two annual plant species (*Biscutella didyma* and *Bromus fasciculatus*) from the Mediterranean region in Israel, we found strong negative relationship between maternal fecundity and offspring germination. Most interestingly, these effects differed between the sites and years, i.e. they were not found in the semi-arid site and in the second year of our study. In the following we discuss these deviations from the generally predicted optimal germination strategy.

The maternal effects were prominent at the more humid Mediterranean site (with 540 mm mean annual rainfall), which is less constrained by rainfall variation than the semi-arid site (with 300 mm). Consequently the main environmental constraint at the Mediterranean site is the higher plant density and biomass (Holzapfel et al. unpublished, Kigel & Konsens unpublished, Schiffers & Tielbörger, in press). In concordance with the classic competition theory (Grime 1973a,b, 1977, Fowler 1986, Linhart 1988), previous studies showed stronger competition pressure in this more productive Mediterranean site (Schiffers & Tielbörger, in press). On the contrary, the interplay of the environmental constraints is less straightforward at the semi-arid site. While rainfall is the main environmental constraint in the semi-arid region, previous studies showed that in this region, facilitation can be more important than competition, depending on annual rainfall amounts. Namely, there are years when having neighbors is beneficial, i.e. facilitation (Holzapfel et al. in press, Tielbörger et al., unpublished) and selection towards reducing their density would become disadvantageous. The site-specific offspring germination patterns are rather interesting considering the fact that maternal fecundity and rainfall were consistently positively correlated at both sites, and even between years. Apparently, maternal fecundity responds to variable rainfall conditions with high phenotypic plasticity. However, the response of offspring germination to maternal fecundity is based on sibling competition. Reviewing the assumptions of the Tielbörger & Valleriani's model (2005), it is not only sibling competition but also generalized competition that under variable environmental settings generate this negative mother-offspring relationship. As previous studies documented (e.g. Tielbörger & Kadmon 1995, 2000), generalized competition is a key pre-requisite for density-dependent seedling survival. Therefore, maternal effects are highly probable to occur in competitive environments.

Our findings indicate that density-dependence/competition can have a tremendous effect on seed dormancy, as predicted also by Tielbörger & Valleriani's (2005) model.

In fact, this study was specifically designed to experimentally test for the Tielbörger & Valleriani's (2005) model. Moreover, our results contribute to the empirical evidence of sibling competition, which has been studied rarely and from different aspects. Hyatt & Evans (1998) provided an empirical evidence exclusively for sibling competition hypothesis (i.e. lower germination fractions of large seed families). Findings of Philippi (1993b) on negatively correlated plant size (seed family size) and offspring germination are qualitatively consistent with our results, although the motivation behind Philippi's (1993b) and our tests was not exactly the same. Finally, Tielbörger & Valleriani (2005) present their own empirical data that actually inspired their model, but this empirical study is not a rigorous test of the model.

In conclusion, integrating the theoretical and empirical results of Tielbörger & Valleriani (2005) and results of our study, maternal effects apparently allow predicting future environmental conditions long before seed germination. Plant densities in the mother environment seem to be a reliable cue for optimal offspring germination. Although the exact mechanism has not been clarified yet, it could be, for example development of a thicker seed coat or higher concentrations of germination inhibitors (Roach & Wulff 1987) in seeds that matured in more humid conditions and higher plant density.

Over the two seasons of rainfall manipulations in a row, maternal environments changed consistently, i.e. the plant densities decreased resp. increased in the same direction (Tielbörger et. al., unpublished). However, the patterns of maternal seed production and offspring germination differed between on the first and second year. While maternal fecundity increased under higher rainfall in both years, germination fractions were negatively related to maternal fecundity only in the first generation. This second-year breaking of the first-year pattern does not support the Tielbörger & Valleriani's (2005) prediction. Furthermore, maternal effects have been predicted to control sibling competition and to maximize maternal and offspring fitness (Roach & Wulff 1987, Wulf 1995, Galloway 2005), however a situation, in which they operate on a sequence of generations has not yet been discussed in detail. While maternal effects represent just one possible way of generalizing the expected behavior of seeds, there is no other theoretical explanation to our current knowledge. In the long run, if the climate changes to a consistently more arid one (i.e. lower and more variable rain), an increase in germination would eventually become disadvantageous. Germination should be low in more variable conditions (Cohen 1966), and as shown by our own results, competition as an environmental constraint should become less important. However, while a long series of increasing arid conditions should, in theory, favor smaller germination fraction, there is no sensible reason for maternal effects being invisible after only two drought (resp. wet) years in a row. Actually, if this pattern is universal, one would need to assume that a 'grandmother' plant might inform its 'grandchildren' about the quality of the environment two years ago.

To summarize, our overall findings on maternal effects reducing sibling competition in competitive environments seem to indicate a short-term advantageous mechanism for predicting future germination success. Our results document that the predicted negative relationship between maternal fecundity and offspring germination breaks already in the second year. This is interesting per se, particularly since there is no current theoretical understanding of maternal effects on subsequent generations. In the long-term, germination should be positively, not negatively correlated with predictability of annual rainfall (Cohen 1966, 1967), as shown by Philippi (1993b), Pake & Venable 1996, Clauss & Venable 2000 and our recent parallel study (Petrů et al., unpublished). However, since this is the very fist rigorous study of maternal effects on predictive germination, we call for more field tests to prove whether the patterns observed here are universal.

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Table 1. Results of ANOVAs for maternal seed production under different maternal environments in 2002-3 (n=8) and controlled-condition offspring germination in 2003-4 for two sites (semi-arid and Mediterranean), three species (*Biscutella*, *Bromus* and *Hymenocarpos*) and three maternal environments (dry, control and wet).

Factors		**2002-3 Maternal seed production**		**2003-4 Offspring germination**	
	df	**F**	**p**	**F**	**p**
Site	1	4.74	**0.031**	21.22	**< 0.001**
Species	2	17.94	**< 0.001**	1158.89	**< 0.001**
Maternal Environment	2	34.24	**< 0.001**	36.81	**< 0.001**
Site x Species	2	3.63	**0.029**	54.95	**< 0.001**
Site x Maternal Environment	2	3.92	**0.022**	22.48	**< 0.001**
Species x Maternal Environment	2	5.78	**< 0.001**	20.19	**< 0.001**
Site x Species x Maternal Environment	4	2.94	**0.023**	16.71	**< 0.001**

Table 2. Results of ANOVAs for maternal seed production under different maternal environments in 2003-4 (n=50) and controlled-condition offspring germination in 2004-5 for two sites (semi-arid and Mediterranean), three species (*Biscutella*, *Bromus* and *Hymenocarpos*) and three maternal environments (dry, control and wet). Note that maternal seed production data was missing for *Hymenocarpos*.

	2003-4			2004-5		
	Maternal seed production			Offspring germination		
Factors	**df**	**F**	**p**	**df**	**F**	**p**
Site	1	19.36	**< 0.001**	1	104.75	**< 0.001**
Species	1	1.53	0.216	2	27.33	**< 0.001**
Maternal Environment	2	40.56	**< 0.001**	2	2.46	0.087
Site x Species	1	31.84	**< 0.001**	2	70.79	**< 0.001**
Site x Maternal Environment	2	2.62	0.074	2	13.63	**< 0.001**
Species x Maternal Environment	2	6.34	**0.002**	4	9.00	**< 0.001**
Site x Species x Maternal Environment	2	0.09	0.914	4	18.79	**< 0.001**

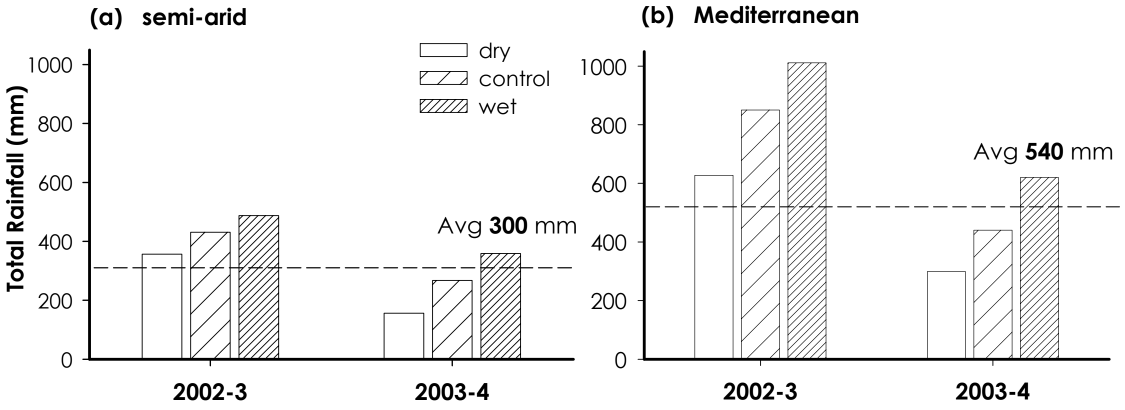

Figure 1. Total annual rainfall in rainfall manipulation plots (dry, control and wet) at **(a)** semi-arid site and at **(b)** Mediterranean site in the seasons 2002-3 and 2003-4. Dashed lines indicate the long-term averages (300 mm in the semi-arid site and 540 mm in the Mediterranean site).

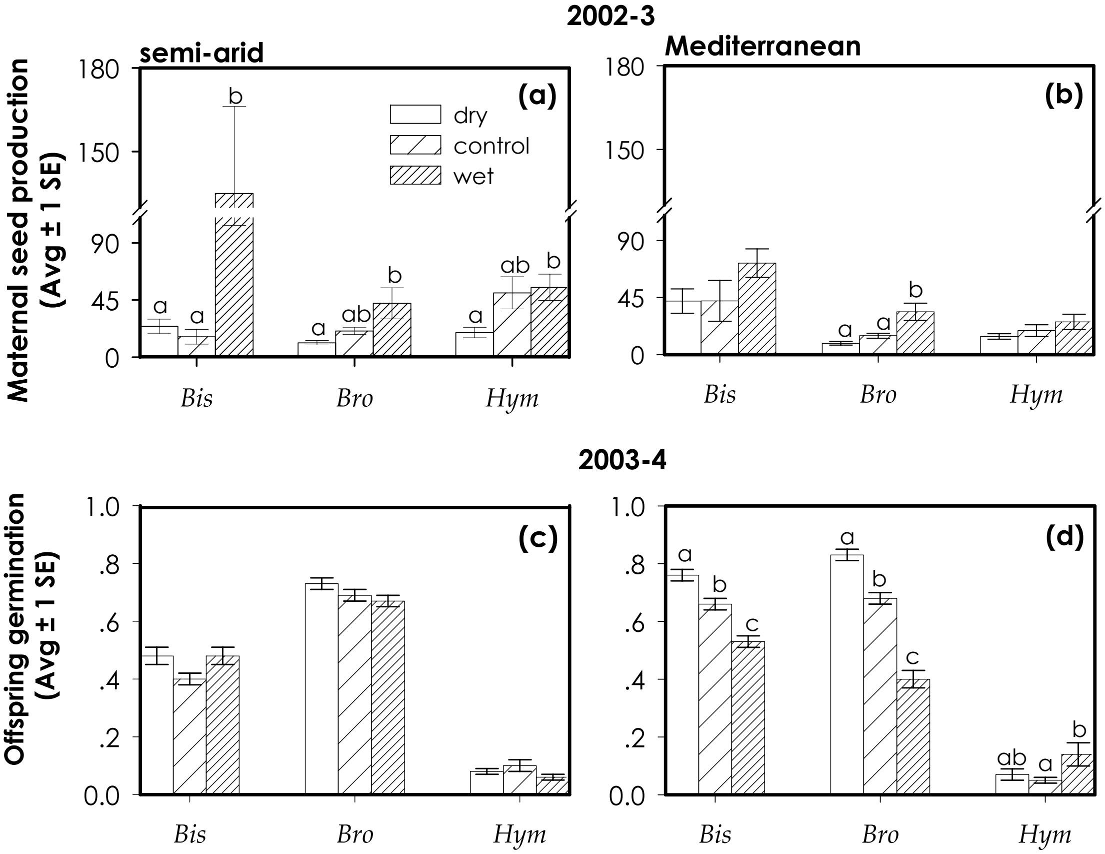

Figure 2. Seed production of plants established under different maternal environments (dry, control and wet) in 2002-3 ('fist generation'): *Biscutella* (=*Bis*), *Bromus* (=*Bro*) and *Hymenocarpos* (=*Hym*) at **(a)** semi-arid and **(b)** Mediterranean sites. Offspring germination of seeds collected in 2002-3 from the maternal environments at **(c)** semi-arid and **(d)** Mediterranean sites, and germinated under controlled conditions in 2003-4. Different letters above error bars indicate significant pair-wise differences between maternal environments within species and site (Tukey's multiple range tests, $p<0.05$).

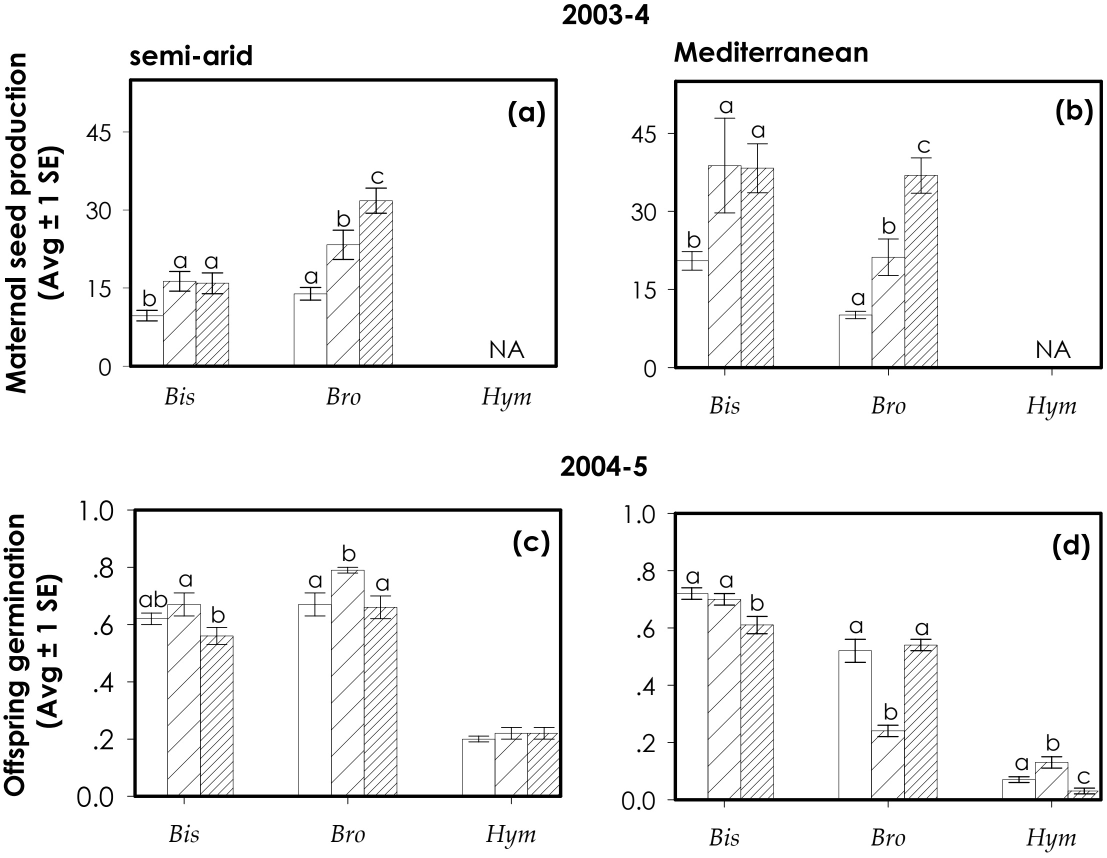

Figure 3. Seed production of plants established under different maternal environments (dry, control and wet) in 2003-4 ('second generation'): *Biscutella* (=*Bis*), *Bromus* (=*Bro*) and *Hymenocarpos* (=*Hym*) at **(a)** semi-arid and **(b)** Mediterranean sites. Offspring germination of seeds collected in 2003-4 from the maternal environments at **(c)** semi-arid and **(d)** Mediterranean sites, and germinated under controlled conditions in 2004-5. Different letters above error bars indicate significant pair-wise differences between maternal environments within species and site (Tukey's multiple range tests, $p<0.05$). Note that maternal seed production data for *Hymenocarpos* was not available (=NA).

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Chapter 3

Germination strategies of annual plants under changing climatic conditions: lessons for future field experiments

Germination strategies of annual plants under changing climatic conditions: lessons for future field experiments

Abstract

In a large-scale field experiment we studied theoretical predictions for germination strategies of annual plants under varying climatic conditions. Simultaneously, we evaluated the importance of separating climatic from other environmental variables. We reciprocally transplanted conspecific seeds of three annual species from and to four sites along a steep aridity gradient in Israel. Based on theory, we predicted higher germination fractions for seeds from wetter origins and at wetter sites. By sowing seeds on both local and standard soil, we separated climatic from edaphic and neighbor effects. We expected germination patterns on local soil to be masked by environmental factors other than climate. Substrate effects were very strong with germination fractions consistently lower on local soil. Climatic effects, on the contrary, were relatively weak. Only two species at the arid site supported the origin prediction, and on-site rainfall effects on germination were inconsistent and insignificant. Local environmental conditions may override effects of climate and should be critically addressed in future studies testing for the potential of species to adapt to changing climate.

Introduction

The acknowledgment of recent climate change has triggered an increasingly large number of studies trying to predict extinction probabilities of plant and animal species under a changing climate. Many of these studies have adopted the so-called 'bioclimatic envelope approach' which is based on the assumption that organisms will actively follow their 'preferred' climate in space resulting in considerable species range shifts (Huntley, 1988; Webb *et al.*, 1993; Davis & Shaw, 2001; Thomas *et al.*, 2004; Thuiller *et al.*, 2005). While this approach has become very popular, it has also been heavily criticized as being rather simplistic and disregarding factors other than climate that determine the abundance of species (Pearson & Dawson, 2003; Gray *et al.*, 2004). For example, this approach unrealistically assumes that the entire set of abiotic conditions and coexisting species may travel to the new range alongside the species of interest. In addition, the fact whether a species is able to persist under climate change, greatly depends on the degree of its local adaptation to current climate and potential pre-adaptation to future conditions (Jump & Peñuelas, 2005). Considering the rapid rate of contemporary climate change, the in situ adaptive capacity increases in importance for the persistence of species, since many species may be unable to migrate fast enough to track their climatic envelope (Davis & Shaw, 2001; Jump & Peñuelas, 2005).

Numerous studies have investigated the role of local adaptations for individual fitness or population growth rates (Harper, 1977; Bradshaw, 1984; Galen *et al.*, 1991; Bell, 1996; Linhart & Grant, 1996). Clearly, climate is an important factor controlling the degree of adaptation of organisms to their local environment (Turesson, 1925; Clausen *et al.*, 1940; Langlet, 1971). Therefore, an increasing number of recent studies have investigated, to which extent local adaptation to climatic conditions may drive the response of species to climate change (Billington & Pelham, 1991; Lynch & Lande, 1993; Potvin & Toussignant, 1996; Etterson & Shaw, 2001; Etterson 2004a,b; Hamrick, 2004; Savolainen *et al.*, 2004).

There are several methods to test for local adaptation in plant populations, which have been used either exclusively or in combination. For example, previous studies have shown genetic differentiation between 'climatic ecotypes' using molecular methods (Linhart & Grant, 1996; Owuor *et al.*, 1999; Volis *et al.*, 2002e; Santamaria *et al.*, 2003). In another approach, apparent ecotypes are transplanted to different climatic conditions and monitored for their performance at home *vs*. at foreign environment (Langlet, 1971; Bradshaw, 1984; Schmid, 1985; Galen *et al.*, 1991; Bell, 1996; Linhart & Grant, 1996). Such an approach has been often used to test for local adaptation along climatic gradients assuming that climate per se is the main evolutionary constraint of ecotypic variation (Volis *et al.*, 2002a-d; Olsson & Ågren, 2002; Sugiyama, 2003; Casler *et al.*, 2004).

Yet, it has been shown that climate affects many other abiotic and biotic variables, such as soil properties (Rustad *et al.*, 2001; Emmett *et al.*, 2004), resource availability (Bowman *et al.*, 1993) or direction and intensity of biotic interactions (Callaway *et al.*, 2002; Schröter *et al.*, 2004; Holzapfel *et al.*, in press). However, many studies investigating ecotypic differentiation along climatic gradients have neglected the role of these confounding variables. This is unfortunate, since the above factors are important constraints for individual fitness. Therefore, conclusions about climate change effects and local adaptation from the above-mentioned studies are questionable. For example, in a set of recent studies on the annual grass *Hordeum spontaneum*, differences between populations were solely related to variation in rainfall along a climatic gradient, while the study sites differed also in bedrock and soil type, exposition (i.e. slopes vs. wadis) and other climatic variables, such as the number of frost days (Volis *et al.*, 2002a-d).

In summary, there are virtually no experimental field studies, which have attempted to test for local adaptation by separating climate from other environmental variables such as soil type or plant community, including inter- and intra-specific interactions.

With this study, we attempt to fill this gap. Namely, we performed reciprocal transplanting of conspecific seeds of three annual plants along a steep rainfall gradient, in which we separated the effects of climate from the local environment by varying the former and keeping the later constant. We achieved this by planting seeds into local natural and standard artificial environments. By examining multiple species, we aimed at critically evaluating the inherent assumption of bioclimatic modeling approaches that the entire community will simultaneously adapt to climate change.

Climate-related variation in life history traits has been frequently found in species along environmental gradients (Del Pozo *et al.*, 2002a,b; Olsson & Ågren, 2002; Santamaria *et al.*, 2003). In these studies, a variety of traits was measured, not all of which represented exclusive adaptation to climate. Since, as explained above, climatic gradients exhibit a complex of associated or independent changes in environmental variables, variation in focal traits may either reflect an adaptation to climate or to other factors. For example, an annual plant *Biscutella didyma* showed variation in reproductive allocation along an aridity gradient that may reflect both climatic conditions as well as response to above-ground competition (Petrů *et al.*, in press). Taking all the above into account, it is desirable to identify traits which are solely constrained by climatic conditions.

Annual plants inhabiting dry and unpredictable environments are an ideal case to implement such an approach. First, they are short-lived and therefore may be able to adapt fast to changing environments (Aronson *et al.*, 1993; Potvin & Tousignant, 1996; Etterson & Shaw, 2001; Etterson, 2004a,b; Jump & Peñuelas, 2005). Secondly, they exhibit seed dormancy, a prominent trait which is clearly and directly related to climatic conditions. Many theoretical models have demonstrated that the potential of plants to produce seeds which spread their germination over several seasons can be selected for by the particular climatic conditions. Namely, in variable and unpredictable environments, the optimal strategy is reflected in a positive correlation between germination fractions and the probability of experiencing a favorable (i.e. rainy) year (Cohen, 1966). Furthermore, germination fractions should positively respond to predicted favorability of the upcoming season (Cohen, 1967), resp. germination increases with increasing actual rainfall amounts.

Based on the above-mentioned we hypothesized:

(i) Seeds originating from wetter sites have higher germination fractions than seeds from drier sites.

(ii) Seeds of the same origin have higher germination fractions when germinating in wetter than in drier sites.

(iii) Germination patterns are more likely to be observed in standard than in natural environment, where they may be confounded by factors other than climate or weather.

Materials and Methods

Study sites

We utilized four stations on similar bedrock and exposition (southern slopes), located on a S-N oriented gradient of increasing rainfall in Israel (further referred to as arid (A), semi-arid (SA), Mediterranean (M) and mesic Mediterranean (MM), Table 1). The growing season in the stations responds to the distribution of rainfall, and germination of annuals and re-growth of most perennials occurs in October-November, soon after the first major rains (~10-20 mm). The plant communities are species-rich, summing up to more than 600 species in all stations. There are few dominant species of perennial shrubs (Table 1) and associated annual plants, typically many species of legumes, crucifers, composites and grasses under the shrub canopies and in shrub inter-spaces (Tielbörger *et al.*, unpublished). The spatial structure of annuals (incl. their soil seed banks) is highly patchy.

Study species

Three study species represent common groups of winter annuals with wide distribution ranges and differential seed dormancy. *Biscutella didyma* (further referred to as '*Biscutella*') is a crucifer with Mediterranean and Irano-Turanian distribution (Zohary, 1966), and it occurs along the entire gradient. *Bromus fasciculatus* (further '*Bromus*') is a grass with Mediterranean, Saharo-Arabian and Irano-Turanian distribution (Feinbrun-Dothan, 1986) growing in the three southern stations. *Hymenocarpos circinnatus* (further '*Hymenocarpos*') is a legume with Mediterranean distribution (Zohary, 1987), present in the three northern stations along the gradient. The species represent different dormancy types: grasses *(Bromus)* with low dormancy, crucifers (*Biscutella*) with intermediate dormancy and legumes (*Hymenocarpos*) with high dormancy (Petrů & Tielbörger, unpublished).

Reciprocal sowing experiment

(a) Diaspores

We collected ripe diaspores (diaspores and seeds are used synonymously) in the field stations between March and May 2003. Our previous trials indicated that seed storing in the field is optimal to naturally break summer dormancy. Therefore, we stored seeds in their original field sites over the summer in bags from organza (permeable transparent synthetic fabric), attached to the ground surface. In September 2003, we collected the seed bags from the field and transferred them to a laboratory for counting into equal portions and subsequent sowing. Sowing took place in the field prior to the onset of the rainy season at the beginning of October 2003. We mixed 20 seeds of *Biscutella*, 30 *Bromus* and 20 fruits of *Hymenocarpos* (each enclosing 2 seeds), and spread them loosely on the surface of a 0.25 x 0.25 m plot area and covered with thin layer of soil. To prevent seed losses due to wind and seed predators before the beginning of the rainy season, we covered the plots with organza sheets, which were stabilized at the edges with stones (Fig. 1a). Previous trials showed that the permeable organza keeps the plots well aerated, it excludes neither rain nor sun and it does not substantially change the microclimate underneath. We kept the plots covered until short after the first rain to prevent secondary seed dispersal out of the plots.

Since seeds stored under natural conditions often loose their viability, we tested seed viability by laboratory germination of one hundred seeds per species and station on moisturized filter paper for ten days (in portions of 20 in five petri dishes of 9 cm diameter). Subsequently, we poked the ungerminated seeds with a needle for examining whether the embryos were fleshy and alive ('poking method' adopted from Pake & Venable, 1996). The final percentage of viable seeds was the sum of the germinating seeds and those with fleshy embryos. Viability was high in all three species and ranged among the stations; in *Biscutella* 0.88–0.98 and in *Bromus* 0.83–0.94, while all *Hymenocarpos* seeds were viable. Germination fractions were estimated on the basis of the number of viable seeds.

(b) Standard substrate

For the field experiment, we used 'standard substrate', a mixture of loess and hamra soil (2:1). We harvested loess (local soil type typical for the semi-arid region) at the experimental farm Gilat in the northern Negev of Israel by excavating the soil from at least 0.5 m depth in a long-term abandoned field. Hamra (sand with lime, developed from 'kurkar' cliffs along the Mediterranean coast) was provided by the Botanical Gardens of the Tel Aviv University in Israel. Preliminary tests showed that this mixture was devoid of seeds. We established standard substrate plots at each site by first excavating and removing the local soil from an area of 0.3 by 0.3 m, to a depth of 0.1 m. A sheet of organza 0.5 x 0.5 m large, spread across the excavated area, physically separated the different substrate types. We filled the standard substrate on the organza sheet and carefully flattened the surface, leaving edges of organza exceeding the outline of the plot. Few small stones placed around the border prevented the organza from touching the soil surface, while few bigger stones were used to stabilize the loose organza around each plot preventing substrate and seed loss due to run-off after the first rain (Fig. 1a). Subsequent experiments were done on standard and on local soil.

(c) General design

The reciprocal transplant experiment involved sowing of seeds to the home site and to the adjacent wetter and drier neighboring sites, respectively. For example, at the semi-arid (SA) station, we added seeds from the drier (A), home (SA) and the wetter (M) stations. In the two extreme stations, we planted seeds from home and one adjacent station, e.g. in the mesic Mediterranean (MM) we planted seeds from drier (M) and home (MM) stations (Fig. 1b). To detect local environmental effects (further also 'substrate' effects) on germination, we performed the experiments both on local substrate and in plots with standard substrate. A control plot with the local substrate and no seed addition controlled for background germination from the local seed bank. A blank standard-substrate plot controlled for germination from local dispersal. The final experimental design with sown and control plots in natural and standard environments resulted in a design with six plot types in the arid resp. the mesic Mediterranean station (without dry resp. wet plots) and eight plot types in the semi-arid and Mediterranean station (Fig. 1c), replicated 10 times. To test for differences among seed origins ('origin' effects), we compared germination of seeds from the home and the drier and/or wetter origins (Fig. 1ba) within each site. To test for germination response to on-site variable rainfall ('site' effects), we compared germination of seeds from each site within the home and at the neighboring drier and/or wetter sites (Fig. 1bb).

Germination census

Initially, we added seeds into the 0.2 x 0.2 m central area of 0.25 x 0.25 m plots to minimize edge effects. However, despite our careful set-up we noticed seed movement within the plots, probably due to runoff during heavy rainstorms, and therefore we decided to score germination in the entire 0.25 x 0.25 m plot area. Since November 2003, we monitored germination in 2-4 seedling counts. In the mesic Mediterranean site, where germination occurred as a single event at the beginning of the season and seedling survival during the season was high, we scored only twice (in November 2003 and January 2004). The germination events and seedling counts were more frequent in the arid stations, and we tracked cohorts after each major rainfall until mid February 2004, when germination ceased. At each census, we recorded and removed all newly emerged seedlings. After completion of germination, we removed all plots and supplemented seeds from all sites to avoid contamination with seeds from alien provenances.

Data analyses

Germination fraction was estimated as the difference between the number of seedlings in sown plots and control plots divided by the number of (viable) supplemented seeds. Due to the specific combinations of species origins and sites in the reciprocal transplant experiment (i.e. no *Hymenocarpos* in the arid station, and no *Bromus* in the mesic Mediterranean station), the overall design was necessarily incomplete. Therefore, we constructed separate ANOVA models for each site, resp. each seed origin and both substrates in every model. Two-way ANOVAs for each species included the fixed factors substrate (= 2) and origin, resp. site (= 2 or 3 depending on the species distribution). Tukey's post-hoc tests were done to compare pairwise differences among the sites resp. origins (when 3) within the species. Data satisfied the assumptions of ANOVA without transformation. To facilitate the visual interpretation of the results, we present the findings for origin and site effects in two separate figures.

Results

Effects of substrate

Germination fractions of all species were consistently higher on standard, compared to the local substrate (Fig. 2 and Fig. 3). The substrate effect was significant in *Bromus* in all origins and at all sites (Table 2 and Table 3, Fig. 2b,d,g and Fig. 3b,d,g) and in few origins and sites of *Biscutella* (Table 2 and Table 3, Fig. 2a,i and Fig. 3f,i) and *Hymenocarpos* (Table 2 and Table 3, Fig. 2e and Fig. 3e,h). Qualitative germination patterns differed between the two substrates for *Biscutella* at the semi-arid site (marginally significant interaction, Table 2, Fig. 2c) and for *Bromus* and *Hymenocarpos* from the arid and semi-arid sites, respectively (Table 3, Fig. 3b,e). Since the germination on standard substrate should reflect mainly the response to climatic variables, in the following we interpret our results related to origin and site effects for the standard substrate only.

Effects of seed origin

Germination fractions of wetter seed origins were significantly higher than of drier origins at the arid site in *Biscutella* and *Bromus* (Fig. 2a,b, Table 2). The same pattern could be seen in *Biscutella* at the semi-arid site (Fig. 2c, Table 2). Differences among seed origins of *Bromus* and *Hymenocarpos* germinating at the semi-arid site (Fig. 2d,e), and of all species at the two Mediterranean sites (Fig. 2f-j) were inconsistent and mostly not significant (Table 2).

Effects of site

Germination trends in response to variable on-site rainfall amounts showed weak support for the predicted pattern of higher germination fractions at wetter sites (Fig. 3). With the exception of the semi-arid seeds of *Biscutella*, which had significantly higher germination at the Mediterranean site compared to the drier sites (Fig. 3c, Table 3), the germination patterns of the other species and their origins at the other sites did not show a consistent trend and were mostly not significant (Table, Fig. 3a,b,d-j).

Discussion

With this study we tested for climate effects on optimal germination strategies of annual plants by improving standard approaches for studying local adaptation of plants to climate in the field. Namely, we separated climatic from other environmental effects and we focused on a trait, which should be largely determined by climate alone.

Our overall results clearly demonstrate that seed germination patterns of annual plants in a reciprocal transplant experiment differed considerably between local and standard environments. This indicates that local environmental effects may virtually override climatic effects in field experiments testing for local adaptation to climate. For example, in the standard environment, arid seeds of *Bromus* had higher germination at the wetter site (consistent with theory), while on local substrate the pattern was reversed. This clearly demonstrates the importance of experimental separation of the environmental variable of interest (i.e. climate) from the multitude of other local variables, such as edaphic or biotic selective forces.

At a first glance, this finding may not appear surprising. For example, numerous studies have documented how germination may vary according to differences in local environmental conditions in general and with respect to climate (Meyer & Monsen, 1991; Mariko *et al.*, 1993; Meyer *et al.*, 1995; Beckstead *et al.*, 1996; Meyer *et al.*, 1997; Vera, 1997; Shimono & Kudu, 2003). Other studies have investigated whether germination and subsequent life history traits (survival, morphology, reproduction, etc.) exhibit local adaptation to climate in field experiments along environmental gradients. For example, Joshi *et al.* (2001) tested for local adaptation of three common and widespread plant species in a cross-continental European transect and they found that environmental factors other than climate explained the greatest fraction of geographic variation in species traits. Similarly, in a set of interrelated studies, Volis *et al.* (2002a-d) found that climate effects could not be separated from other environmental effects when examining several life history traits of the annual grass *Hordeum spontaneum*. Therefore, while previous studies have found that many selective factors may operate simultaneously with climate, some of the very same studies did not succeed to experimentally separate the factor of interest from the 'noise' (e.g. Volis *et al.*, 2002a-d). Thus, while our findings may not appear unexpected per se, they explicitly demonstrate the potentially overriding effects of confounding factors in previous experiments designed to test for local adaptation to climatic conditions. We therefore advocate more careful experimental design for similar future field studies.

We suggest that in addition to climate, there are two dominant environmental variables which may have determined the response of the species to local environmental conditions: soil and neighbor conditions. While we aimed at mimicking the general soil conditions with the standard substrate, differences between local and artificial soil occurred automatically due to the different soil types at the stations. Field germination may vary considerably between soil types (Ohlson, 1999; Schutz *et al.*, 2002; Kubo *et al.*, 2004; Villagra & Cavagnaro, 2005) with available soil moisture being the most critical soil property for seed germination of annual plants. Therefore, higher germination fractions on standard soil may have been the result of differences in soil moisture. Interestingly, when we compared moisture levels between the local and standard soil in a parallel experiment, we consistently found higher moisture content in the local soil across all stations (Petrů, unpublished). This indicates, that the differences between the germination on local vs. standard soil could not be explained by soil moisture conditions. We therefore suggest that neighbor effects, i.e. effects of the local plant community may be responsible for the higher germination on standard soil. For example, seed density has been demonstrated to affect fraction of germinating seeds in several field and greenhouse experiments (Bergelson & Perry, 1989; Dyer *et al.*, 2000; Goldberg *et al.*, 2001; Turkington *et al.*, 2005). In a system similar to ours, Goldberg *et al.* (2001) and Turkington *et al.* (2005) have documented a negative correlation between germination fractions of annual plants and the density of surrounding seeds in the soil. While the mechanism behind such a behavior is still unclear, negative density dependence in seed germination may explain the pattern found in our study. Namely, germination from the control plots indicated that the total seed densities were up to three-fold higher on local soil than on standard soil (with only the supplemented seeds). Clearly, additional experiments are needed to establish the ultimate reason for the observed pattern. However, our findings demonstrate the potential importance of neighbors as opposed to the factor of interest (i.e. climate) for the performance of plants. This is important since direction and intensity of plant-plant interactions may vary greatly along aridity gradients (Holzapfel et al., in press;

Schiffers & Tielbörger, in press) and therefore, may be always confounded with climatic conditions.

In addition to the methodological aspect of our study, we aimed at testing theoretical predictions of bet-hedging germination of annuals in variable environments. The main finding from these tests is a relatively weak support for the predictions, but large differences among the species. While *Hymenocarpos* germinated almost independently of the local environment and climate, the other species showed trends supporting the results of theoretical models (Cohen, 1966; Cohen, 1967). Namely, *Biscutella* and *Bromus* seeds from the arid (drier) origin had lower germination than seeds of the semi-arid (wetter) origin when both origins germinated within the arid site. At the other sites, *Bromus* showed high and conservative germination, and *Biscutella* increased germination fractions progressively from the arid to the mesic Mediterranean site, supporting our predictions derived from models of bet-hedging germination (Cohen, 1966; Cohen, 1967). In this and later similar models, it was found that seed dormancy should be a ubiquitous mechanism to cope with uncertainty and therefore, the fraction of dormant seeds should decrease with increasing predictability of the environment. It is therefore surprising that the climatic uncertainty constraint was relatively unimportant in determining seed germination behavior of our focal species.

In addition to the weak origin effect, there was no obvious germination response related to on-site variable rainfall. The theory predicts that optimal germination should be positively correlated to a cue, which indicates the favorability of the upcoming season (Cohen, 1967; Venable & Lawlor, 1980). In our system, this cue is most likely the first rainfall of a season. We tested this prediction by sowing seeds of the same origin into sites with different climatic conditions, assuming that the first rainfall of the season would be positively correlated with the total rainfall of the upcoming season at each site. Previous empirical studies were indeed able to document such a positive correlation and, subsequently, predictive germination (Pake & Venable, 1966; Clauss & Venable, 2000). Yet, in our study, no such correlation could be found. Namely the first rainfall was largest in the semi-arid (and not the mesic Mediterranean) site and additionally, the number of effective rainfalls differed between sites. The apparent contradiction of our findings with theory should therefore be interpreted with caution.

A correct test for the on-site effects on seed germination should aim at both manipulating the amount of the first as well as of total rainfall in a given year.

In summary, our results on climatically determined germination represent a rather weak support for the optimal germination theory. Therefore, conclusions about the potential responses of the study species to climate change must remain tentative. The main lesson is the difference among species, indicating that climate change will likely lead to species-specific extinctions rather than to a range shifts in all component species. With this finding, our study adds to the wealth of studies indicating the severe limitations of the climate envelope approach (e.g. Pearson & Dawson, 2003; Gray *et al.*, 2004; Jump & Peñuelas, 2005). Regarding the single species, *Biscutella* appears to exhibit a greater level of local adaptation than the other two species, as supported also in our parallel study (Petrů & Tielbörger, unpublished). However, in order to predict whether the species may persist under climate change, detailed investigation about the within-population genetic variation related to the adaptive traits is needed.

Conclusions

Our study points to some major methodological constraints of field experiments studying local adaptation within the context of climate change research. Namely, future experiments should explicitly separate climatic effects from other selective forces along climate gradients. In particular, the neighbor conditions at the introduction site may play a critical role in determining the behavior of plant in reciprocal transplant experiments. This and the fact that we found large differences in species of the same life form, calls for a community-wide approach to climate change studies as opposed to the reductionist approach of bioclimatic modeling.

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Table 1. Environmental conditions at four stations on a S-N oriented rainfall gradient in Israel.

Station	Latitude	Longitude	Location	Elevation (a.s.l.)	Avg Temp (°C)	Geology & Soil	Avg rainfall (mm)	Rainfall 2003/4 (mm)	Vegetation description
Arid	30° 52´N	34° 46´E	north of Sde Boqer (central Negev)	470	19.1	limestone & desert Lithosol	90	75	annuals and small shrubs: *Zygophyllum dumosum, Artemisia sieberi*
Semi-**A**rid	31° 23´N	34° 54´E	northeast of Lahav (northern Negev)	590	18.4	limestone & Brown Rendzina	300	240	dwarf shrubland: *Sarcopoterium spinosum, Thymelaea hirsuta, Coridothymus capitatus* and annuals
Mediterranean	31° 42´N	35° 03´E	south of Matta (Jerusalem Mts)	620	17.7	limestone, hard chalk & Terra Rossa	540	446	dwarf shrublands: *S. spinosum* and annuals
Mesic **M**editerranean	33° 00´N	35° 14´E	east of Ein Ya'akov (Northern Galilee)	500	18.1	limestone, hard chalk & Terra Rossa	780	828	Mediterranean maquis to garrigue: *Calicotome villosa* and annuals

Table 2. Results of ANOVAs for germination of *Biscutella*, *Bromus* and *Hymenocarpos* at arid, semi-arid, Mediterranean (= Mediter.) and Mesic Mediterranean (= Mes. Mediter.) stations (separate analyses for each species and station) as a function of seed ORIGIN (= dry, home and wet, respectively; see methods for differences in number and types of seed origin for each site and species) and the substrate (= local vs. standard). Significant differences are in bold.

at **Station**	**Species**	**Factors**						
		ORIGIN		**Substrate**		**ORIGIN** x **Substrate**		**Error**
		F_{df}	**p**	**F_{df}**	**p**	**F_{df}**	**p**	**MS_{df}**
at **A**rid	*Biscutella*	13.57_1	**0.001**	5.18_1	**0.029**	0.79_1	0.378	0.02_{36}
	Bromus	30.40_1	**< 0.001**	20.31_1	**< 0.001**	0.22_1	0.640	0.03_{36}
at **S**emi-**A**rid	*Biscutella*	4.45_2	**0.020**	3.35_1	0.070	3.04_2	0.056	0.01_{54}
	Bromus	3.56_2	**0.040**	95.04_1	**< 0.001**	0.99_2	0.380	0.03_{54}
	Hymenocarpos	6.20_1	**0.018**	28.36_1	**< 0.001**	0.02_1	0.890	0.01_{36}
at **M**editer.	*Biscutella*	0.79_2	0.460	2.94_1	0.090	0.69_2	0.930	0.07_{54}
	Bromus	2.85_1	0.100	40.19_1	**< 0.001**	0.86_1	0.360	0.06_{36}
	Hymenocarpos	4.40_2	**0.020**	3.46_1	0.070	0.36_2	0.700	0.01_{54}
at **M**es. **M**editer.	*Biscutella*	0.67_1	0.420	7.06_1	**0.010**	0.05_1	0.840	0.05_{36}
	Hymenocarpos	2.78_1	0.090	0.15_1	0.700	1.02_1	0.320	0.06_{36}

Table 3. Results of ANOVAs for germination of *Biscutella*, *Bromus* and *Hymenocarpos* originating from arid, semi-arid, Mediterranean (= Mediter) and Mesic Mediterranean (= Mes. Mediter.) stations (separate analyses for each species and seed origin) comparing the SITES of germination (= at dry, at home and at wet, respectively; see methods for differences in number and types of seed origin for each site and species) and the substrate (= local vs. standard). Significant differences are in bold.

from **Station**	**Species**	**Factors**						
		SITE of germination		**Substrate**		**SITE of germination** x **Substrate**		**Error**
		F_{df}	p	F_{df}	p	F_{df}	p	MS_{df}
from **A**rid	*Biscutella*	0.71_1	0.410	3.49_1	0.070	0.20_1	0.650	0.02_{36}
	Bromus	0.43_1	0.530	94.39_1	**< 0.001**	9.30_1	**0.004**	0.02_{36}
from **S**emi-**A**rid	*Biscutella*	9.82_2	**< 0.001**	2.14_1	0.150	2.37_2	0.100	0.03_{54}
	Bromus	4.16_2	**0.020**	44.60_1	**< 0.001**	2.37_2	0.070	0.06_{54}
	Hymenocarpos	11.02_2	**< 0.001**	28.71_1	**< 0.001**	6.73_2	**0.002**	0.01_{54}
from **M**editer.	*Biscutella*	3.56_2	**0.040**	10.37_1	**0.002**	0.76_2	0.470	0.06_{54}
	Bromus	0.44_2	0.650	32.03_1	**< 0.001**	0.86_2	0.430	0.06_{54}
	Hymenocarpos	3.47_2	**0.040**	9.63_1	**0.003**	1.36_2	0.270	0.03_{54}
from **M**es. **M**editer.	*Biscutella*	1.28_1	0.270	4.61_1	**0.040**	1.04_1	0.320	0.08_{36}
	Hymenocarpos	0.29_1	0.590	0.29_1	0.590	2.04_1	0.160	0.05_{36}

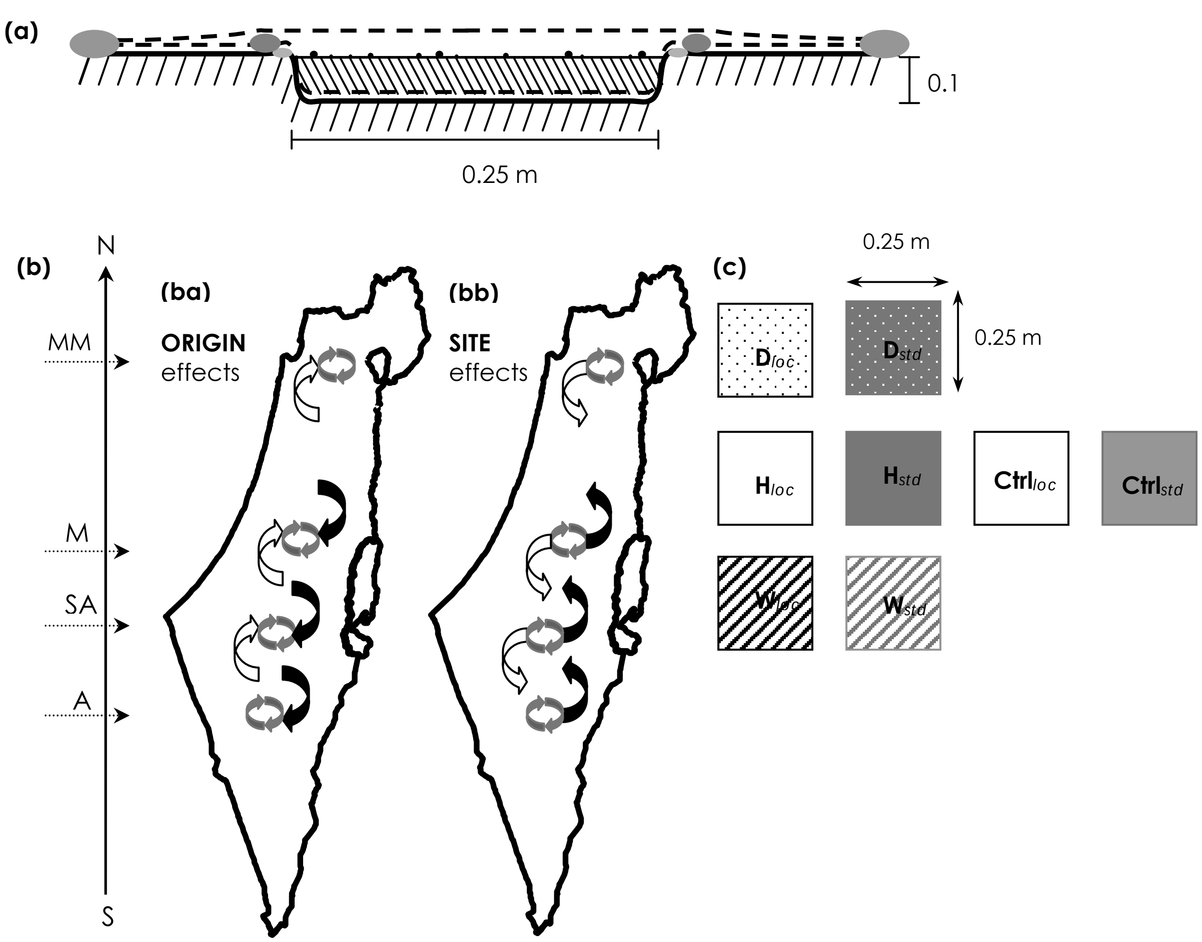

Figure. 1. (a) Cross-section of a standard substrate plot: a quadrate of 0.25 x 0.25 m, 0.1 m deep with the local soil removed. A layer of organza (dashed line) separated the local from the standard substrate (center) that was filled into the depression and leveled evenly. Seeds of 3 species (dots of different sizes) were spread on the standard soil surface and the whole plot covered with a layer of organza fixed with stones on the edges. **(b)** Design of reciprocal sowing experiment among four stations on a S-N aridity gradient in Israel with arid (= A), semi-arid (= SA), Mediterranean (= M) and mesic Mediterranean (= MM) stations. **(ba)** Set-up testing the effects of ORIGIN: seeds of different origins: dry (white arrows), home (gray) and wet (black) translocated to the respective sites. **(bb)** Set-up testing the effects of SITE: seeds from each station were transplanted to: drier (white arrows), home (gray) and wet sites (black). **(c)** Layout of a representative group of plots in the reciprocal sowing experiment. Plots of 0.25 x 0.25 m with local soil (= loc, white) and standard substrate (= std, gray) with diaspores originating from the drier site (= D, dotted pattern), home site (= H, blank) and wetter site (= W, striped), and control plots (= Ctrl) for background germination. Positions of plots within each group and site were randomized. At the arid site were 'home' and 'wet' plots only, at the mesic Mediterranean site only 'home' and 'dry' plots only. *Bromus* was not found in the mesic Mediterranean and *Hymenocarpos* not in the arid station.

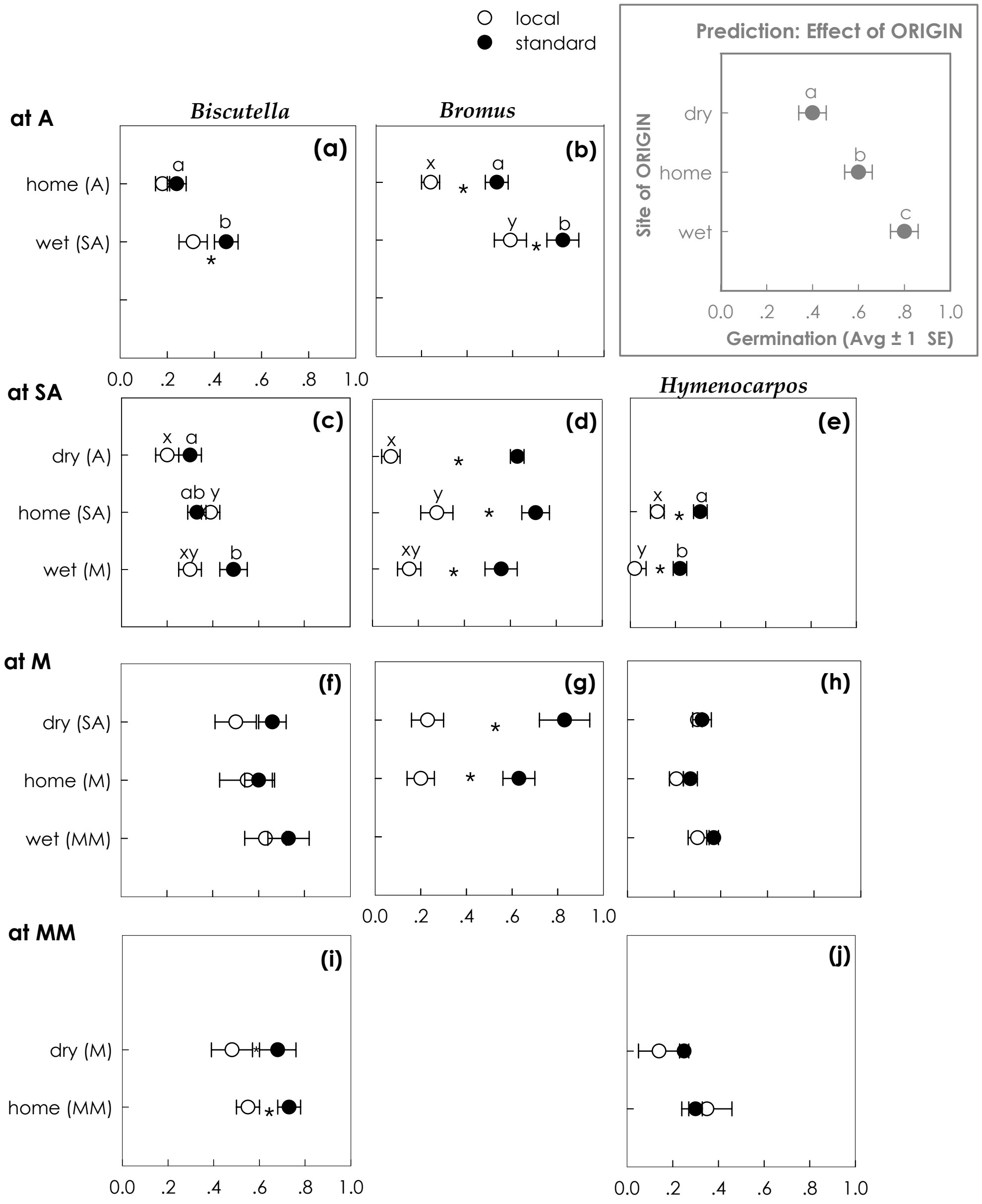

Figure 2. Effects of seed ORIGIN on germination. Germination proportions (Avg ± 1 SE on X-axis) of species (in columns): *Biscutella* – **(a)**, **(c)**, **(f)**, **(i)**, *Bromus* – **(b)**, **(d)**, **(g)** and *Hymenocarpos* – **(e)**, **(h)**, **(j)**. Seeds of different origins (on Y-axis: dry, home and wet – specific for each species, seed origin and station) germinated at the stations (in rows): at A = arid – **(a)**, **(b)**, at SA = semi-arid – **(c)**, **(d)**, **(e)**, at M = Mediterranean – **(f)**, **(g)**, **(h)** and at MM = Mesic Mediterranean – **(i)**, **(j)** on local (empty circles) and standard (full black circles) substrates. The predicted pattern on standard substrate is shown in the upper right panel. Note the reverse axes for species origin (Y-axis) and germination proportions (X-axis). Different letters above error bars indicate significant differences among origins with substrates; asterisks indicate significant differences between the two substrates (Tukey's multiple range tests, $p<0.05$).

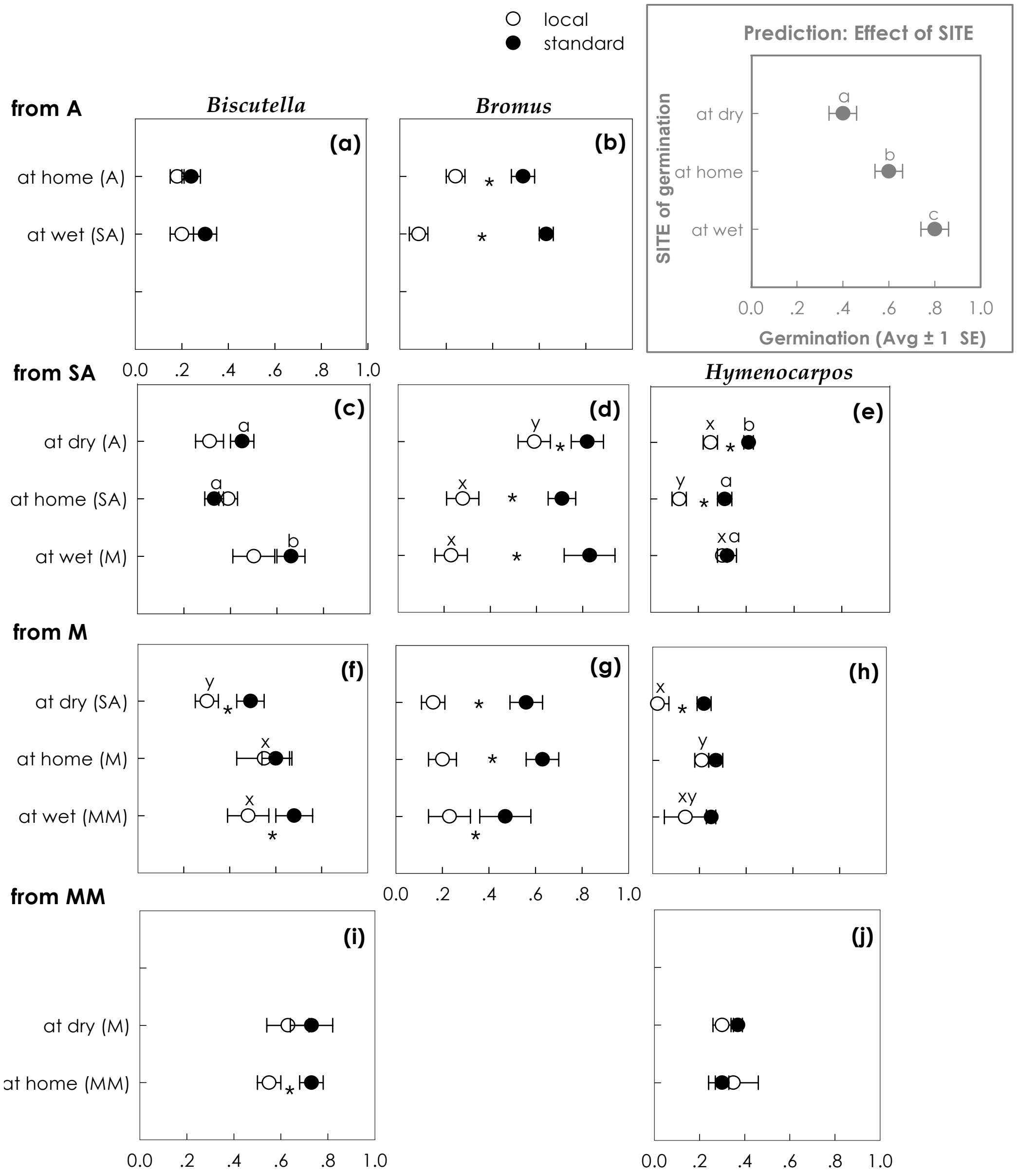

Figure 3. Effects of SITE on germination. Germination proportions (Avg ± 1 SE on X-axis) of species (in columns): *Biscutella* – **(a)**, **(c)**, **(f)**, **(i)**, *Bromus* – **(b)**, **(d)**, **(g)** and *Hymenocarpos* – **(e)**, **(h)**, **(j)**. Seeds of a single origin (in rows): from A = arid – **(a)**, **(b)**, from SA = semi-arid – **(c)**, **(d)**, **(e)**, from M = Mediterranean – **(f)**, **(g)**, **(h)** and from MM = Mesic Mediterranean – **(i)**, **(j)** germinated at different sites (on Y-axis): at dry, at home and at wet – specific for each species and seed origin on local (empty circles) and standard (full black circles) substrates. The predicted pattern on standard substrate is shown in the upper right panel. Note the reverse axes for site of germination (Y-axis) and germination proportions (X-axis). Different letters above error bars indicate significant differences among origins within substrates; asterisks indicate significant differences between the two substrates (Tukey's multiple range tests, $p<0.05$).

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Chapter 4

Life history variation in an annual plant under two opposing environmental constraints along an aridity gradient

Life history variation in an annual plant under two opposing environmental constraints along an aridity gradient

Abstract

Environmental gradients represent an ideal framework for studying adaptive variation in the life history of plant species. However, on very steep gradients, largely contrasting conditions at the two gradient ends often limit the distribution of the same species across the whole range of environmental conditions. Here, we study phenotypic variation in a winter annual crucifer *Biscutella didyma* persisting along a steep gradient of increasing rainfall in Israel. In particular, we explored whether the life history at the arid end of the gradient indicates adaptations to drought and unpredictable conditions, while adaptations to the highly competitive environment prevail at the mesic Mediterranean end. We examined several morphological and reproductive traits in four natural populations and in populations cultivated in standard common environment. Plants from arid environments were faster in phenological development, more branched in architecture and tended to maximize reproduction, while the Mediterranean plants invested mainly in vertical vegetative growth. Differences between cultivation and field in diaspore production were very large for arid populations as opposed to Mediterranean ones, indicating a larger potential to increase reproduction under favorable conditions. Our overall findings indicate two strongly opposing selective forces at the two extremes of the aridity gradient, which result in contrasting strategies within the studied annual plant species.

Introduction

Widely distributed plant species are often exposed to a broad range of environmental conditions (Joshi et al. 2001). In order to persist in different environments, plants often vary considerably in their life history and the expression of morphological traits. This phenotypic variation may be due to plasticity, or genetically determined ecotypic differentiation (Clausen et al. 1940, Schilchting 1986, Dudley and Schmitt 1995).

Environmental gradients provide a useful framework for evaluating the relative importance of differential selection for adaptive traits maximizing species fitness (Lande 1977, Endler 1986). For example, extensive work has been done along environmental gradients looking at shifts in phenology, growth and sexual reproduction (Hauser and Weidema 2000, Olsson and Agren 2002, Stinson 2004), reproductive allocation (Callahan and Waller 2000, Sugiyama 2003), and phenotypic plasticity and genetic variation (Fritsche and Kaltz 2000, Galloway and Fenster 2000, Joshi et al. 2001, Volis et al. 2001, 2002 a-d, Santamaria et al. 2003, Casler et al. 2004). These studies covered a wide range of spatial scales (from local and regional to a global scale) and a range of environmental conditions.

Optimally, the environmental gradients should include a wide range of conditions in order to allow the detection of clear patterns of phenotypic or genetic differentiation. Therefore, many previous studies have been conducted across relatively large spatial scales (e.g. Hauser and Weidema 2000, Galloway and Fenster 2000, Santamaria et al. 2003, Casler et al. 2004). However, if the gradient is too steep, studies of trait variation may be hampered by the fact that only few species may persist along the whole spectrum of environmental conditions. Another shortcoming of large-scale gradient studies is that habitat conditions are very likely to differ in many different aspects, and confounding of the environmental factor of interest with other factors becomes more likely (Santamaria et al. 2003, Sugiyama 2003, Casler et al. 2004).

Several studies, so far, have focused on climatic gradients (e.g. Volis 2001, 2002a-d, Del Pozo et al. 2002a,b, Olsson and Agren 2002, Santamaria et al. 2003, Sugiyama 2003). Yet, only scarce data exist on climatic gradients, which occur over a relatively short geographic distance. This is unfortunate, since first, climate is one of the most important factors determining the geographical distribution and abundance of plant species (Woodward 1987), and secondly, such gradients represent an ideal case for studying adaptive shifts in traits within a given species. In particular, rainfall gradients have only been investigated by a limited number of researchers (Aronson et al. 1990, 1992 and 1993, Volis et al. 2001, Volis et al. 2002a-d, Del Pozo et al. 2002a, 2002b). Climate gradient studies further increase in significance as the degree of local adaptation of plants to climatic conditions may determine the vulnerability of species to climate change (Rehfeldt 2002).

In summary, the above studies emphasize the need for studying adaptive variation in plant life history traits to a variety of climatic conditions. Here, we evaluate the phenotypic performance of an abundant Eastern Mediterranean annual plant species along a steep climatic gradient, which varies both in amount as well as predictability of water availability. The abiotic conditions represent two opposing environmental constraints at the two extremes of the gradient, which should generate distinct plant responses. Specifically, at the arid end of the precipitation gradient, rainfall events are highly fluctuating and unpredictable from one year to the next. Adaptation to such conditions results often in great plasticity in plant size and phenological development (Went 1949, Lewontin 1957, Levins 1963, Jain 1978, Evenari et al. 1982, Aronson et al. 1990). For example, it has been found that arid ecotypes reproduce earlier and produce more diaspores per plant biomass than conspecific plants growing under more humid conditions (Aronson et al. 1990, Volis et al. 2002a), since the shorter growing season and the unpredictability of environmental conditions requires fast development and rapid response to episodic rainfall. At the Mediterranean end of the gradient, climatic constraints are less prominent but neighbor densities are much higher (Holzapfel et al., unpubl.). Therefore, the main environmental constraint is most likely the density of neighboring plants, and shifts in plant life history traits towards the wet end of the gradient should reflect competitive ability. This expectation is in concordance with the theory of Grime (1973a, 1973b, 1977), who predicted dominance of superior competitors at the favorable end of productivity gradients. Specifically, plants growing under more productive conditions should invest more into survival under intense neighbor competition rather than to fast reproduction and allocate more biomass into vegetative growth (Aronson et al. 1993, Nachreiner 2005). According to Newman (1973) and Tilman (1988), it is mainly above-ground competition intensity which increases along productivity gradients. Therefore, vegetative investment at the wet end should be predominantly into shoot mass and height, rather than into belowground biomass.

Morphological, phenological and reproductive traits have been previously compared between two contrasting extremes – arid and humid - on Eastern Mediterranean climatic gradients for a number of species (Aronson et al. 1990, 1992 and 1993). However, little is known whether the observed differences between the studied populations change gradually along the gradient. Such gradual and directional change would be a strong indication for adaptive variation. In the notable exception, where rigorous tests were done on adaptive plant trait variation across a steep climatic gradient, the rainfall differences were confounded with between-site differences in presence of frost, topographic position, and bedrock (Volis et al. 2001, Volis et al. 2002a-d).

Here, we explore variation in phenotypic traits of an annual plant species, the crucifer *Biscutella didyma*. Specifically, we search for trends in trait variation reflecting the changes of rainfall along a steep climatic gradient in Israel. These trends are studied both under natural conditions as well as in a standard environment, for evaluating natural (i.e. in situ) *vs*. genetically based differences between the climatic populations (see e.g. Clausen et al. 1940, Hauser and Weidema 2000). We focus on growth morphology and architectural traits, phenological shifts and changes in allocation patterns, and their relation to the opposing environmental conditions along the gradient.

In particular, we test the following hypotheses:

(i) Plant morphology shifts from small, dense plants in the arid region to tall, loosely branched plants in the Mediterranean region.

(ii) Reproductive allocation decreases from the arid to the Mediterranean populations.

(iii) Onset of flowering and completion of the life cycle is faster for arid than for Mediterranean populations.

(iv) Differences between optimal conditions in cultivation and conditions in the field result in large positive response of desert populations to the cultivation and small response of Mediterranean populations.

Methods

Study species

Biscutella didyma (further referred to as *Biscutella*) is a winter annual crucifer. Under natural conditions, the plant grows 15-50 cm high and it has 5-10 radical leaves and several fruiting branches. Fruits are double silicles fragmenting easily, and dispersing when ripe (further referred to as diaspores). The species has a Mediterranean and Irano-Turanian distribution and occurs in a wide range of habitats from dwarf shrublands, fallow fields, steppes and deserts (Zohary 1966).

Study system

We studied *Biscutella* populations from four stations on similar bedrock and exposition, located along a S-N oriented gradient of increasing rainfall in Israel (further referred to as an arid, semi-arid, Mediterranean and mesic Mediterranean, Table 1). In these stations, the growing season responds to the distribution of rainfall, and germination of annuals and re-growth of most perennials occurs in October-November, soon after first major rains (~10-20 mm). The length of the rainy season increases from the desert (December- March) to the Mediterranean (October- May).

Plant cultivation

In June 2002, we collected ripe *Biscutella* diaspores from approx. 300 randomly selected individuals in each of the natural populations. Previous trials have indicated that oversummering in the field is the optimal method for naturally breaking seed dormancy. Therefore, we stored the fresh diaspores from May to September 2002 in the respective field stations, in bags made from organza (light transparent synthetic fabric), which we attached to the ground surface. In September 2002, we collected the bags in the field and transferred them to a laboratory for counting diaspores into equal portions. At the end of November 2002, we planted the diaspores in pots (7 x 7 cm x 15 cm deep) filled the with a mixture of garden soil and sand (2:1) with added one handful of Osmocot Scott® fertilizer (15% N, 9% P_2O_5, 9% K_2O) per 15 l soil mixture. We planted approximately 30 diaspores in each pot to assure substantial germination. A week after the bulk germination, we thinned seedlings randomly to one seedling per pot. We placed the pots in a screen-house at the Botanical Garden at Tel Aviv University (further referred to as cultivation) under identical light and water regime, where we kept them until the end of the experiment, randomizing the pot position three times during the course of the experiment. Except for rainy days, plants got irrigated daily with an automatic irrigation system, so that growth was unlikely to be water-limited. Shortly before the onset of flowering, we prevented cross-pollination by fully isolating the populations in organza enclosures constructed around the flowering plants grouped by station (all plants from one station under one enclosure). We hand-pollinated flowers within each station to standardize pollination success and to maximize diaspore production, and kept plants in cultivation irrigated until all plants from all the stations finished completely with flowering and produced ripe (brown) diaspores.

Morphological traits

We quantified growth morphology and plant architecture of 24 individuals per *Biscutella* population both in the field and under cultivation (also referred to as field and cultivated groups). In the field, we randomly marked 24 individuals from the wild populations; in cultivation we used all potted plants. Due to plant loss and damage (especially in cultivation), and due to early diaspore dispersal, sample sizes were not equal for all parameters (see Table 2). We measured plant height during diaspore maturation when the first diaspores on a given plant began drying out and we evaluated plant growth by using plant height as a nondestructive measure for biomass. Parallel trials indicated that height is highly correlated with total plant biomass (Schiffers 2003). We used these nondestructive measurements since we harvested the ripe diaspores and therefore, could not harvest the plants. We further counted number of fruiting stems (the main stem bearing fruits and all branches with fruits) during diaspore maturation. We defined plant 'growth architecture' as number of fruiting stems per plant height, with many branches per height reflecting a more compact architecture and few branches defining a loose shape.

Fitness-related traits

To evaluate reproductive output in cultivated and field groups, we counted number of diaspores per plant prior to dispersal, i.e. when fruit maturation began and all diaspores were still attached to the fruiting stems. We harvested all ripe brown diaspores from plants both in cultivation and in the field and measured the diaspore diameter of a subset of plants in both growth conditions. A previous study has indicated that diaspore diameter and weight are good surrogates for diaspore quality (Schiffers 2003). We estimated reproductive allocation as the number of diaspores produced per unit plant height. We further documented phenology (dates of first flowering and end of fruiting) in cultivation and in the field.

Data analyses

We analyzed the differences between populations for all parameters using one-way ANOVAs separately for the populations in cultivation and in the field. Due to plant loss and damage in cultivation and due to time constraints, our sample sizes for number of diaspores and diaspores per plant height were unbalanced. We run unbalanced design ANOVAs with small sample sizes including all available data (see Underwood 1997). Tukey's post-hoc tests compared pairwise differences in population means within the cultivated and field groups. All data conformed to the assumptions of ANOVA without transformation. We further compared the parameters of plant performance in single populations under cultivation vs. in the field pairwise using separate t-tests with Bonferroni adjusted significance level to 0.0125 (Sokal and Rohlf, 2000).

Results

Growth and plant architecture

Both field and cultivated populations showed a clear and significant trend of gradually increasing plant sizes along the gradient (Fig. 1a, Table 2). The cultivated plants in all populations were 2 to 3 times larger than the plants in the field (Fig. 1a). The relative increase in plant size from arid to mesic Mediterranean was greater in the field (3-fold) than in cultivation (Fig. 1a). Cultivated plants produced significantly more fruiting stems in cultivation compared to the field in all populations (Fig. 1b, Table 3), with an increase towards the wet end of the gradient, while field populations did not differ in their number of fruiting stems (Fig. 1b, Table 2). The growth architecture, i.e. the number of fruiting stems per plant height, showed divergent patterns in the different growth conditions (Fig. 1c, Table 2, 3). Field plants had significantly more fruiting stems per plant height (i.e. a more tight architecture) in the arid region, declining towards mesic Mediterranean (Fig. 1c, Table 2). This morphological difference disappeared under common growth conditions in cultivation (Fig. 1c, Table 2). Note that the field populations showed a similar number of fruiting stems (Fig. 1b, Table 2) and the differences in fruiting stems per plant height among the field populations were mainly correlated with plant height (Fig. 1a). In the cultivated plants, the fruiting stem number and plant height increased proportionally and the fruiting stem per plant height was constant across the different populations (Fig. 1c).

Fitness-related traits

Diaspore number was significantly higher in the cultivated plants compared to the field in the arid and semi-arid regions (Fig. 1d, Table 2,3). Diaspore number of cultivated plants declined significantly from the arid towards the mesic Mediterranean (Fig. 1d, Table 2), but increased the opposite direction in the field. Despite the overall significant population difference, there were no pairwise significant differences among the field populations (Fig. 1d). Cultivated plants produced larger diaspores than plants in the field, except the semi-arid population (Fig. 1e, Table 2,3) and diaspore diameter followed a declining trend from the arid towards the mesic Mediterranean. In field populations, diaspore diameter varied non-directionally (Fig. 1e, Table 2). The diaspore number per plant height showed a sharp decline from the arid towards the mesic Mediterranean in cultivation, but was indifferent in the field (Fig. 1f, Table 2,3).

Phenology

There were marked phenological shifts in timing of flowering along the climatic gradient. Arid populations both in the field and in cultivation began flowering earlier in the season both in the field as well as under common growth conditions. Onset of flowering gradually progressed to semi-arid, Mediterranean and mesic Mediterranean populations (Table 4). In the cultivated plants, first flowering occurred with intervals of 8 – 10 days between each of two neighboring populations along the gradient (Table 4). The favorable conditions in the cultivation extended the length of the reproductive period (from the first flowering until no more flowering occurred and all diaspores were set) compared to the field, and the reproductive period prolonged from the arid towards the mesic Mediterranean region, especially in the cultivation (Table 4). In the field this period lasted 40, 62, 56 and 54 days (arid – mesic Mediterranean respectively), while in the cultivation it was 63, 92, 98 and 99 days, Table 4).

Discussion

In this study, we found distinct directional trends in growth morphology, architecture, and fitness-related traits across four populations of the winter annual crucifer *Biscutella didyma* growing along a steep rainfall gradient in Israel. Based on our comparison of trait variation between field and common environments, we suggest that these trends may be genetically based (Turesson 1922, Clements et al. 1950, Langlet 1971, Rehfeldt et al. 1999, 2002 and Hauser and Weidema 2000). Our findings confirm recent findings on directional phenotypic variation of multiple plant species in the same system (Nachreiner 2005) and complement Aronson et al.'s findings (1990, 1992 and 1993) of contrasting trait values on the two extreme edges of a different rainfall gradient in the region. We thus empirically support previous theoretical work suggesting that life history traits should vary in a clinal fashion along continuous gradients with gradually changing environmental factors (Cooper 1963, Weber and Schmid 1998, Rehfeldt et al. 1999, Li et al. 1998).

Selective forces for traits maximizing species fitness have been documented to change along gradients (e.g. Hickman 1975, Aronson et al. 1992, 1993). Our findings indicate that in our system, there are two major selective forces which are negatively correlated and which shift simultaneously and steadily along the climatic gradient. The first force, which apparently dominates the wet end of the gradient, is competition. The second force, which prevails at the arid end, is scarcity and unpredictability of rain. In the following, we discuss how our results relate to our hypotheses of these two opposing forces.

Competition

There has been an intensive debate about whether competition intensity increases along productivity gradients (Grime 1973a) or whether it stays constant (Newman 1973, Tilman 1988). Though this so-called Grime-Tilman conflict is far from being resolved, both theories agree that above-ground competition is more intense at the favorable end of a resource gradient. Therefore, we hypothesized that shifts in traits towards the Mediterranean should reflect adaptations to above-ground competition. Our findings corroborate this hypothesis in two aspects. First, plant architecture changed from small, compact plants in the arid regions to tall, loosely branched plants in the Mediterranean region, where biomass, height and density of neighbors are relatively high (Holzapfel et al. unpubl.). Secondly, there were differences in reproductive allocation, which can be related to changes in competition intensity. It has been suggested that Mediterranean plants should be selected to cope with competition with neighbor plants (Shmida et al. 1986, Shmida and Burgess 1988) and consequently, are ‘programmed’ for relatively greater allocation to vegetative growth at the expense of lower reproductive output (Weiner 1988). Results of our study fully support this prediction, as our cultivated plants exhibited a dramatic decrease in reproductive allocation from the arid to the Mediterranean.

Climate

Climatic differences between the arid and the humid end of the gradient do not only include the average amount of annual rainfall but more important, the rainy season is much shorter in the arid region and availability of water is highly variable and unpredictable between years.

Adaptive shifts in timing of flowering are known to occur in annuals along longitudinal and altitudinal climatic gradients and have been related to the length of the growing season (Turesson 1922, Ray and Alexander 1966, Lacey 1988, Li et al. 1998, Callahan and Waller 2000, Olsson and Agren 2002). In our system, rains start usually later and finish earlier in the arid region compared to the Mediterranean (Shmida and Burgess 1988), resulting in a much shorter growing season. Therefore, we hypothesized that desert annuals would flower earlier and accomplish their life cycle faster, according to the "living fast and dying young" - principle (Aronson et al. 1990, 1993). The flowering schedule observed in our study confirmed this hypothesis: plants began flowering earlier in the arid region and progressed towards the Mediterranean. Interestingly, this pattern was stable even under varying onset of rain. Flowering phenology was similar across populations both in cultivation as well as in the field, indicating that this behavior may be genetically determined (Turesson 1922, Clements et al. 1950, Langlet 1971, Hauser and Weidema 2000).

The largest constraint on long-term persistence of plants in the desert is the high and unpredictable variation in resource availability (water). A major mechanism which allows annual plants to persist even after years with no reproduction is the maintenance of a persistent seed bank (Cohen 1966). In addition, plants may develop strategies, which enable them to maximize the fitness gain from an occasional favorable season. For example, theory predicts that in low-density areas, such as our arid site, plants should allocate much more to reproduction in order to be able to maximize reproductive output as soon as the environment allows it (Levins 1963, Jain 1979). Therefore, the pattern we have found here, with high reproductive allocation towards the arid populations, may be explained similarly by the two selective forces competition and climate.

Another strategy which helps to cope with the unpredictability of favorable years is to maximize reproduction during good years and to 'store' these fitness gains in the between-year seed bank during unfavorable years. Therefore, we would expect that desert populations would exhibit a larger potential to rapidly respond to varying rainfall conditions than Mediterranean ones. In order to test this prediction, we need to compare our findings from the field and the cultivation: Here, large differences between cultivation and field would suggest a large environmental component in trait expression, since in the field, plant performance is not only determined by genetic effects but by actual climatic, edaphic and biotic conditions. One of our most intriguing findings is that under cultivation (i.e. optimal conditions), desert plants produced almost three times as many seeds as plants from the mesic Mediterranean, while in the field, the opposite pattern was found. This suggests that desert plants have a much higher potential to respond plastically to differing rainfall conditions. Support for this observation also stems from a recent study which showed higher *Biscutella* seed production in the arid than in the semi-arid populations during a relatively rainy season (Nachreiner 2005). Clearly, this ability to 'bet' on favorable years represents an advantage in habitats, where such years occur only very rarely.

In addition to maximizing seed number under optimal conditions, desert plants also showed an increase in diaspore size, when irrigated. It is clear that this pattern may be similarly advantageous as increasing the number of offspring. However, this finding contradicts one of the fundamental principles of life history theory: the seed size - seed number trade-off. In cultivation, diaspore number and size were positively correlated and decreased markedly from the arid towards the Mediterranean. This trend has found support in parallel studies on additional annual species in our system (Nachreiner 2005), while another study in the region has indicated that the annual grass *Stipa capensis* produced higher number of smaller diaspores in arid populations compared to the Mediterranean ones (Aronson 1990). In the same study, diaspore size of another annual grass, *Brachypodium distachyon*, were significantly larger than the Mediterranean, but their number did not differ between the two populations. In our study, diaspores of desert annuals were - unexpectedly- either much larger than (cultivation) or similar in size to those of plants growing at the humid end of the gradient.

In summary, our findings suggest that the observed clinal trends in life history traits along the climatic gradients indicate the response of plants to two opposing selective forces at the two climatic extremes. Above-ground competition intensity at the wet end apparently favors taller plants, which invest more into vegetative growth. On the other hand, low resource availability and highly pulsed resources at the arid end select for rapid development, high reproductive allocation and high plasticity in seed production with respect to water availability. An intriguing consequence of our study is that although basic plant strategy theory has been developed for explaining between-species differences in habitat-trait relationships (e.g., Grime 1973b, 1977, Harper 1977), our findings suggest that largely opposing strategies may be found even within a single species.

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Table 1. Environmental conditions at four stations with *Biscutella* populations on a S-N oriented rainfall gradient in Israel.

Station	**Latitude**	**Longitude**	**Location**	**Altitude**	**Avg Temp (°C)**	**Geology & Soil**	**Avg rainfall (mm)**	**Rainfall 2001/2 (mm)**	**Rainfall 2002/3 (mm)**	**Vegetation description**
Arid	30° 52´N	34° 46´E	north of Sde Boqer (central Negev)	470	19.1	limestone & desert Lithosol	90	100	86	annuals and small shrubs: *Zygophyllum dumosum, Artemisia sieberi*
Semi-arid	31° 23´N	34° 54´E	northeast of Lahav (northern Negev)	590	18.4	limestone & Brown Rendzina	300	305	342	dwarf shrubland: *Sarcopoterium spinosum, Thymelea hirsuta, Coridothymus capitatus* and annuals
Mediterranean	31° 42´N	35° 03´E	south of Matta (Jerusalem Mts)	620	17.7	limestone, hard chalk & Terra Rossa	540	736	795	dwarf shrublands: *S. spinosum* and annuals
Mesic Mediterranean	33° 00´N	35° 14´E	east of Ein Ya'akov (Northern Galilee)	500	18.1	limestone, hard chalk & Terra Rossa	780	905	1031	Mediterranean maquis to garrigue: *Calicotome villosa* and annuals

Table 2. Results of ANOVAs for parameters of plant performance in cultivation and in the field (separate ANOVAs) comparing the four populations (A = arid, SA = semi-arid, M = Mediterranean and MM = mesic Mediterranean). Note the different sample sizes within groups for the various parameters and the unbalanced design for ANOVAs in few groups.

	Factors	**Cultivation**		**Field**	
Parameters	**N**	df = 3)		(df = 3)	
		F	**p**	**F**	**p**
Height	24	65.2	**< 0.001**	22.8	**< 0.001**
Fruiting stems	20	18.8	**< 0.001**	0.3	0.787
Fruiting stems/Height	16	1.5	0.229	32.6	**< 0.001**
Number of diaspores	f: 8, c: 7 A; 8 SA, M; 5 MM	6.2	**0.003**	3.4	**0.032**
Diaspore diameter	20	37.2	**< 0.001**	11.3	**< 0.001**
Diaspores/Height	f: 8, c: 7 A, M, MM; 6 SA	17.2	**< 0.001**	0.7	0.556

Table 3. Results of t-tests for parameters of plant performance comparing single populations in cultivation vs. in the field. Pairwise difference tested with Bonferroni adjusted significance level (0.0125). Note the different sample sizes within populations for the various parameters.

Population	Arid			Semi-Arid			Mediterranean			Mesic Mediterranean		
Parameters	**df**	**t**	**p**	**df**	**t**	**p**	**df**	**t**	**p**	**df**	**t**	**p**
Height	46	35.7	**< 0.001**	46	20.9	**< 0.001**	46	11.6	**< 0.001**	46	18.0	**< 0.001**
Fruiting stems	38	17.0	**< 0.001**	38	12.5	**< 0.001**	38	12.0	**< 0.001**	38	12.6	**< 0.001**
Fruiting stems/Height	30	5.4	**< 0.001**	30	3.8	**< 0.001**	30	1.0	0.320	30	6.5	**< 0.001**
Number of diaspores	13	6.3	**< 0.001**	14	10.3	**< 0.001**	14	2.7	0.019	11	1.7	0.117
Diaspore diameter	38	7.7	**< 0.001**	38	0.7	0.447	38	6.6	**< 0.001**	38	5.5	**< 0.001**
Diaspores/Height	13	5.9	**< 0.001**	12	7.8	**< 0.001**	13	1.8	0.09	13	2.0	0.063

Table 4. Dates of the first flowering and dates of the end of fruiting (no more flowering and all diaspores set) of *Biscutella didyma* from the four stations Arid (Sde Boqer), Semi-Arid (Lahav), Mediterranean (Matta) and Mesic Mediterranean (Ein Ya'akov) in the cultivated and field groups (data from field stations according to Holzapfel & Parag, pers. comm.). Plants in cultivation were irrigated from 20 November 2002. First effective rain in the field (~10 mm), after which germination occurred.

Plant Population	First flowering in cultivation	End of fruiting in cultivation	First flowering in the field	End of fruiting in the field	First rain in the field
Arid	19 Jan 2003	24 Mar 2003	4 Feb 2003	16 Mar 2003	9 Dec 2002
Semi-Arid	27 Jan 2003	29 Apr 2003	8 Feb 2003	11 Apr 2003	31 Oct 2002
Mediterranean	6 Feb 2003	15 May 2003	18 Feb 2003	15 Apr 2003	9 Dec 2002
Mesic Mediterranean	17 Feb 2003	27 May 2003	2 Mar 2003	25 Apr 2003	12 Nov 2002

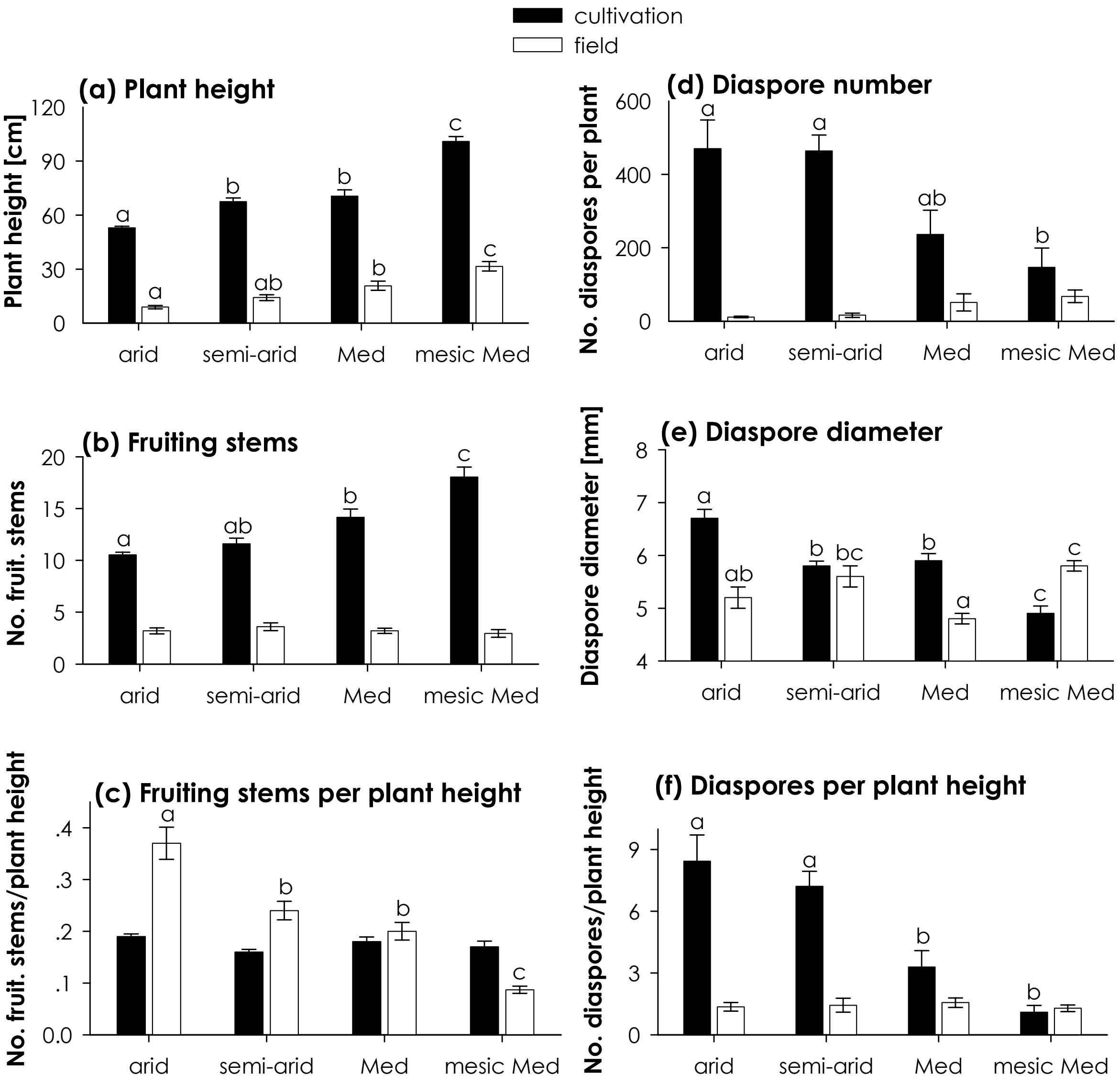

Figure 1. Architectural and fitness parameters of four populations of *Biscutella didyma* under common (cultivation) and field conditions (arid, semi-arid, Mediterranean (Med) and mesic Mediterranean (mesic Med); **(a)** plant height, **(b)** number of fruiting stems, **(c)** number of fruiting stems per plant height, **(d)** diaspore number, **(e)** diaspore diameter, and **(f)** diaspore number per unit plant height. Mean values ± 1 SE. Different letters above error bars indicate significant differences among populations within a given treatment (cultivation vs. field, Tukey's test, $p<0.05$).

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Epilogue

Once upon a time, you formulated a hypothesis (or Somebody did) and you designed the experiments to test it. You applied for a grant (or Somebody did). You were awarded for the money (or Somebody did and employed you) to pursue your line of inquiry. You did the work. You wrote the paper (together with Others). Your colleagues reviewed your work and found it to be true. You published your paper. The conclusions of the paper joined the cannon of scientific knowledge. THE END.

This platonic idea of the scientific method is, sadly, at best science fiction and at worst history of science. The reality, as everyone knows, is much less linear – simultaneously more frustrating and more exciting (Parthasarathy, www.plos.org, 2005).

Curriculum Vitae

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1985-89	Primary school in Stříbřec u Chlumu u Třeboně
1989-92	Secondary school in Třeboň
1992-97	High school (Gymnázium) in Třeboň
1997-99	BSc. biology studies at the Department of Botany, University of South Bohemia, České Budějovice, Czech Republic. BSc. Thesis title: Inter- and intraspecific interactions in populations of *Pedicularis palustris* and *P. sylvatica*, two rare species of wet grasslands. Advisor: Dr. Jan Lepš.
1999-02	MSc. studies in Geobotany at the same Department. MSc. Thesis title: The effects of experimental disturbances on microsites and plant demography. Research project conducted in the Czech Republic (advisor: Dr. Jan Lepš) and at Archbold Biological Station in Florida (advisor: Dr. Eric Menges).
2002-06	Ph.D. candidate at the Department of Plant Ecology, University of Tübingen, Germany. Thesis topic (within the GLOWA Jordan River project): Life-history expressions of annual plants in unpredictable environments: From theoretical models to empirical tests. Advisor: Prof. Dr. Katja Tielbörger.

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