Ontogenetic shifts in interactions among annual plants

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Summary

1 Interactions among plants strongly influence the structure and dynamics of plant populations and communities. However, most empirical studies of plant–plant interactions failed to make repeated measures of responses to neighbouring individuals and thereby neglected possible changes in interactions throughout the life history of the plants.

2 We tested the hypothesis that competition between annual species intensifies from early to late life-history stages, by sequentially measuring interactions in neighbour-removal experiments at three study sites located along a rainfall gradient in Israel.

3 Two annual species, *Biscutella didyma* and *Hymenocarpos circinnatus*, grew with and without neighbours in their natural habitats. Five response variables representing consecutive life-history stages (seedling survival, juvenile biomass, adult survival, number of seeds and final biomass) were recorded throughout the whole growing season.

4 The direction and intensity of interactions varied considerably between environments and life stages. On average, growth-related response variables indicated higher competition intensity at the productive end of the climatic gradient, while survival indicated either facilitation at the dry end or no trend along the gradient.

5 Temporal changes occurred, with moderate facilitation soon after germination shifting to strong competition at the end of the growing season.

6 Our results demonstrate that the outcome of experimental studies on plant–plant interactions may depend not only on the environmental productivity but more so on the life stage at which the target plant is studied.

Key-words: annuals, competition, facilitation, life-history stages, plant–plant interactions, productivity gradient, removal experiment, RII, semi-arid environment, water-stress

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Introduction

The importance of interactions among plants for the structure and functioning of natural plant communities has attracted large numbers of empirical and theoretical studies investigating interactions under different environmental conditions. However, most of these studies have narrowly focused on a single life-history stage (usually biomass) for evaluating the response of plants to the presence of neighbours (Goldberg *et al*. 2001). Only a few studies have used two response variables (e.g. Sarukhan & Harper 1973; Ehrlén 1995a,b; Howard & Goldberg 2001) and even fewer have explicitly tested for differences between life-history stages. This is

unfortunate as the measure of interaction intensity at only a single point in time is obviously insufficient for drawing conclusions about lifetime fitness or population dynamics (McPeek & Peckarsky 1998).

The necessity to study interactions throughout the life cycle of a plant has been highlighted before (Tielbörger & Kadmon 2000; Goldberg *et al*. 2001). The few relevant studies point out that density dependence and underlying mechanisms of competition may vary considerably among life-history stages (De Steven 1991a,b; Goldberg *et al*. 2001; Howard & Goldberg 2001). For example, it has been shown that seedlings appear to be more sensitive to competition than adult plants (Foster 1999; Nash-Suding & Goldberg 1999). Other studies have indicated that negative effects are relatively rare early after germination, but may be important in determining the final biomass of the

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plants (Goldberg *et al.* 2001). Such contradictory findings highlight the need to explicitly investigate how interactions among plants depend on life stage and abiotic environment.

This study was designed to test explicitly for shifts in interaction intensity throughout the life-history stages of two annual plant species that grow along a steep climatic gradient in Israel. By combining neighbour removal experiments with different environmental conditions, we enhanced the probability of covering the full spectrum of interactions among plants (from facilitation to competition) and, hence, of detecting shifts in interactions.

In particular, we tested the hypotheses that (a) net interactions shift from positive or neutral at early life stages to negative at later stages, and that (b) the effect of life stage interacts with the environmental gradient in that the intensity of negative interactions increases with site productivity.

Methods

STUDY SITES AND SPECIES

Our experiments were conducted from November 2001 to April 2002 at three study sites located along a steep precipitation gradient in Israel. All sites share the same calcareous bedrock and were established on south-facing slopes. The overall climate of the region is Mediterranean, with hot, dry summers and cold, rainy winters. The amount and distribution of annual rainfall is subject to high fluctuations, with increasing variability towards the arid site (Katsnelson 1964). The rainy season (the growing season of annual plants) extends from approximately October until May, with a delayed start and earlier end in the southern desert. During our study, the first effective rains occurred at the end of November 2001 in the north of Israel and at the beginning of December in the centre and the south.

The three sites differ primarily in variation and amount of annual precipitation and, accordingly, in habitat productivity. Mean annual temperatures are similar at all sites and range between 17 °C and 18 °C (M. Sternberg *et al.*, unpublished data).

Lahav, the southernmost station, which is located about 20 km north of Be'er Sheva in the periphery of the Negev desert, has a mean annual precipitation of 300 mm (305 mm in the study season) and can be classified as semi-arid. Vegetation is open shrubland dominated by annual plants. Shrub cover is approx. 10%, with the dominant shrub species *Sarcopoterium spinosum* (L.) Spach. and *Thymelaea hirsuta* (L.) Endl. Annuals may reach a cover of 15% (J. Kigel, unpublished observations) and often grow under the canopy of shrubs.

The second station, Matta, is located about 15 km south-west of Jerusalem in the Judean Mountains. Climate at this station is Mediterranean, with 540 mm average annual rainfall (above average, 736 mm, in 2001–02). Shrub cover in the area is approximately

30%, with *Sarcopoterium spinosum* and *Calycotome villosa* (L.) Link. as dominant species. In the open patches between shrubs, annuals reach a cover of 60%.

Ein Ya'acov, the northernmost station, is located about 10 km east of Nahariya at the edge of the Western Galilee, on Terra Rossa soil. Annual precipitation in this humid-Mediterranean environment averages 780 mm and was 905 mm in the study period. The natural vegetation of this area is characterized by a dense cover (60%) of different tree species (e.g. *Quercus calliprinos* Webb) and shrubs such as *Sarcopoterium spinosum* and *Calycotome villosa*. In the remaining area, annual vegetation has an average cover of 70%.

Our experiments were conducted in open patches between shrubs and trees to avoid confounding shrub–annual and annual–annual interactions. Though this design may result in a higher overall probability of detecting negative interactions, directional shifts should not be affected. As study species, we selected the two annuals, *Biscutella didyma* (L.) (Brassicaceae) and *Hymenocarpos circinnatus* (L.) (Fabaceae), that are frequent in all study sites (except *B. didyma* in Ein Ya'acov) and easily recognizable by their cotyledons. During the study season, these two species germinated in a single large cohort soon after the first major rainstorm at each site.

EXPERIMENTAL DESIGN

At the beginning of December 2001, shortly after germination of the annuals, 40 pairs of single plants, separated by at least 30 cm, were randomly selected at each site and for each species, except that sufficient seedlings of *B. didyma* could be found in Ein Ya'acov. Two treatments were applied: for the removal treatment (R) all neighbours around the target plant were removed carefully within a radius of 15 cm throughout the growing season, whereas the second plant was used as a control (C), with neighbour plants left intact.

LIFE-HISTORY STAGES

Five fitness parameters were documented for each target and control plant at different points in time. As phenology differs greatly between sites and is delayed towards the productive end of the gradient (Petrů *et al.* 2006), measurements were taken at similar life stages for all focal plants but not at the same date at each station.

Seedling survival was recorded 3 weeks after the first removal of the neighbours at each of the stations.

Juvenile weight was measured around the fifth week after the beginning of the experiment. Thirty randomly selected plants of each species were collected in the surroundings of the study areas to identify the most suitable non-destructive measure for dry mass during early life-history stages. Multifactorial regression models were constructed, with dry mass as dependent variable and linear combinations of the measurements for height, length of branches, number of leaves and diameter as

independent variables. The number of fully developed leaves was the best predictor for the dry weight of the plants (*B. didyma*, $P < 0.01$, $r^2 = 0.87$; *H. circinnatus*, $P < 0.01$, $r^2 = 0.72$) and was used to estimate juvenile weight.

Survival to seed production was recorded between the third week after the first removal of neighbours and harvesting of the target plants.

Number of seeds produced per plant was determined at seed set (in the beginning and middle of March in Matta and Lahav, and in the middle of April in Ein Ya'acov).

Weight was determined after seed set. Plants were harvested and weighed after drying at 80 °C for 48 hours. In the few cases, where *B. didyma* had dispersed some of their seeds, we added the average weight of dispersed seeds as determined from independent seed collections. The number of dispersed seeds could be easily determined by the number of extant pedicels.

We also harvested and dried the above-ground parts of all neighbouring plants in the control plot up to a distance of 15 cm from the target, as a measure of productivity of the site.

DATA ANALYSES

Each of the five response variables was tested for significant differences between treatments for each site and species separately. We used analyses of variance with a paired design for juvenile weight, number of seeds and adult weight and analyses of deviance, based on generalized linear models, for the remaining, binomially distributed survival data.

We used 'relative interaction index' (RII, *sensu* Armas *et al*. 2004) as our measure of interaction intensity, as it reflects consequences of interactions for the target plants without the impact of the abiotic environment. Moreover, it has desirable statistical properties such as symmetry around zero, linearity and continuity. RII was calculated as $RII = (X_{+N} - X_{-N})/(X_{+N} + X_{-N})$, where X_{-N} is the plant performance without neighbours and X_{+N} the plant performance with neighbour plants.

To test the hypothesis that RII changes with increasing productivity, we conducted analyses of variance for both species. 'Life stage' of the annuals and 'site' were used as independent, fixed factors, and the relative interaction index as dependent variable.

As measurements of all five response variables were conducted on the same plants throughout the life cycle, repeated-measures ANOVAs should be used. However, data sets for plants that died before seed set would be excluded from the analyses and survival could not be investigated in that kind of model. Therefore, we used an analysis of variance with life stage as independent factor taking into account a potential loss of power.

Results

The measurements of standing crop indicate a significant decline of productivity with increasing aridity (Fig. 1; $P < 0.001$ one-way ANOVA).
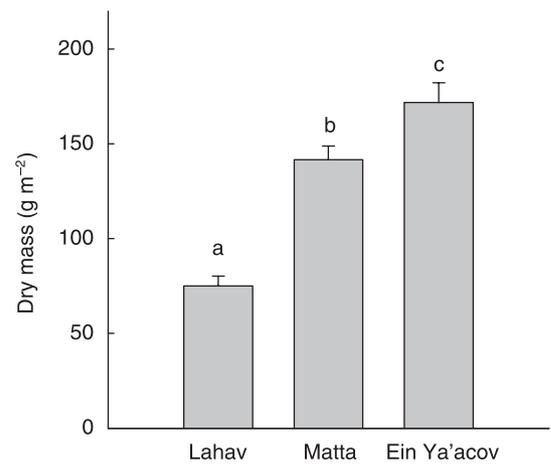

Fig. 1 Mean values for the dry mass of standing crop for the three study sites. Error bars indicate 95% confidence intervals; different letters indicate significant differences between sites (Student-Newman Keuls Tests, $P < 0.001$).

The further analyses show the occurrence of both facilitative and competitive effects in response to the presence of neighbours (Fig. 2). Significant positive neighbour effects were found in Lahav at the seedling survival stage for both species (*B. didyma*, $P < 0.001$; *H. circinnatus*, $P = 0.049$). Highly significant negative effects ($P < 0.001$) could be shown for both species in the Mediterranean site (Matta) and the humid Mediterranean site (Ein Ya'acov), regarding the number of seeds and the final biomass. The same applied for *B. didyma* in Matta for the juvenile weight.

There were highly significant ($P < 0.001$) differences in interaction intensity between life-history stages for both species (Table 1). Differences between sites were highly significant for *H. circinnatus* ($P = 0.001$) and less so for *B. didyma* ($P = 0.046$).

In general, the two species responded similarly to the treatments, tending towards higher competition intensity with increasing productivity, and towards increasingly negative neighbour effects as plant age increased (Fig. 2). In other words, positive neighbour effects were more common at the semi-desert site and at early life stages. In contrast to the rather negative neighbour effects on growth-related variables, the effects of neighbours on juvenile and adult survival were either positive or neutral (Fig. 2).

There were no significant interactions between the variables 'site' and 'life stage', indicating that the productivity level had no significant effect on the general temporal progression of interaction intensity. However, visual inspection of RII indicates that the switch from positive or neutral interactions towards competition occurred at a later stage at the drier sites (Fig. 2).

Discussion

Our overall results suggest that the direction and intensity of interactions among annual plants depend not only on habitat productivity but even more so on the
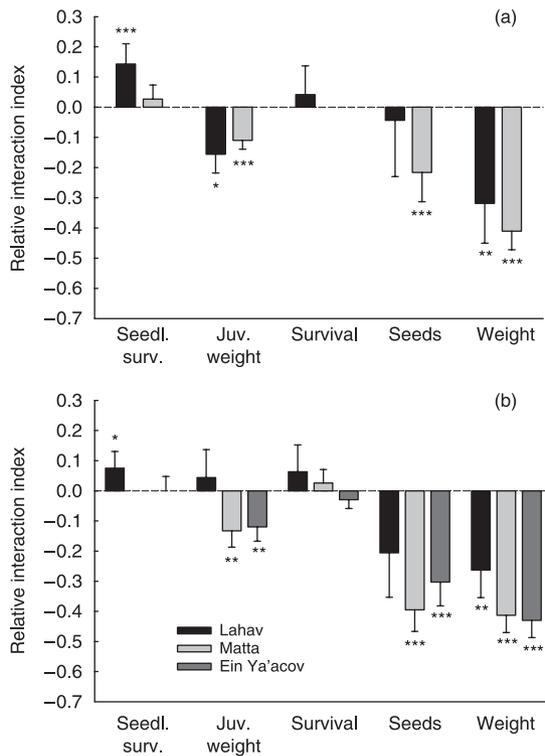
Fig. 2 Mean (+ 1 SE) values of the relative interaction index for different response variables (seedling survival, juvenile weight, survival, number of seeds and adult weight) and sites for (a) *Biscutella didyma* and (b) *Hymenocarpos circinnatus*. Asterisks indicate significant differences in the response variable between isolated plants and plants growing with all neighbours (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Table 1 *F*-values and significance levels for ANOVA with the RII as dependent variable and site and life stage as independent variables for the species *Biscutella didyma* and *Hymenocarpos circinnatus*

Species	Source of variation (d.f.)	F	P
B. didyma	Site (1)	4.032	0.046*
	Life stage (4)	9.495	< 0.001***
	Site × life stage (4)	0.685	0.603
H. circinnatus	Site (2)	6.676	0.001***
	Life stage (4)	22.892	< 0.001***
	Site × life stage (8)	0.435	0.900

life stage at which a plant is observed. In general, the interaction type shifted from facilitation or neutral net interactions towards intensive competition with increasing productivity and time.

COMPETITION DOMINATES IN HIGH-PRODUCTIVE HABITATS, INDEPENDENT OF THE PLANTS' LIFE STAGE

Most previous discussions about the importance of accounting for life-history stages in competition experiments have been motivated by the so-called Grime-Tilman (Newman) debate. This debate stems from the

unresolved question of whether competition intensity increases with increasing habitat productivity (Grime 1973, 1979; Huston 1979; Keddy 1990) or stays constant (Newman 1973; Tilman *et al.* 1982). As empirical work has supported both views equally, many efforts have been made to explain the contradictory findings. One such explanatory hypothesis claims that the use of different response variables may lead to opposing results, e.g. the response of mortality to the presence of neighbours might change with productivity while the response of growth stays constant (Goldberg & Novoplansky 1997). We can now evaluate whether our experiment would have yielded differential support to either of Grime's or Tilman's views, depending on the life stage under consideration.

On average, our findings indicate facilitative or neutral interactions at the drier end of the gradient and increasingly negative interactions with increasing rainfall. With one exception (juvenile weight of *B. didyma*), competition was more intense in the two wetter sites (Matta and Ein Ya'acov) compared with the semi-arid site (Lahav), for both species in all life stages (Fig. 2). As productivity at our sites increases strongly with rainfall, this pattern corroborates the model of Bertness & Callaway (1994) and between-site differences for RII values were similar for all response variables, irrespective of life-history stage. Therefore, the time at which interactions among plants are studied does not necessarily affect the qualitative outcome of experiments designed to test for the effect of interaction with the environment on relationships.

INTERACTION INTENSITY SHIFTED FROM SLIGHTLY POSITIVE TO STRONGLY NEGATIVE OVER TIME

Our overall results show clearly that there were differences between life stages in direction and intensity of interactions. Intriguingly, these differences did not vary randomly across the plants' life cycle but exhibited a consistent trend through time: RII shifted from positive or neutral at early life stages towards extremely negative values at the time of seed production.

Several reasons for the shift from facilitation to competition have been discussed before. For example, Goldberg *et al.* (2001) observed in a study on desert annuals that negative density dependence was most intense at the germination stage. Seedling density and total standing crop were therefore low, resulting in less frequent negative interactions at early stages of the growth of the vegetation. However, in our case, density-dependent germination is unlikely to explain the positive neighbour effects at the seedling stage, as seed germination of *B. didyma* is markedly accelerated in the presence of seeds of other species (K. Tielbörger, unpublished results), suggesting that facilitation is critical even at the earliest life stages.

Other hypotheses concerning changes in interaction type and strength with time suggest that seedlings

might be more sensitive to harsh conditions than to competition. Therefore, at this stage, both importance and intensity of competition (Brooker *et al.* 2005) may be low and the effect of neighbours predominantly positive. For example, bigger plants may facilitate seedlings by non-trophic effects, such as reduction of temperature and evapotranspiration (Callaway *et al.* 1995; Holmgren *et al.* 1997), particularly in water-limited systems such as ours. As there is no apparent reason why positive interactions (e.g. via shading) should become less intense at later life stages, the increasingly negative RII values during development can only be explained by more intense competition. The most straightforward explanation may be that increasing standing crop (i.e. growth of the plants) leads to an elevated demand for resources and thus greater competition. This idea is consistent with the hypothesis that exploitation competition is the primary mechanism underlying growth patterns (Goldberg *et al.* 2001).

Despite the general trend of increasingly negative density dependence along the gradient and across life stages, plant survival always responded either positively or not at all to the presence of neighbours. As with other variables, survival was more positively affected by neighbours in seedlings than in adults, but both showed markedly different values from those for juvenile weight, number of seeds and adult weight.

These findings are consistent with a series of previous studies, which attempted to distinguish between growth and survival as responses to competition (Kadmon 1995; Callaway & King 1996; Goldberg & Novoplansky 1997; Goldberg *et al.* 1999; Howard & Goldberg 2001; Hastwell & Facelli 2003; Sher *et al.* 2004). These studies found that neighbour effects on growth are more likely to be negative than effects on survival. Such results might be related to the fact that both mortality and facilitation occur more often during time periods of high water-stress. Therefore, positive neighbour effects may be more likely to be observed in survival than in growth.

For two reasons, interpretation of our findings should explicitly take into account the fact that both species we used are annuals. First, such 'within life-form' interactions may be more likely to be negative than, for example, the effect of shrubs on annual vegetation (Callaway 1995; Holmgren *et al.* 1997), as plants interact at similar life stages. This may be an explanation for why we detected facilitative effects rather rarely. However, our main hypothesis, i.e. a directional shift in interaction intensity through time, can be tested irrespective of the presence of positive interactions.

A more important limitation of our study system may be that annual plants follow strategies that are different from those of perennial plants. For instance, their short life cycles, and the variable environment of sites such as ours, mean that annuals invest much more into reproduction than into growth (Petrů *et al.* 2006) and clonal growth or retrogression does not occur at all. Another important difference is that annuals survive the most stressful periods (summer drought at our site) in the form of seeds and we cannot therefore observe neighbour effects in the season where positive interactions are most likely to occur. In coexisting perennial species, such facilitative interactions may repeatedly occur throughout the life cycle. Therefore, while annual plants are a simple and useful system to investigate directional shifts in plant–plant interactions throughout a complete life cycle, future studies with perennial plants are needed to test the generality of our findings.

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