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# Plastic bet-hedging in an amphicarpic annual: an integrated strategy under variable conditions

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**Abstract** Amphicarpy is a form of diversified bet-hedging expressed mostly in annual plants, where two types of offspring are produced with two distinct ecological roles: long-range aerial dispersers and highly competitive subterranean, sedentary fruit. *Emex spinosa* is a semi-arid, amphicarpic annual, inhabiting habitats with different levels of environmental variation. We tested the hypothesis that, in *E. spinosa*, bet-hedging may be "fine-tuned" by plasticity in the phenotype ratio (aerial/subterranean fruit mass) as a function of environmental conditions. We conducted a greenhouse experiment, manipulating nutrient availability and intraspecific density, to determine the pattern of ratio shifts. In order to determine whether the integrated strategy is an adaptation to variable habitats, a similar common garden experiment was conducted, comparing two natural populations differing in environmental variability. The offspring ratio shifted in response to both nutrient availability and plant density. In pots containing single plants the ratio increased steeply with nutrient availability, while in pots containing eight plants a more moderate increase occurred. These shifts were the result of plasticity in allocation to both achene types, as well as ontogenetic effects on aerial achene production. The degree of response increased with the heterogeneity of the habitat of origin. We found evidence for an adaptive integrated strategy, with bet-hedging "fine-tuned" by phenotypic plasticity. Strenuous conditions tended to shift the offspring ratio towards securing subterranean reproductive success, while favorable conditions resulted in a shift towards dispersible achenes.

**Keywords** Bet-hedging · Seed dispersal · *Emex spinosa* · Integrated strategies · Life history · Phenotypic plasticity · Sibling competition · Variable environments

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The authors Asaf Sadeh and Hagai Guterman contributed equally to this study.

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## Introduction

Unpredictable environments confront organisms with the challenging task of reproducing and surviving under variable conditions. Stochasticity in conditions such as weather (Blaustein and Schwartz 2001), resource availability (Nager and Van-Noordwijk 1995) floods (Satake et al. 2001) and fires (Cheplick 1987) is often the cause of increasing variance in fitness and therefore strongly influences the life-history of an individual organism (Murphy 1968; Philippi et al. 2001; Roff 2002). Evolutionary ecologists traditionally define four different strategies for coping with variable environments (Dewitt and Langerhans 2004): (a) specialization; (b) generalization; (c) bet hedging—an organism produces either several phenotypes or a single phenotype probabilistically; (d) phenotypic plasticity—environmental factors trigger the production of different phenotypes from a single genotype. According to recent theoretical developments in evolutionary biology, these "pure" strategies should not be perceived as discrete, but rather as special cases within a general strategy space which includes different combinations of these strategies (Dewitt and Langerhans 2004).

Out of these four strategies, we here focus on bet-hedging and its fine tuning via phenotypic plasticity. Bet-hedging is defined as the expression of several phenotypes (strategies) by a single individual, reducing the between-generations variance of reproductive output, minimizing the probability of complete reproductive failure and thus increasing the genotype's geometric mean fitness over generations (Cohen 1966; Gillespie 1977). Some theorists require that the reduction in the between-generation variance comes at the expense of the maximal within-generation possible output (Seger and Brockmann 1987), but this restriction has not been fully adopted (e.g., Dewitt and Langerhans 2004). Most studies concerning bet-hedging theory in plants have focused on seed dormancy and dispersal (Cohen 1966; Gillespie 1981; Bulmer 1984; Philippi 1993; Venable 2007). Seed dormancy was experimentally shown to act as a form of bet-hedging (Philippi 1993). Further studies have suggested that bet-hedging strategies can be observed in additional traits such as timing of reproduction (Satake et al. 2001) and propagule heteromorphism (Venable 1985; Mandák and Pyšek 1999).

Amphicarpy is a form of propagule dimorphism displayed mostly by annual plants, where two types of flowers are produced on each individual: (a) subterranean flowers that usually self-pollinate and start developing early in the season into subterranean propagules that germinate in situ (b) aerial flowers that have the ability to cross-pollinate but start developing later in the season into aerial propagules, for long range dispersal, (Zohary 1962; Cheplick 1987, 1994). Adopting Philippi and Seger's (1989) terminology of phenotypes, between years subterranean fruit represent a "bad" year, low-risk phenotype, germinating in a proven site, and facilitated by the mother plant (Weiss 1980). On the other hand, following rainy seasons, numerous new patches become available for colonization, making the dispersible aerial fruit a "good" year phenotype. The latter phenotype involves risk proportional to the scarcity of good patches in the otherwise hostile matrix. Within years, there is also temporal variation in the distribution of rainfall, thus the early production of subterranean fruit is a "timid" strategy of ensuring reproduction, while the later production of aerial fruit is a "bold" strategy. These two distinct strategies are similar to the ones described in Satake's et al. (2001) model of reproduction timing under unpredictable catastrophes, with both pure strategies here displayed in a single individual.

Thus, amphicarpic species manage the variability of their habitats using the two fruit types, with their two qualitatively distinct strategies. These two strategies expressed by each individual are in fact a form of bet-hedging. To be consistent with Cohen's (1966) approach, we define a plant's *amphicarpic ratio* as its total aerial divided by its total subterranean fruit mass. This index is a response-sensitive equivalent to the germination fraction that reached a fixed optimum in Cohen's model, and it serves to illustrate the frequencies of the two offspring strategies, rather than reproductive allocation per-se. We evaluated the extent to which bet-hedging, a primary organismal trait, can be "fine-tuned" by secondary plasticity in the phenotype ratio. According to model predictions for pure plasticity, the degree of plasticity should be positively related to the strength of divergent selection (e.g. Bell et al. 1993; Alpert and Simms 2002; De Witt and Langerhans 2004; but see Volis et al. 2002). In order to test for plastic fine-tuning of a bet-hedging strategy, we studied the effect of nutrient availability and intra-specific density, on the amphicarpic ratio of the semi-arid annual, *Emex spinosa*.

Our study consisted of two greenhouse experiments. The first experiment was designed to determine the general strategy of *E. spinosa* by testing for pattern-shifts and plasticity in amphicarpic ratio as a function of nutrient availability and intra-specific density. We hypothesized that *E. spinosa* will display plasticity in amphicarpic ratio as a "fine-tuning" mechanism for its classic bet-hedging strategy. Specifically, we predicted that the amphicarpic ratio should decrease under strenuous conditions, thus increasing the relative investment in subterranean achenes as the "bad year" phenotypes. Similarly, under favorable conditions there should be an increase in amphicarpic ratio. We therefore predicted that amphicarpic ratio should be positively correlated with nutrient availability but negatively correlated with intra-specific density.

The second experiment was a similar common garden (fully factorial crossing nutrient availability and intraspecific density) designed to determine whether the strategy delineated in the first experiment is adaptive. We compared the population reaction norms of plants taken from two natural habitats that are significantly different in environmental variability. We hypothesized that while amphicarpy is a fixed bet-hedging strategy for this species, the level of its plasticity should be greater in populations inhabiting more variable habitats. We predicted that population reaction norms of amphicarpic ratio should display similar trajectories between the two habitats of origin with greater slopes for the population that originated from the more variable habitat.

## Materials and methods

### Natural history

In the semi-arid amphicarpic annual, *Emex spinosa* (L.) Campd. (Polygonaceae), aerial achenes are smaller than subterranean ones, and morphologically adapted to dispersal either by animals, water, or wind via spines, buoyancy, and low specific weight, respectively. These fruits co-develop with vegetative growth along the shoot internodes. In contrast, subterranean achenes are produced at the base of the plant, lack spines, are never shed from the mother plant (Evenari et al. 1977), and produce seedlings that are more competitive compared to aerial achenes (Weiss 1980). After germination, the plant grows into a rosette, at which stage subterranean flowering occurs. As far as conditions allow (Weiss 1980), the plant continues to grow, while maturing subterranean fruit and developing a shoot that carries aerial flowers on the internodes.

The environments experienced by *E. spinosa* in its natural habitats range in their degree of variability. The northern Negev, a semi-arid region in Southern Israel, is the species center of distribution in the country. This environment is more variable both spatially

(Shachak et al. 1998) and temporally (Table 1), compared to that of its peripheral habitats in northern Israel. Arid and semi-arid systems are characterized by pulses of high and low biotic activity driven by temporal variation in water availability (Evenari et al. 1982). This temporal variability may interact with landscape variability, leading to high spatio-temporal variation in nutrient availability (Peters and Havstad 2006; Snyder and Tartowski 2006). The precipitation at the beginning of the growing season is not always followed by enough rainfall for annuals to complete their life cycle and mature seeds. The northern habitat is characterized by relatively more uniform vegetative cover as well as rainfall (Table 1). Two representative populations were selected to test whether plasticity in amphicarpic ratio is correlated with environmental variability. The first population is located in the northern Negev (Ofakim), a semi-arid area in southern Israel (31°16′ N, 34°49′ E), and the second in the coastal plains (Hadera), a Mediterranean area in northern Israel (32°26′ N, 34°53′ E). These regions differ significantly in amounts and patterns of rainfall, as well as in spatial heterogeneity. Ofakim displays significantly lower values for annual rainfall, amount of rain in a single rain event and maximum number of dry days within a season. Variance of these values is greater in Ofakim, especially within seasons (Table 1). Spatially, distribution of rainfall is more heterogeneous in semi-arid regions of Israel (Evenari et al. 1982), which includes Ofakim. Additionally, Ofakim area is patchily vegetated, while Hadera area is characterized by more uniform rainfall and a continuous vegetative cover.

## Experiment 1: plasticity and amphicarpic ratio shifts

In November 2004, we collected seedlings of *E. spinosa* from a field near Ofakim. The seedlings, approximately 1 month after germination and in a state of small leaf rosette with no apparent subterranean flowering, were brought to a greenhouse located in the Ben-Gurion University campus, Be'er Sheva (31°14′ N, 34°48′ E). The seedlings were planted in plastic 1.5 l pots using inert vermiculite as substrate. We implemented a fully factorial design with four levels of nutrient availability (Hoagland solution at 0, 10, 50 and 100% strength, applied via irrigation; Hoagland and Arnon 1950), four levels of intra-specific density (1, 2, 4 and 8 plants per pot) and 10 replicates for each of the 16 treatment

**Table 1** Rainfall data for Desert vs. Mediterranean regions in Israel

| Region | Sample size ($n = 23$ yrs) | (a) Amount rainfall/rain event (mm) | (b) Number of rainy days/season | (c) Maximum number of dry days between rain events | (d) Mean annual rainfall (mm) |
|---|---|---|---|---|---|
| Desert | Average ± SD | 4.97 ± 7.04 | 39.78 ± 11.61 | 34.48 ± 18.90 | 197.75 ± 66.18 |
| | CV | 142% | 29% | 55% | 33% |
| Mediterranean | Average ± SD | 9.86 ± 11.63 | 52.52 ± 10.26 | 29.15 ± 11.70 | 516.54 ± 147.52 |
| | CV | 118% | 20% | 40% | 29% |
| Bartlett's test homogeneity of variance | $\chi$ value | 241.08 | 0.324 | 4.970 | 12.553 |
| | $P$-value | $P < 0.001$ | $P = 0.569$ | $P = .026$ | $P < 0.001$ |

Data were collected from the nearest weather stations to Hadera and to Ofakim, all within 10 km radius of each. Coefficient of variation (CV = [SD/average]*100%) is significantly higher in the Desert in both intra-generational (columns a, c) and inter-generational scales (column d). (Data were provided by the Israel Meteorological Service and includes 23 consecutive years of measurements, i.e., 1982–2004) combinations. The pots were randomly distributed in ten blocks on the greenhouse tables. Nutrient and water availability are often coupled. Specifically, plant mechanisms of nutrient uptake and soil biotic activity that produces these nutrients are both water-dependent. Our choice of manipulating nutrient availability allowed us to better differentiate the effect of nutrients from the effect of water and to compare our results with those obtained in an earlier study on *E. spinosa* (Weiss 1980). We irrigated the plants for the first 10 days with tap water for root system establishment before commencing with the different nutrient treatments. During the rest of the experiment we irrigated the plants two to three times a week, as needed, each pot receiving 100 ml of the treatment solution. Once every 2 weeks, the pots were washed with 200 ml of distilled water in order to prevent nutrient accumulation in the substrate, followed by 100 ml of the treatment solution. We terminated and harvested the experiment at the end of the plants' growing season (end of March 2005). At the harvest, all the plants from each pot were pooled together and dismantled into root systems, shoots, aerial achenes and subterranean achenes, and were dried in paper bags for 48 h at 80°C. Once dry, all parts were weighed on an analytical scale to the nearest milligram (CP224S, Sartorius AG, Goettingen, Germany) and both aerial and subterranean achenes were counted. Some plants in 14.5% of the pots died during the experiment and disappeared before harvesting. In all cases, intraspecific density is considered the initial per-pot density of established plants. In calculating root/shoot ratio, we excluded pots where the numbers of shoots and roots differed due to plant death and breakdown.

### Experiment 2: Comparison of population reaction norms

Germination in northern Israel occurs earlier than in southern Israel. In late November 2005, we collected *E. spinosa* seedlings from a field near Hadera (northern Israel, hereafter "Hadera population"). In late December, we collected seedlings from the same site as for the previous experiment (southern Israel, hereafter "Ofakim population"). The seedlings used from both populations were at the same age, developmental stage and size when the manipulations were initiated and they all withered at the same time. In other words, beginning the experimental manipulation on both population samples at the same time would have produced different manipulations with respect to the plants' developmental stage, which could lead to biased results (Rice and Bazzaz 1989). We planted the seedlings in plastic pots in the same greenhouse and using the same procedures as in the previous experiment, with the following differences: we used rinsed sand as substrate, and tap water irrigation for root system establishment was applied for 3 weeks prior to beginning nutrient treatments. We implemented a fully factorial design with two levels of nutrient availability (Hoagland solution at 5% and 50% strength, applied via irrigation), two levels of intraspecific density (1 and 4 plants per pot), two populations of origin (Ofakim and Hadera), and 12 replicates for each of the eight treatment combinations. The pots were randomly distributed in 12 blocks on the greenhouse tables. In early April, after all of the plants of both populations withered, the experiment was terminated and all the plants were harvested and measured in the same manner as with the previous experiment.

### Statistical analysis

Data for both experiments were analyzed using Systat 9.0 (Systat Software, Inc. CA, USA). To avoid pseudoreplications all analyses were done using mean pot values, with each pot serving as a replication of its specific combination of nutrient and density levels (Hairston 1989). All analyses were done using ANOVA.

In the first experiment, we started by analyzing the effects of nutrients and density on amphicarpic ratio, including analyses of the total mass, numbers and average mass of both achene types.

Next, to clarify the nature of the observed shifts in amphicarpic ratio and to differentiate between "true" and "apparent" plasticity (Sultan 1995; McConnaughay and Coleman 1999), we conducted an additional analysis examining reproductive allocation patterns, measured as achene mass/vegetative mass (Bazzaz 1997). A correlation between reproductive output and plant size is a well known phenomenon which can lead to reproductive responses to experimental manipulations due to "ontogenetic drift" (Evans 1972). In our case such pattern is caused by the co-development of aerial flowers along the shoot internodes throughout ontogeny. To control for such possible ontogenetic effects while testing for true plasticity in achene production we examined only a subset of our data in which there was a substantial overlap in the range of total plant mass (total mass <4 g, therefore reducing our sample size from 156 to 128 only for this analysis). By doing so, we were able to rule out the possibility that treatment effects resulted only from size differences. Our null hypothesis that only ontogenetic drift causes the observed plasticity in reproductive allocation should be rejected if one or both experimental treatments are significant. This is because it implies that the relationship between the two components comprising reproductive allocation (e.g., reproductive mass and vegetative mass) is not consistent among the different levels within the treatment. Such significant treatment effects indicate that true plasticity exists. Qualitatively similar results were obtained when applying an ANCOVA on reproductive mass with vegetative mass as a covariate, but here rather than testing for treatment effects we tested for significant treatment × vegetative mass interaction terms. We present here only the former and more intuitive analysis.

In the second experiment, seven amphicarpic ratio values were excluded from the data set as significant outliers. These outliers were caused by cases of extremely low average subterranean achene mass, resulting in exceptionally high ratio values. The outliers occurred in four different treatments and were subtracted from a data set of 88 pots. Again, we first analyzed the effects on amphicarpic ratio and the two components comprising it. Second, to differentiate between "true" and "apparent" plasticity, we examined reproductive allocation patterns. However, this time there was a substantial overlap in the range of total plant mass within and among treatments and the entire data set was used.

## Results

### Experiment 1: plasticity and amphicarpic ratio shifts

We first present the effects of our experimental treatments on the amphicarpic ratio. Next, we present the two components comprising this ratio, including analyses for both achene types regarding mass, numbers and average achene mass. To clarify the nature of the observed shifts in amphicarpic ratio and to differentiate between "true" and "apparent" plasticity (Sultan 1995; McConnaughay and Coleman 1999) we then present data regarding reproductive allocation patterns. In other words, we distinguish between the plant's ultimate performance using the measures of amphicarpic ratio and absolute production, and its relative reproductive efforts using the measures of allocation. Finally, we present the effects of our manipulations on the root/shoot ratio in order to account for shifts in vegetative allocation.

The amphicarpic ratio (Fig. 1) was positively affected by nutrient availability ($F_{3,136} = 6.52$, $P < 0.001$) and negatively affected by intraspecific density ($F_{3,136} = 8.592$, $P < 0.001$), but the interaction term between these two treatments was not significant ($F_{9,136} = 1.407$, $P = 0.191$).

Total aerial achene mass (Fig. 2a) was positively affected by nutrient availability ($F_{3,140} = 32.086$, $P < 0.001$) and negatively affected by density ($F_{3,140} = 54.498$, $P < 0.001$). However, the positive effect of nutrients weakened in high density treatments (density by nutrient interaction; $F_{9,140} = 4.846$, $P < 0.001$).

The total number of aerial achenes was positively affected by nutrient availability ($F_{3,140} = 26.452$, $P < 0.001$) and negatively affected by density ($F_{3,140} = 33.651$, $P < 0.001$). Again, this positive effect of nutrients weakened in high density treatments (density by nutrient interaction; $F_{9,140} = 2.999$, $P = 0.003$).

Average aerial achene mass was negatively affected by density ($F_{3,123} = 3.944$, $P = 0.01$), but we could not detect a significant effect of nutrient availability ($F_{3,123} = 1.809$, $P = 0.149$) nor was there a significant interaction ($F_{9,123} = 1.635$, $P = 0.112$).

Total subterranean achene mass (Fig. 2b) was negatively affected by both nutrient availability ($F_{3,140} = 4.938$, $P = 0.003$) and density ($F_{3,140} = 10.273$, $P < 0.001$). No significant interaction was found ($F_{9,140} = 0.727$, $P = 0.684$).

The total number of subterranean achenes was negatively affected by density ($F_{3,140} = 5.76$, $P = 0.001$), but there was no significant effect of nutrient availability ($F_{3,140} = 1.348$, $P = 0.262$) nor was there a significant interaction ($F_{9,140} = 1.056$, $P = 0.399$).

Average subterranean achene mass was negatively affected by both nutrient availability ($F_{3,131} = 8.914$, $P < 0.001$) and density ($F_{3,131} = 5.28$, $P = 0.002$), but the interaction term was not significant ($F_{9,131} = 1.397$, $P = 0.196$).

Total reproductive mass and total plant mass were positively correlated ($r = 0.926$, $P < 0.001$). Similarly, total aerial achene mass and total plant mass were positively correlated ($r = 0.926$, $P < 0.001$). However, we could not detect any correlation between

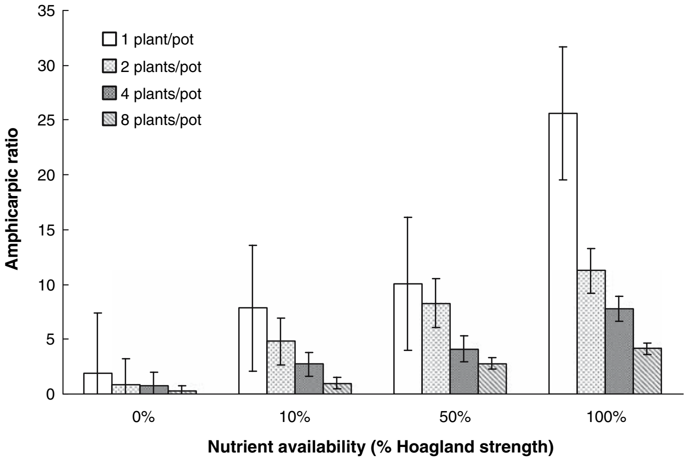

**Fig. 1** The effects of nutrient availability on amphicarpic ratio (total aerial/subterranean achene mass) for different plant densities. Mean values for each treatment ±1 SE are shown

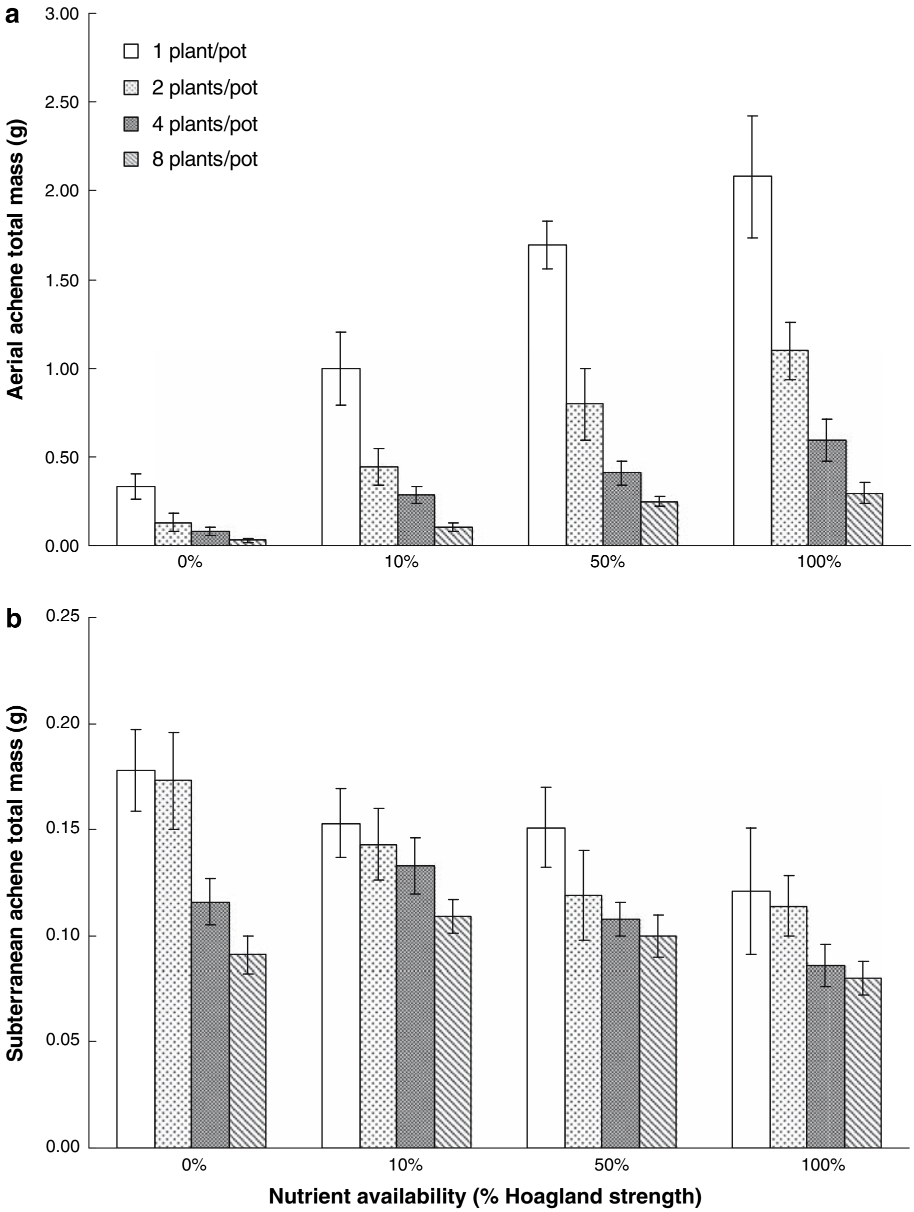

**Fig. 2** The effects of nutrient availability on achene mass for different plant densities. (**a**) Aerial achenes. (**b**) Subterranean achenes. Error bars are 1 SE

total subterranean achene mass and total plant mass ($r = 0.130$, $P = 0.635$). This pattern of correlation between reproductive output and plant size is caused by the co-development of aerial flowers along the shoot internodes throughout its growth, resulting in an effect known as ontogenetic drift (Evans 1972). To control for this ontogenetic effect, while testing for true plasticity in achene production we first examined mass allocation to reproduction in the aggregate and to each achene type separately.

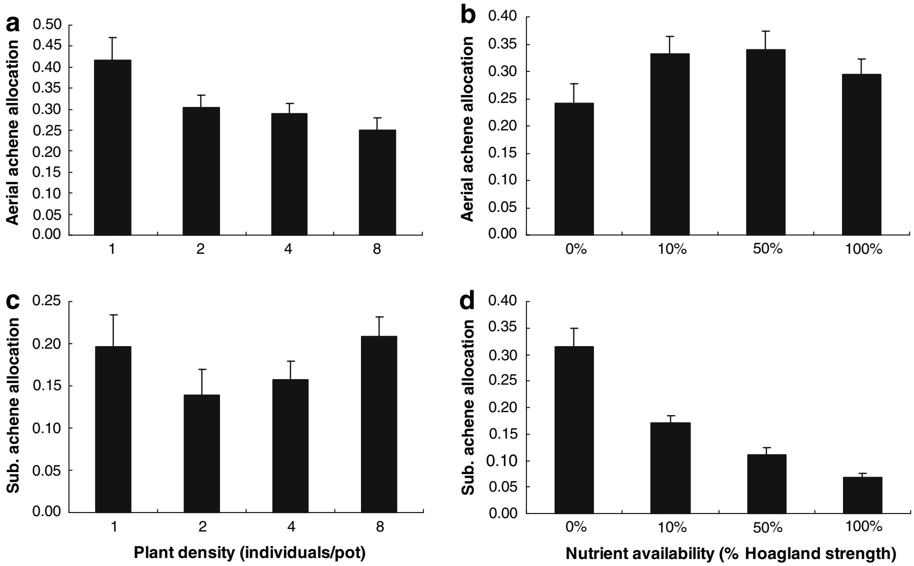

**Fig. 3** The effects of plant density and nutrient availability on allocation to aerial (**a**, **b**) and subterranean achenes (**c**, **d**). Error bars are 1 SE. Note that only a subset of the data in which there was a substantial overlap in the range of total plant mass was included in the analysis (total mass <4 g, consequently sample size was reduced from 156 to 128), to rule out the possibility that treatment effects resulted only from size differences

Total reproductive allocation (i.e., total achene mass/vegetative mass) was negatively affected by nutrient availability ($F_{3,106} = 3.299$, $P = 0.023$). However we could not detect a significant effect of density ($F_{3,106} = 0.349$, $P = 0.790$) nor was there a significant interaction between these two factors ($F_{9,106} = 0.524$, $P = 0.854$). Aerial achene allocation (Fig. 3a,b) was positively affected by nutrient availability ($F_{3,106} = 3.378$, $P = 0.021$). However we did not detect a significant effect of density ($F_{3,106} = 1.738$, $P = 0.164$), nor was there a significant interaction ($F_{9,106} = 0.942$, $P = 0.493$). Allocation to subterranean achenes (Fig. 3c,d) was negatively affected by nutrient availability ($F_{3,106} = 21.153$, $P < 0.001$) and positively affected by density ($F_{3,106} = 2.771$, $P = 0.045$), but the interaction term was not significant ($F_{9,106} = 0.085$, $P = 1.00$). To test if allocation to aerial achenes occurred at the expense of allocation to subterranean achenes or vice versa, we calculated allocation (only for this single test) as achene type mass/total plant mass. We found that the relative allocations to aerial and subterranean achenes were negatively correlated ($r = -0.538$, $P < 0.001$), suggesting that allocation to aerial achenes in nutrient rich conditions occurred at the expense of allocation to subterranean achenes.

We found the root/shoot ratio (Fig. 4) to be negatively affected by nutrient availability ($F_{3,134} = 20.466$, $P < 0.001$) and positively affected by intraspecific density ($F_{3,134} = 13.096$, $P < 0.001$) with no significant interaction between the two ($F_{9,134} = 1.677$, $P = 0.100$).

## Experiment 2: Comparison of population reaction norms

Nutrient availability had a positive effect on amphicarpic ratio in both populations, ($F_{1,72} = 11.73$, $P = 0.001$). There were no significant density or population effects

($F_{1,72} = 0.233$, $P = 0.631$; $F_{1,72} = 1.891$, $P = 0.173$, respectively) nor could we detect a significant three-way interaction ($F_{1,72} = 0.094$, $P = 0.761$). However, a steeper reaction norm was evident for the Ofakim population in low plant density (Fig. 5). Indeed, when testing for interactions within the low density treatment, a significant population by nutrient interaction was found ($F_{1,29} = 6.29$, $P = 0.018$). Note that this interaction is significant after applying a Bonferroni correction for multiple testing, reducing the significance threshold to 0.025.

We could not detect significant differences in either total plant mass ($F_{1,79} = 0$, $P = 0.989$) or total reproductive mass ($F_{1,79} = 0.562$, $P = 0.456$) between the two populations.

Total aerial achene mass was positively affected by nutrient availability ($F_{1,73} = 86.550$, $P < 0.001$) and negatively affected by density ($F_{1,73} = 48.554$, $P < 0.001$). However, the effects of both nutrient availability and plant density were stronger in the Ofakim population (nutrient by population interaction $F_{1,73} = 7.922$, $P = 0.006$, and density by population interaction $F_{1,73} = 3.801$, $P = 0.055$).

Total subterranean achene mass was negatively affected by density ($F_{1,73} = 20.877$, $P < 0.001$), however this effect was stronger in the Ofakim population (density by population interaction $F_{1,73} = 4.461$, $P = 0.038$). Furthermore, nutrient availability had a positive effect on subterranean achene mass in the Ofakim population while no such trend was detected for the Hadera population (nutrient by population interaction $F_{1,73} = 8.071$, $P = 0.006$).

Allocation to aerial achenes was positively affected by nutrient availability ($F_{1,73} = 9.153$, $P = 0.003$), negatively affected by intraspecific density ($F_{1,73} = 5.357$, $P = 0.023$), and was higher in the Ofakim population ($F_{1,73} = 8.924$, $P = 0.004$). None of the interaction terms was significant. Nonetheless, a non-significant trend did exist suggesting that the response of the Ofakim population to nutrient availability might be stronger.

Allocation to subterranean achenes was negatively affected by nutrient availability ($F_{1,73} = 39.850$, $P < 0.001$), but there was no significant effect of density ($F_{1,73} = 0.256$, $P = 0.615$). In addition, allocation to subterranean achenes tended to be smaller in the

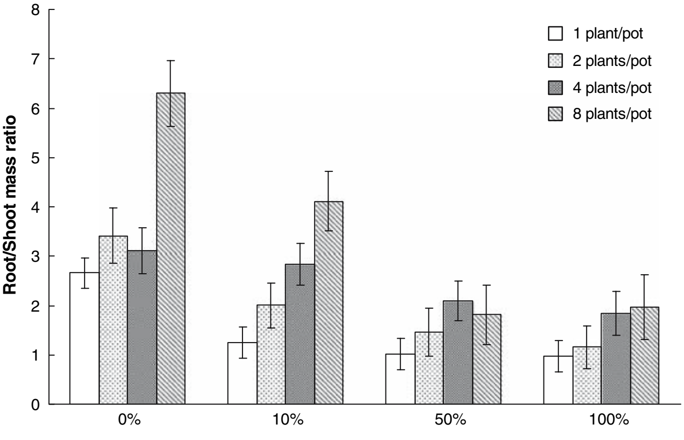

**Fig. 4** The effects of nutrient availability on root/shoot mass ratio for different plant densities. Mean values for each treatment ±1 SE are shown

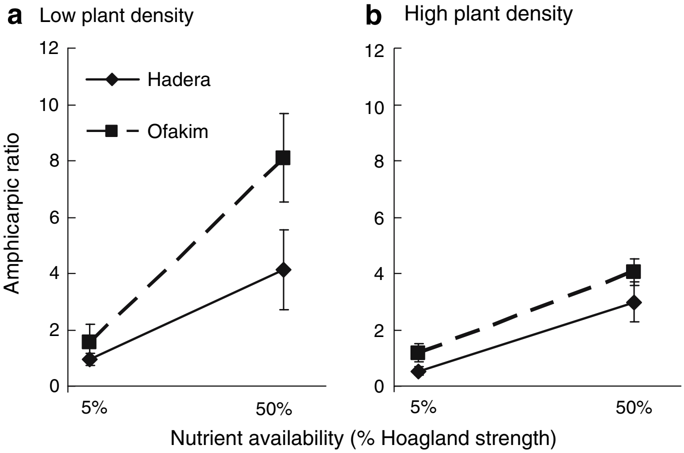

**Fig. 5** Reaction norms of the amphicarpic ratio compared between Hadera (stable environment; solid line) and Ofakim (variable environment; dashed line). (**a**) one plant per pot; (**b**) four plants per pot. A significant population by nutrient availability interaction was found within low density ($P = 0.018$). Error bars are 1 SE

Ofakim population, however, this trend was not significant ($F_{1,73} = 0.929$, $P = 0.338$). Again, none of the interaction terms was significant.

Although we could not detect any differences in the plasticity patterns of allocation to subterranean achenes between the two populations, such differences did arise when comparing subterranean achene numbers (Fig. 6). While the Hadera population retained a constant number of subterranean achene between densities, the Ofakim population was negatively affected by density (population by density interaction, $F_{1,73} = 12.2582$, $P < 0.001$). There was no significant effect of nutrient availability on the number of subterranean achenes ($F_{1,73} = 0.1088$, $P = 0.743$).

## Discussion

Amphicarpy is a classic example of diversified bet-hedging where offspring produced by each individual express two distinct ecological strategies for coping with variability between two types of environmental conditions. Our study revealed that the bet-hedging strategy possessed by *E. spinosa* is fine tuned via plasticity when confronting variable conditions. This pattern was evident in the allocation to and production of both aerial and subterranean achenes. The combined effects of both "true" plasticity and ontogenetic drift work in concert to shift the plant's amphicarpic ratio, enabling the plant to display different combinations of the two reproductive strategies along a continuum. In other words, the "good year"/"bad year" phenotype ratio is not rigidly fixed according to the probability of such years occurring, as predicted by classic bet-hedging models. Instead, it changes according to environmental signals predicting the quality of the present year. Although environmentally-induced ratios of flower or seed morphs have been previously reported and described (Harper 1977; Zeide 1978; Weiss 1980; Cheplick and Quinn 1983; Cheplick 1994; Venable 2007), to the best of our knowledge, this phenomenon has never been discussed within the context of integrated bet-hedging and phenotypic plasticity as an adaptive strategy to variable conditions. Cheplick (1994) summarized possible benefits and

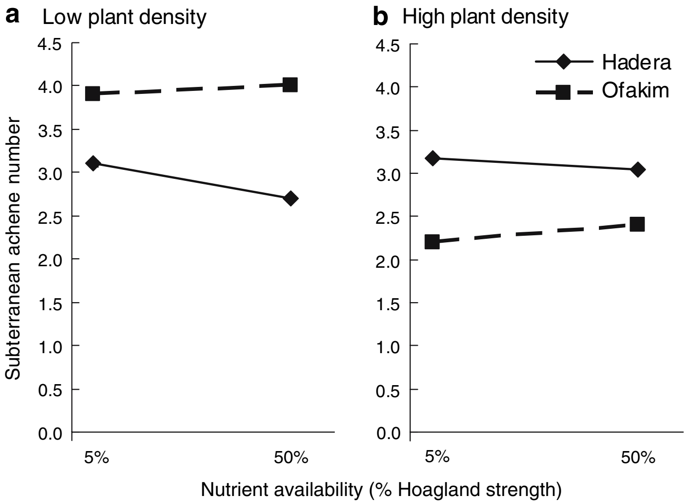

**Fig. 6** Reaction norms of subterranean achene numbers compared between Hadera (stable environment; solid line) and Ofakim (variable environment; dashed line). (**a**) one plant per pot; (**b**) four plants per pot. A significant interaction was found between population of origin and intraspecific density ($P < 0.001$). Error bars are 1 SE but are too small to be visible

constraints of subterranean reproduction. These include herbivory, protection from environmental extremes at the soil surface and fire avoidance, as well as ecological mechanisms that are discussed below: retention of offspring in the proven mother-site, limited dispersal, and sib-sib competition. In the context of environmental variability, considering the role of information and risk-management is relevant to understanding ultimate mechanisms of life-history strategies. The unpredictability of factors such as fires and herbivory is expected to select for fixed amphicarpic ratios, as in Cohen's (1966) bet-hedging model. However, habitat conditions such as nutrient availability and intraspecific density, that may have a degree of predictability through correlated local environmental cues, are expected to select for plasticity (Cohen 1967; Moran 1992; Alpert and Simms 2002; DeWitt and Langerhans 2004) and therefore give rise to adjustable amphicarpic ratios.

Three forms of evidence exist for the adaptiveness of an observed strategy (Lytle and Poff 2004): (1) experimental manipulation and the direct measurement of the organism's fitness components, (2) comparison of the performance of a species across a gradient of natural conditions (e.g. Rood et al. 2000), and (3) cross-species comparisons in a phylogenetic context. Our experiment comparing the population reaction norms of two natural populations falls under the second category. This experiment indicated a similar plastic bet-hedging pattern among populations. Plasticity in amphicarpic ratio of the Ofakim population tended to be greater than that of Hadera. These results strongly suggest that the observed strategy is an adaptation to the higher variability characterizing the southern population.

The general reproductive strategy observed in *E. spinosa* was the insuring of reproductive success in the form of subterranean achenes (i.e., low amphicarpic ratio) under strenuous nutrient and density conditions, and increasing investment in aerial achenes (i.e., high amphicarpic ratio) as far as more favorable conditions allow. This may seem counter-intuitive, as one might expect that plants experiencing difficult conditions will "escape" by investing in dispersing offspring. Still, it is consistent with model predictions and empirical studies showing that plants under unfavorable conditions tend to allocate relatively more resources into seeds with low dispersal ability (Venable and Lawlor 1980; Venable and Brown 1988; Cheplick 1994; Venable et al. 1995). Indeed, dispersal is a luxurious enterprise. Since a small fraction of the propagules usually make it to successful germination, the plant has to produce extremely high numbers of them to allow some success, thus rendering the "escape" strategy economically unfeasible under strenuous conditions.

Using the results of our first experiment, we suggest an analysis of the strategy of *E. spinosa* for coping with conditions that may be experienced by individuals in the field. The four combinations of the extreme levels of our experimental manipulations represent four soil-patch types that may be encountered in the field: nutrient-rich and vacant, nutrient-rich and crowded, nutrient-poor and vacant, nutrient-poor and crowded. In this system, such abstractions as "good/bad year" phenotypes can be specified in terms of real ecological trade-offs (see Table 2): *colonization opportunities* vs. *risk of dispersal* are factors acting on the aerial achenes, while *mother-site control* (Cheplick 1987) vs. *sibling competition* act on subterranean achenes. Additionally, reproductive allocation may be traded-off against root-competitiveness. We shall discuss how these closely related trade-offs, acting independently on each achene type, work together to produce the observed shifts in amphicarpic ratio. The pattern of achene production does not necessarily follow the allocation patterns to that achene type, especially in the subterranean achenes. We emphasize the production of each achene type as ultimate performance measurements, and as components of the amphicarpic ratio.

High nutrient treatments generally increased the production of aerial achenes at the expense of subterranean achenes, compared to low-nutrient treatments. This is to the extent that subterranean achenes of a nutrient-rich plant are smaller than those produced by a nutrient-deprived one, all else being equal. We suggest that the plants assess within-patch conditions, and respond pertaining to the entire habitat. A plant invests in greater numbers of long range dispersing propagules when it perceives the habitat as nutrient-rich and holding an opportunity to colonize new patches. However, when it perceives the habitat as nutrient-poor where the risk of dispersal is high, it should increase its allocation to subterranean achenes at the expense of aerial ones.

**Table 2** Ecological factors relevant for aerial achenes, subterranean achenes and root system, in each of four representative patch types, and the emerging amphicarpic ratio

| Patch type | Environmental conditions | Factors relevant for aerial achenes | Factors relevant for subterranean achenes | Factors relevant for root system | Amphicarpic ratio |
|---|---|---|---|---|---|
| Nutrient-rich and vacant | Luxurious | Colonization opportunity | Mother-site control | No immediate competition | High |
| Nutrient-rich and crowded | Intermediate | Risk of dispersal: conspecifics | Sibling competition | Moderate immediate competition | Intermediate |
| Nutrient-poor and vacant | Intermediate | Risk of dispersal: poor habitat | Mother-site control | Low resources, no competition | Intermediate |
| Nutrient-poor and crowded | Strenuous | Risk of dispersal: poor habitat and conspecifics | Sibling competition | Acute immediate competition | Low |

Examining the effect of plant density among the nutrient-rich treatments, the low densities of a vacant patch exert a positive effect on the production of both achene types, but a stronger response occurred in the aerial achenes, thus increasing the amphicarpic ratio. This may imply, again, that the plant responds to the within-patch density it experiences as an indicator of the environment at large. In this case, a habitat rich in nutrients and available for colonization should invoke increased dispersal. However, in a habitat where other plants are expected to be investing in dispersal as well, it may be important to strengthen control over the proven mother-site and resist conspecific invasion. This may explain the smaller increase in subterranean achene production in the vacant, nutrient-rich patches.

The crowded-rich patches, on the other hand, reduce production of both achene types, mainly the aerial ones. This reduction can be attributed to higher allocation of resources to the root system, to increase competitive ability. Such root system proliferation as a result of intra-specific density has been shown before (e.g., Gersani et al. 2001; Weigelt et al. 2005). Investment in subterranean achenes would intensify sib–sib competition, which could be fiercer than conspecific competition among individuals that are unrelated (Cheplick and Kane 2004). Therefore, subterranean achene production is reduced. If conspecific density within the patch reliably reflects that of the general habitat, the probability of successful dispersal should drop, thus explaining the considerable reduction in aerial achene production.

In both nutrient-poor cases, aerial achene production is low and relatively rigid, since the general habitat is sensed as hostile for dispersal. This rigidity is *not* displayed in subterranean achene production, where plasticity does occur: in vacant-poor patches, subterranean achene production is stronger than in the crowded-poor patches. This, again, can be attributed to fierce root competition that is magnified by the low levels of available nutrients and diverts resource allocation. In a nutrient-poor environment, where the risk of dispersal is high, a plant growing in a sparsely inhabited patch invests more in subterranean achenes. By doing so it increases the probability of its descendants dominating the patch. In contrast, when the patch is densely populated, root competition is much higher, and investment in subterranean achenes decreases to avoid intensification of sib–sib competition, since *E. spinosa* patches tend to include descendants of one or a few founder plants (Evenari et al. 1977).

A comparison of the levels of investment in numbers of subterranean achenes between the Ofakim and the Hadera populations reveals, besides the higher plasticity in Ofakim, another pattern that may be counter-intuitive, but supports the mother-site control hypothesis. Under low intraspecific density, the Ofakim individuals invest more in subterranean achenes than do the Hadera individuals, despite the fact that their habitat is more limited in resources, thus driving their stay-home offspring to fierce sib–sib competition. We suggest that in the spatially variable and generally hostile habitat of Ofakim, the benefits of securing the mother-site by a single individual for its offspring may outweigh the costs of increased sib–sib competition, therefore displaying a higher level of optimal investment in subterranean achenes.

Many of the amphicarpic ratio patterns found in *E. spinosa*, as in other studied amphicarpic species (reviewed in Cheplick 1994), can be explained by plant size effects. Specifically, aerial achene production is positively correlated with shoot growth, accounting for a considerable part of the amphicarpic ratio shift. However, our results show that when controlling for the effect of ontogenetic drift, “true” plasticity is still evident. Moreover, shifts in subterranean achene production and allocation were independent of shoot size, indicating that they are driven by true plasticity. Furthermore, physiologically simple, proximate explanations, such as ontogenetic drift can be viewed as mechanisms through which evolutionary strategies are “hard-wired” into an organism (Mangel and Clark 1988). In *E. spinosa*, there is a coupling of aerial flowering to shoot vegetative growth, which is not a characteristic of all annuals. Such co-development of floral and vegetative organs transforms reproduction from a single committing event into a series of short-term events based on continuously updated cues from the surroundings (see also Cohen 1971). This “step-by-step” strategy may enable plants to better predict and respond to within-season environmental variations and thus plays an important role in the survival of plants growing under such conditions.

In conclusion, we bring evidence for the integration of adaptive strategies for coping with environmental variability, consistent with recent theoretical development in evolutionary biology (DeWitt and Langerhans 2004) and point out specific ecological mechanisms that drive the observed phenotype shifts. We suggest that this concept should be a useful framework that will potentially improve the general understanding of life-history strategies of organisms inhabiting variable environments.

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