



Maternal effects alter progeny's response to disturbance and nutrients in two *Plantago* species

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Environmental stress leading to a decrease in growth may be compensated for later in ontogeny by a growth plastic response. Such response could be also transmitted to the next generation, which is called transgenerational plasticity. In this study, two *Plantago* species were used to test whether compensation for biomass loss after disturbance is driven by maternal effects (ME) due to nutrients and disturbance, i.e. by the form of transgenerational plasticity. Additionally, we tested whether ME could contribute to a different performance of progeny having different disturbance histories. We also tested whether ME are adaptive and whether they differ between related species.

The degree of (over)compensation for biomass loss was affected by ME. Maternal effects resulted in different performance of disturbed over undisturbed progeny in relation to nutrient status of the progeny environment along with disturbance and nutrients experienced by mothers. Progeny of *P. lanceolata* grew more leaf biomass when grown in the same nutrient conditions as experienced by their mothers suggesting that maternal effects might be adaptive. Although overall, there was a consistent role of ME in biomass compensation after disturbance among the two species, there were also some species-specific effects.

We conclude that compensation for biomass loss is driven both by maternal effects and by progeny environment. This may lead to the different success of regenerative strategies in environments with contrasting nutrient levels. The different role of ME even between related species may contribute to ecological diversity among species.

Plants and animals may adjust their phenotype in response to biotic and abiotic changes. This is known as a phenotypic plasticity. During the past three decades it has been repeatedly shown that the phenotype expressed by an individual can also be affected by the parental environment (reviewed by Jablonka and Raz 2009), which is called transgenerational plasticity. Maternal effects (ME) often present an important form of this plasticity.

Maternal effects are the case when the phenotype of progeny is determined by maternal environment. ME may be mediated through the embryo via storage reserves, hormones, enzymes, toxins and alike (Roach and Wulff 1987, Rossiter 1996, Huxman et al. 2001). Such mediated ME should notably influence early stages of ontogenic development being less relevant during maturity as other environmental factors outweigh the importance of storage (Breen and Richards 2008). Epigenetic mechanisms may also affect progeny phenotype (Rossiter 1996, Bossdorf et al. 2008) due to changes in gene expression that are not conditioned by changes in DNA sequence (Richards 2006, Bird 2007). This could be facilitated by reversible enzymatic methylation of cytosine in the DNA, modification in chromatin structure or by regulatory processes associated with small RNA molecules (Wagner 2003, Vanyushin 2006).

Maternal effects are considered as an important factor contributing to population dynamics and evolution. For instance, Plaistow and Benton (2009) showed that high food availability in parents of soil mites *Sancassania berlessei* affects their offspring's reproduction, which has an impact on population growth rate. Bertin et al. (2009) showed that disturbance caused by human activity experienced by mothers of Japanese quail *Coturnix japonica* affects the behavior of their offspring, altering thus whole population structure and dynamics. Different food quality experienced by parents and grandparents could be responsible for the varying risk for cardiovascular and diabetes mellitus mortality in humans (Kaati et al. 2002). Light conditions experienced by maternal plants of *Campanulastrum americanum* affect life cycle of their descendants resulting in better performance of progeny growing in maternal light conditions (Galloway and Etterson 2007).

Plants may overcome loss of above-ground tissue due to disturbance (e.g. mowing or grazing) by compensatory or even overcompensatory growth (Owen and Wiegert 1976). In other words, they may alleviate the negative consequences of disturbance and perform equally or better than undisturbed individuals. This is mediated by plastic changes in photosynthetic rate, longevity of remaining biomass, resource allocation

patterns and resprouting from reserve buds (Maschinski and Whitham 1989, Trumble et al. 1993, Strauss and Agrawal 1999). The degree of compensation, although expected to be higher under lower environmental stress (Oosterheld and McNaughton 1991, Strauss and Agrawal 1999, Huhta et al. 2000), was found also to be context specific (Wise and Abrahamson 2007). This unexplained result could be, however, modulated, among other reasons, by maternal effects.

In our recent study on offspring of two *Plantago* species (Latzel et al. 2009), we demonstrated that maternal effects due to nutrient availability and/or disturbance shape the phenotype of progeny. The response of progeny to differently productive environments was altered by the disturbance regime experienced by their mothers (Latzel et al. 2009). In addition, nutrient shortage experienced by mothers resulted in a higher photosynthetic rate, longer leaves and higher fitness of the progeny in the year of their establishment (Latzel et al. 2009). We proposed that epigenetics originated in response to stress by mothers (i.e. disturbance and nutrient shortage) and/or inherited resource allocation patterns towards photosynthesis might be responsible for the observed effects (Latzel et al. 2009).

Considering the fact that ME may alter the photosynthesis and growth of progeny (Latzel et al. 2009), which are parameters potentially important in compensatory growth, we hypothesize that (1) the degree of compensation for biomass loss depends not only on the progeny's environment but also on maternal nutrient availability and/or disturbance history and that (2) an increase in photosynthetic rate is the mechanism responsible for biomass compensation. We also hypothesize that (3) maternal effects are adaptive, i.e. progeny performs better in maternal than non-maternal environments.

It has been demonstrated that the species regenerating mainly vegetatively after severe disturbance (referred to as resprouters in the text) are favored in nutrient poor environments while the species regenerating only from seeds after severe disturbance (referred to as seeders in the text) are favored in nutrient rich environments (Iwasa and Kubo 1997, Bellingham and Sparrow 2000, García and Zamora 2003, Buhk et al. 2007). However, the mechanisms for different performance of seeders and resprouters in differently productive environments are poorly understood. In light of our previous study (Latzel et al. 2009) illustrating different progeny's performance in relation to the disturbance history of their mothers we also hypothesize that (4) different ME due to disturbance and nutrients are responsible for different performance of (un)disturbed progeny.

There is only limited knowledge about different ecological aspects of maternal effects between species (Fox et al. 1997, Donohue and Schmitt 1998) and comparative experiments testing key environmental factors are lacking (Sultan et al. 2009). Hence, we compared the role of ME due to nutrients and disturbance between selected plantains and hypothesize that (5) maternal effects due to nutrients and disturbance alter growth of progeny differently in selected species.

To summarize we tested the following hypotheses: H1 - the degree of compensation for biomass loss depends on ME. H2 - an increase in photosynthetic rate is the mechanism responsible for biomass compensation. H3 - maternal effects are adaptive. H4 - ME are responsible for different performance of (un)disturbed progeny. H5 - ME differ among related species.

To test the hypotheses we established a long term experiment (Latzel et al. 2009) using two plantain species – *Plantago lanceolata* and *P. media*. These species are facultative resprouters, i.e. regenerate from seeds as well as roots after severe disturbance (Klimešová and Klimeš 2006) allowing thus comparisons of different regenerative strategies within a species.

Mother plants were grown in either undisturbed or severely disturbed environments that were either nutrient poor or nutrient rich. Half of the progeny of disturbed and undisturbed mothers, respectively, were severely disturbed during their second year of life. Using a full-factorial design, we measured the effect of maternal nutrient availability and disturbance history in relation to progeny nutrient availability and disturbance on the aboveground biomass and photosynthesis of the progeny.

Material and methods

Design of the study

Maternal plants

To avoid undesirable carry-over environmental effects on species performance, we used seeds from a commercial production garden where more than 10 000 plants had been cultivated for over ten years in similar conditions without severe disturbance events or nutrient limitation (Planta Naturalis, Czech Republic). At the beginning of the experiment, plants of mothers' line (80 per species) were cultivated in nutrient poor (40 plants per species) and nutrient rich conditions (40 plants per species) in 2005 and 2006 (see Fig. 1 and Latzel et al. 2009 for details). Half of the plants from both nutrient levels (i.e. 20 plants from nutrient poor and 20 plants from nutrient rich conditions) were severely disturbed in May 2006 – each plant was removed from a pot and its tap root was cut into a single 8 cm fragment with the shoot base and apical end removed. The root fragment was subsequently replanted in a pot containing same nutrients as prior to disturbance. Pollination was not controlled and ripe seeds were continuously collected during the 2006 season and used for the establishment of offspring in 2008.

Progeny plants

On 28 April 2008, seeds of a subgroup of five randomly selected mother plants from every treatment group (four treatments – nutrient poor/rich environment × disturbed/undisturbed mother) and species were chosen (Fig. 1). From those seeds, 20 offspring of each mother plant were grown (i.e. 100 progeny individuals from each maternal treatment group and species). The offspring of each mother plant was divided in half into either nutrient rich or nutrient poor conditions. Hence, half of the offspring experienced a nutrient level that differed to that of the mother plant and 800 plants were grown in total.

On 27 April 2009, half of the randomly selected plants (400) chosen across treatments and species were severely disturbed by clipping off the aboveground biomass at the shoot base. Therefore, half of the offspring of mothers that experienced disturbance were disturbed and half of the offspring of undisturbed mothers were disturbed as well. Thus,

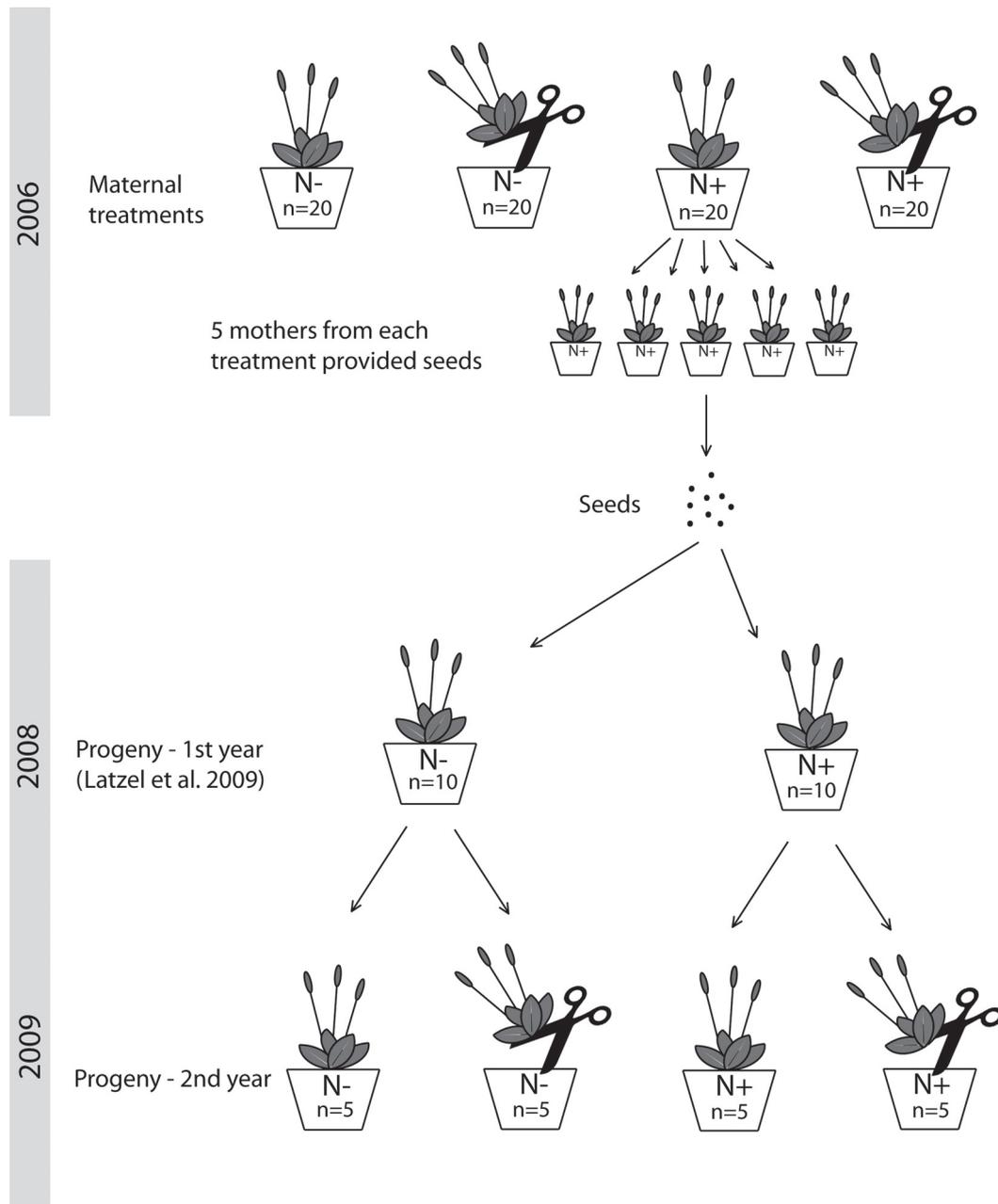


Figure 1. Scheme of experimental design of the study. N- low nutrients, N+ high nutrients.

the experiment consisted overall of 16 different treatment groups (four maternal treatments \times four progeny treatments in full factorial design, Fig. 1).

Plant material

All plants were grown separately in pots ($15 \times 15 \times 20$ cm) that were placed into shallow pits filled with sand to avoid frost damage during overwintering and to maintain homogeneous conditions among individuals. A mixture of commercial soil substrate and sand in a 2:3 ratio was used. The nutrient rich group was fertilized with slow-release fertilizer three times per season by adding 1.2 g N, 0.80 g P and 0.57 g K per plant and season. No fertilizer was added to the nutrient poor group. Plants were reared individually and treatments were randomized in cultivation.

Measurements

Overwintering survival between seasons 2008 and 2009 was recorded. Resprouting success was monitored in all disturbed plants after the disturbance event in 2009.

Net CO_2 assimilation rate (photosynthesis) and stomatal conductivity of *P. lanceolata* leaves was measured using a portable photosynthesis system equipped with a standard 6 cm^2 leaf chamber. Due to the small leaf size of *P. media*, a non-destructive measurement was not possible and thus, this species was not measured. Measurements were performed on 22 and 23 July 2009. We randomly selected eight individuals per treatment combination and performed the measurements on a single, fully developed non-senescent intact leaf. The chamber conditions were the following: CO_2 concentration of 370 ppm, leaf temperature of $25.0 \pm 0.5^\circ\text{C}$, air flow rate

of 500 $\mu\text{mol s}^{-1}$ and mean relative humidity of 66%. A built-in LED light source maintained a saturating photosynthetic active radiation irradiance at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Each measurement took about 150 s, which allowed for the stabilization of CO_2 exchange. We expressed the light-saturated photosynthetic rate (i.e. photosynthetic capacity) on a per leaf area basis.

All plants were randomly harvested between 4 and 14 August 2009 and aboveground dry biomass was divided into leaf biomass and spike biomass. Leaves were dried at 80°C for 24 h, spikes were dried in the greenhouse for two weeks (average temperature 28°C). For assessment of compensatory growth only biomass at harvest was considered, i.e. when final biomass of disturbed versus undisturbed plants did not differ, this was considered as equal compensation.

Statistical analyses

The effect of maternal (maternal nutrients, MN) and progeny nutrient conditions (progeny nutrients, PN), disturbance of mother plants (maternal disturbance, MD) and disturbance of progeny (progeny disturbance, PD) on the overwintering capacity and regeneration of disturbed plants was analyzed using a generalized mixed effect model with assumed binomial distribution of the dependent variable (regenerated: yes/no). Maternal plant identity was a random factor whereas species identity, maternal and progeny treatments were crossed fixed effects in the model.

A linear mixed effect model was used to test the effects of all treatments on the aboveground dry biomass of progeny and net photosynthetic rate of *P. lanceolata*. Included random effect of mother plant created a split-plot structure of the model, reflecting dependence of the offspring coming from particular mother plant. Maternal as well as progeny treatments were defined as fixed effects in the model. Analyses were performed on a combined dataset as well as for individual species separately. To meet the assumptions of homoscedasticity and normality, all variables were log transformed prior to analyses. Linear mixed-effect models were estimated using nlme package and generalized mixed-effect models with the lme4 package in the R program (2008). All treatment interactions were included into the statistical model - the treatment interactions considered as the most relevant to our hypotheses are marked in the Table 1.

In case of significant interaction terms, post-hoc test (unequal N HSD) was performed to demonstrate which differences between means of individual treatment combinations are significant. These post-hoc tests were performed in Statistica 8 software (StatSoft Inc. 2008) using the same mixed effect model definitions as specified in R program, where implementation limitations prevented correct performance of post-hoc tests.

Results

Some mortality occurred during overwintering in 2009: 26 individuals of *P. lanceolata* and 19 of *P. media* out of 780 (20 plants excluded from the study due to herbivore damage in 2008) individuals did not survive the winter. Neither

disturbance nor nutrient level experienced by mothers or offspring had a significant effect on this (data not shown).

Resprouting success after disturbance

There was no effect of maternal disturbance or maternal and offspring nutrient level on regeneration of disturbed plants in the cumulative or individual species datasets. Forty-one out of 200 disturbed plants of *P. lanceolata* and 12 out of 183 disturbed individuals of *P. media* failed to regenerate (data not shown).

Disturbance – compensation for biomass loss

Progeny of *P. lanceolata* grew more leaves if their mothers were disturbed as compared to progeny of undisturbed mothers (MD, Table 1) but this was not the case in *P. media* (S×MD, Table 1).

Disturbed progeny of both species overcompensated for leaf biomass loss (PD, Table 1) but did not compensate for total aboveground biomass or spike biomass loss. The degree of compensation for total and spike biomass loss was significantly affected by the nutrient level in the progeny environment (PN × PD, Table 1) both in the combined dataset as well as for the individual species data sets (*P. lanceolata* marginally significant for spike biomass, $p = 0.062$). No significant differences between species were apparent (S × N × PD, Table 1). Undisturbed plants produced more above-ground biomass in nutrient poor conditions when compared to disturbed ones (post-hoc test, $p = 0.044$, Fig. 2). There were no significant differences in biomass production in nutrient rich conditions between disturbed and undisturbed plants (Fig. 2).

Overcompensation for leaf biomass loss was marginally significantly (combined dataset, $p = 0.056$) or significantly (*P. media*) affected by nutrients and disturbance experienced by mother plants (interaction MN × MD × PD, Table 1, Fig. 3). There were no significant differences between species (S × MN × MD × PD). In the combined dataset, disturbed progeny overcompensated for leaf biomass if the mother was undisturbed and grown in nutrient poor conditions (post-hoc test, $p = 0.028$) or if the mother was disturbed and grown in nutrient rich conditions ($p < 0.0001$). In other maternal conditions disturbed progeny only compensated for leaf biomass (Fig. 3). The same trend was apparent if *P. media* was considered alone. Post hoc comparisons revealed that significant overcompensation of disturbed over undisturbed progeny occurred only for disturbed progeny of undisturbed mothers grown in nutrient poor conditions and undisturbed progeny of disturbed mothers grown in nutrient rich conditions (post-hoc test, $p = 0.034$).

Nutrient effect

A higher nutrient level enhanced above ground biomass production of progeny (PN, Table 1). However, the effect of nutrients was conditioned by disturbance and nutrients experienced by mothers (total biomass and spike biomass, MN × MD × PN, Table 1, combined dataset, Fig. 4). Post-hoc comparisons in the combined dataset revealed that progeny produced marginally significantly ($p = 0.075$) more above-ground biomass in nutrient poor conditions if

Table 1. Effects of maternal and progeny nutrient levels and maternal and progeny disturbance on the performance of *P. lanceolata* and *P. media* progeny. Results for combined (both species included) and individual species datasets. ANOVA in general linear models, Statistical significance – # $p < 0.065$, * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $0.001 > p$, ns = $p > 0.05$. Error of DF for each significant level is presented in parentheses – 1 = 32, 2 = 625, 3 = 301, 4 = 16, 5 = 324. In brackets are labeled the hypotheses relevant to a given treatments' interaction. For the number of hypothesis see Introduction.

| | Combined dataset | | | | | | | | | | <i>P. lanceolata</i> | | | | | <i>P. media</i> | | | | | | | | | |
|--------------------------------|------------------|---------|--------------|---------|---------------|---------|---------------|--------|--------------|---------|----------------------|---------|---------------|---------|--------------|-----------------|---------------|---------|---------------|-----|--------------|-----|---------------|-----|--|
| | Total biomass | | Leaf biomass | | Spike biomass | | Total biomass | | Leaf biomass | | Spike biomass | | Total biomass | | Leaf biomass | | Spike biomass | | Total biomass | | Leaf biomass | | Spike biomass | | |
| | ns | *** | ns | *** | ns | *** | ns | *** | ns | *** | ns | *** | ns | *** | ns | *** | ns | *** | ns | *** | ns | *** | ns | *** | |
| Maternal nutrients (MN) | 775.1737 | *** (2) | 291.51216 | ** (2) | 229.83375 | *** (2) | 432.6878 | ** (3) | 239.08333 | *** (3) | 164.24598 | ** (3) | 346.2799 | *** (5) | 107.78885 | *** (5) | 74.88806 | *** (5) | | | | | | | |
| Progeny nutrients (PN) | ns | | ns | | ns | | ns | | 5.24849 | # (4) | ns | | ns | | ns | | ns | | | | | | | | |
| Maternal disturbance (MD) [H4] | 5.5483 | # (2) | 19.06713 | *** (2) | 34.21644 | *** (2) | ns | | 17.57784 | *** (3) | 23.07304 | *** (3) | 3.3459 | # (5) | 6.43833 | # (5) | 12.31419 | *** (5) | | | | | | | |
| Progeny disturbance (PD) | ns | | ns | | ns | | ns | | 5.98331 | # (3) | ns | | ns | | ns | | ns | | | | | | | | |
| MN × PN [H3] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| MN × MD [H4] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| MD × PN [H4] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| MN × PD [H4] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| PN × PD [H1] | 4.3497 | # (2) | ns | | 6.76582 | ** (2) | ns | | ns | | 3.51770 | # (3) | 6.1485 | # (5) | ns | | 3.41989 | # (5) | | | | | | | |
| MD × PD [H3, H4] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| MN × MD × PN [H4] | 6.9228 | ** (2) | ns | | 4.54835 | # (2) | ns | | ns | | 3.50990 | # (3) | 10.3453 | *** (5) | ns | | 3.74231 | # (5) | | | | | | | |
| MN × PN × PD [H4] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| MN × MD × PD [H1, H4] | ns | | 3.67518 | # (2) | ns | | ns | | ns | | ns | | ns | | 4.82998 | # (5) | ns | | | | | | | | |
| MD × PN × PD [H4] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| MN × MD × PN × PD [H3, H4] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| Species (S) | 10.1968 | ** (1) | 4.72482 | # (1) | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MN | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × PN | ns | | ns | | 9.77592 | ** (2) | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MD | ns | | 5.35914 | # (1) | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × PD | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MN × PN [H5] | ns | | 4.14099 | # (2) | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MN × MD [H5] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MD × PN | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MN × PD [H5] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × PN × PD | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MD × PD | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MN × MD × PN [H5] | 3.8656 | # (2) | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MN × PN × PD [H5] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MN × MD × PD [H5] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MD × PN × PD | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |
| S × MN × MD × PN × PD [H5] | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | ns | | | | | | | | |

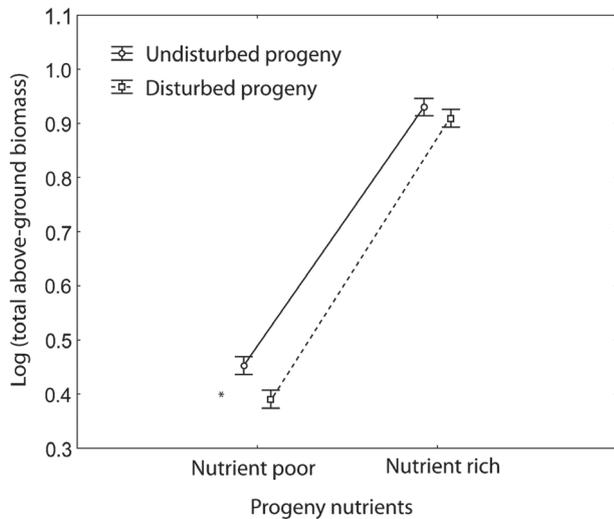


Figure 2. Above-ground biomass of disturbed and undisturbed progeny of both species in relation to nutrients of the progeny environment (PN×PD, for significance test see Table 1, means and SE are shown, significant post-hoc contrasts are marked: # $p < 0.1$, * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $0.001 > p$).

their mother was also grown in nutrient poor conditions and did not experience disturbance as compared to progeny of undisturbed mothers grown in nutrient rich conditions (Fig. 4A). No other significant differences were found. Despite the fact that the interaction was significant in the

combined dataset the results should be attributed mainly to *P. media* as the progeny nutrient level in relation to maternal conditions showed no significant effect in *P. lanceolata* ($S \times MN \times MD \times PN$, Table 1). In the case of spike biomass, a post-hoc test on the combined dataset showed a marginally significant ($p = 0.077$) higher spike production by progeny grown in nutrient poor conditions if mothers experienced disturbance when compared to progeny of undisturbed mothers (Fig. 4B). Such pattern did not differ between species ($S \times MN \times MD \times PN$, Table 1).

Progeny nutrients also affected leaf biomass in *P. lanceolata* differently in relation to nutrient status of mothers (MN × PN, Fig. 6) but this was not the case for *P. media* ($S \times MN \times PN$). Progeny of *P. lanceolata* produced more leaf biomass if it was grown in nutrient rich conditions and mothers were also grown in nutrient rich conditions ($p = 0.017$). If progeny was grown in nutrient poor conditions, an opposite trend was apparent (i.e. progeny of mothers from nutrient poor conditions produced more spikes) but a post-hoc test revealed this effect to be not significant ($p = 0.23$).

Photosynthesis – *P. lanceolata*

Overall, there were no significant differences in photosynthetic rate between individual treatments. However, some nearly significant differences were identified. On average, disturbed progeny had a marginally significantly lower photosynthetic rate than undisturbed progeny (Table 2). The photosynthetic rate of progeny was marginally significantly affected by dis-

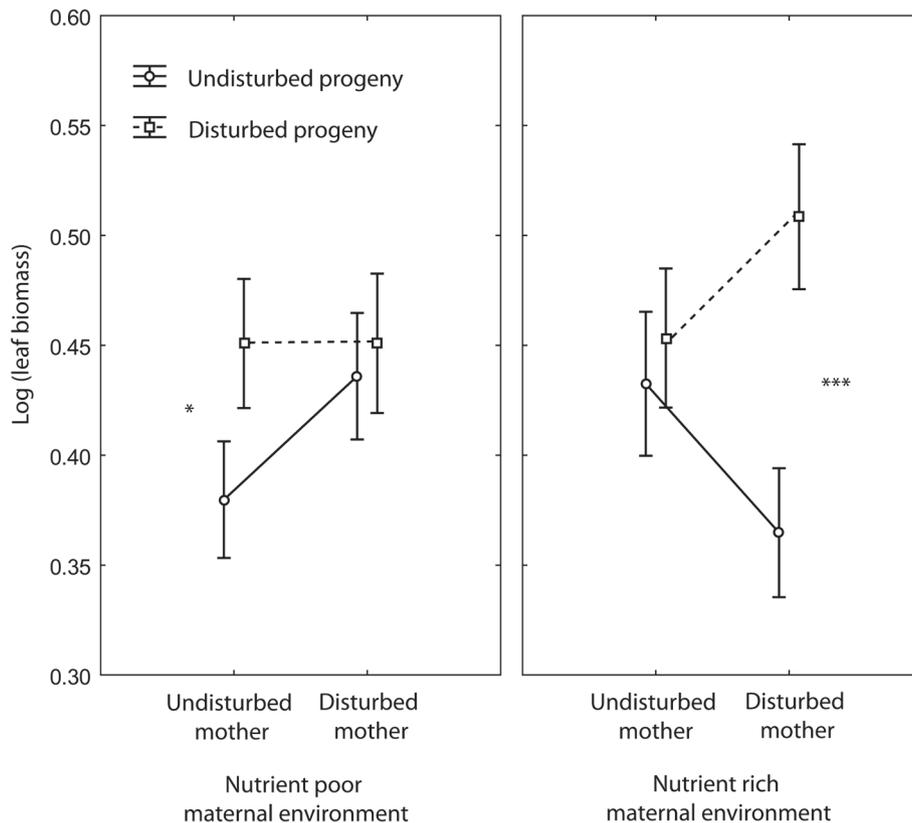


Figure 3. Leaf biomass of disturbed and undisturbed progeny of both species in relation to maternal nutrient levels and disturbance (MN×MD×PD, for significance test see Table 1, means and SE are shown, significant post-hoc contrasts are marked: # $p < 0.1$, * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $0.001 > p$).

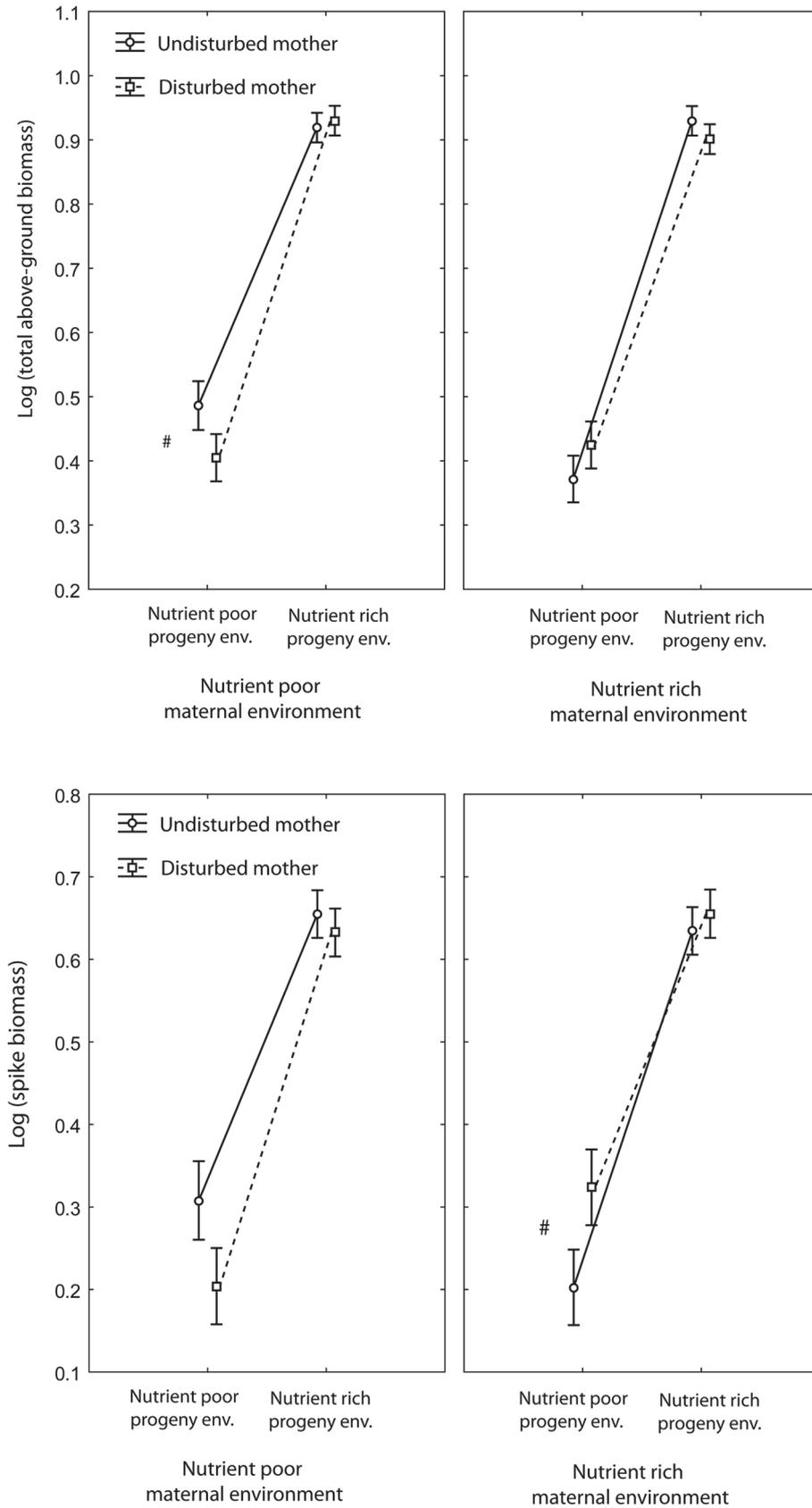


Figure 4. Above-ground biomass (A) and spike biomass (B) of both species in relation to nutrients of maternal and progeny environment and maternal disturbance (MN×MD×PN, for significance test see Table 1, means and SE are shown, significant post-hoc contrasts are marked: # $p < 0.1$, * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $0.001 > p$).

Table 2. Effects of maternal and progeny nutrient levels and maternal and progeny disturbance on photosynthetic rate of *P. lanceolata*. General linear models, ANOVA, only marginally significant interactions are presented. Mean values, \pm SE and significance are shown. MN: maternal nutrient levels, PN: progeny nutrient levels, MD: maternal disturbance level, PD: progeny disturbance level. Error of Df for each significant level is presented in parentheses – 1 = 96, 2 = 16.

| Interaction | MN | PN | MD | PD | Net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) | ANOVA test |
|-------------------------------|------|------|-------------|-------------|---|----------------------|
| PD(1) | | | | | | |
| | ... | ... | ... | undisturbed | 9.552 \pm 0.359 | F = 2.984, p = 0.087 |
| | ... | ... | ... | disturbed | 8.701 \pm 0.315 | |
| MN \times MD(2) | | | | | | |
| | low | ... | undisturbed | ... | 8.920 \pm 0.475 | F = 4.306, p = 0.055 |
| | low | ... | disturbed | ... | 9.195 \pm 0.551 | |
| | high | ... | undisturbed | ... | 10.060 \pm 0.488 | |
| | high | ... | disturbed | ... | 8.333 \pm 0.374 | |
| MN \times PN \times PD(1) | | | | | | |
| | low | low | ... | undisturbed | 10.824 \pm 0.828 | F = 3.795, p = 0.054 |
| | low | low | ... | disturbed | 7.669 \pm 0.652 | |
| | low | high | ... | undisturbed | 9.005 \pm 0.702 | |
| | low | high | ... | disturbed | 8.721 \pm 0.477 | |
| | high | low | ... | undisturbed | 9.356 \pm 0.720 | |
| | high | low | ... | disturbed | 9.848 \pm 0.704 | |
| | high | high | ... | undisturbed | 8.990 \pm 0.567 | |
| | high | high | ... | disturbed | 8.574 \pm 0.608 | |

turbance and nutrients experienced by mother plants (MN \times MD, Table 2). Post-hoc tests revealed that progeny had a marginally significantly higher photosynthetic rate if their mother was kept undisturbed and grown in nutrient rich conditions in comparison to progeny of disturbed mothers grown in nutrient rich conditions ($p = 0.070$). Photosynthesis of progeny was also nearly significantly affected by nutrients experienced by mothers and disturbance and nutrients experienced by progeny (MN \times PN \times PD, Table 2). Post-hoc tests showed that the only significant differences in photosynthesis are between disturbed (lower photosynthetic rate) and undisturbed (higher photosynthetic rate) progeny if their mothers did not experience disturbance and were grown in nutrient poor conditions ($p = 0.018$, Table 2).

Discussion

Our study demonstrates that the degree of compensation for biomass loss was affected not only by nutrients experienced by progeny but also by nutrients and disturbance experienced by mother plants, i.e. by maternal effects. Contrary to our expectations, higher photosynthesis was not the mechanism responsible for compensation. The growth of *P. lanceolata* supported our hypothesis that ME may be adaptive, as progeny showed enhanced growth in maternal conditions. The results also support our hypothesis that ME play a role in shaping the regenerative strategies in recurrently disturbed habitats and that ME due to nutrients or disturbance may play different roles between related species.

Maternal effects and compensatory re-growth

Although neither species compensated for spike production and total above-ground biomass after disturbance, they over-compensated for leaf biomass. Our study demonstrates for the first time that the degree of compensation for biomass

loss is driven both by the maternal as well as by the progeny environment. We showed that (over)compensatory growth of disturbed plants was affected not only by current nutrient availability (the more nutrients the more compensatory growth; see also Huhta et al. 2000, Latzel and Klimešová 2009), but also by maternal nutrients and disturbance history (Fig. 3). This aspect of ME is potentially important in the ecology of disturbed communities and might be partly responsible for nonconsistent results concerning nutrient availability and compensatory growth. This is also supported by a recent study by Bossdorf et al. (2009) showing that ME due to disturbance and nutrients may influence competition between species.

In contrast to other studies (Whitfield et al. 1980, Trumble et al. 1993, Mabry and Wayne 1997, Thomson et al. 2003, Schutz et al. 2009), compensatory growth of disturbed plants was not facilitated by increased photosynthetic activity. It is possible that other mechanisms such as changes in resource allocation patterns (e.g. higher above ground tissue production at the expense of root growth) or increased branching patterns that could be affected by maternal as well as by offspring conditions played a role in the compensation for biomass loss in our study.

Are maternal effects adaptive?

Maternal effects can be adaptive if the progeny has higher fitness in its maternal environment (Galloway 2005). Nevertheless, adaptiveness of ME should be considered in the mother-offspring context (Marshall and Uller 2007) and studies may fail to properly identify adaptive ME when consider only limited components of ME on offspring performance (see discussion in Plaistow et al. 2007). Some studies, notably those engaged in invertebrates and plants, have demonstrated adaptive maternal effects (Fox et al. 1997, Donohue and Schmitt 1998, Agrawal et al. 1999, Galloway and Etterson 2007, Allen et al. 2008, Sultan et al.

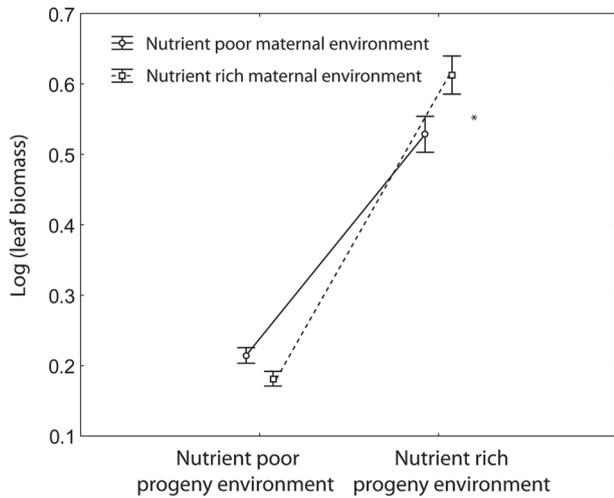


Figure 5. Leaf biomass of *P. lanceolata* progeny in relation to maternal and progeny nutrient levels (MN×PN, for significance test see Table 1, means and SE are shown, significant post-hoc contrasts are marked: # $p < 0.1$, * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $0.001 > p$). Resulted pattern is independent on disturbance experienced by progeny.

2009). Our study provides some evidence that ME may be adaptive for *P. lanceolata* since progeny produced more leaf biomass when grown in the same conditions of their mother (Fig. 5).

Interestingly, better performance of progeny in its maternal environment was not observed in the first year of the study (Latzel et al. 2009). In the year of progeny establishment there was a strong effect of nutrient shortage experienced by mothers seen as a higher photosynthetic rate, higher production of spikes and leaves both in maternal as well as non-maternal environments (Latzel et al. 2009). Such results implied that ME were not adaptive, which agreed with the studies by Sultan (1996) on plants and Laugen et al. (2005) on animals showing pronounced effects of ME when resources are limited. However, the progeny performance changed in the second year when a better performance of progeny was limited to its maternal environment (nutrients). This is in agreement with our suggestion that an interaction of different underlying mechanisms governing ME (e.g. seeds quality and epigenetics), or costs associated with ME, might be responsible for changing ME expression during a plant's lifetime.

Galloway (2005) suggested that if seeds fall into a non-maternal environment, ME may disadvantage progeny, however, fitness should be reduced only during a single generation. Plastic responses of individuals in a new environment will result in the appropriate ME in the next generation (Galloway 2005). This could be particularly the case for species with short life cycles such are annuals or biennials, but not for perennials. As we demonstrated, ME in perennials may affect offspring performance differently during their ontogeny having a different role in their establishment phase as compared to their maturity. We suggest that the changing effect of ME during ontogeny could be a mechanism to buffer environmental stochasticity on a longer time scale.

Even though ME in our study had a weaker effect on progeny fitness than showed by Galloway and Etterson

(2007), who documented more than three times higher progeny fitness in their maternal environment, ME may still play a significant role in population dynamics and evolution of *Plantago*. Despite the absence of physical barriers and presence of gene flow, the spatial distribution of plants could be affected by ME since progeny grows better in its maternal environment. Even in a case scenario where seeds from distant populations have the same probability to reach a site as seeds from the maternal population, local progeny will be highly probably in advantage also due to ME. Such aspect would allow for phenotypic adaptation to local conditions and ME may alter the evolutionary dynamics of gene frequencies in populations. However, our study also pointed out on some difficulties in estimation of the role of ME in the evolution of populations of long lived organisms as the role of ME could be changing during life span. The effect of ME on population dynamics in the year of the individual establishment was different (Latzel et al. 2009) compared to the second year. Such changing expression of ME clearly demonstrates that the analysis of a single season provides only a snapshot in time and long term studies are needed to obtain a more complex understanding of the role of maternal effects in evolution.

The role of maternal effects on regenerative strategies

When the disturbance event in the progeny generation is not considered, it is apparent that the growth of the progeny could differ in relation to the regenerative strategy of its mother and nutrient status of maternal and progeny environment (Fig. 4). Such ME demonstrate that progeny of seeders (mothers regenerated from seeds only) and progeny of resprouters (mothers regenerated vegetatively) may have different success in differently productive environments.

Moreover, the performance of disturbed and undisturbed progeny differed in relation to the disturbance history and nutrient status of their mothers (Fig. 3) demonstrating thus that ME may contribute to different success of diverse regenerative strategies in differently productive environments. Leaf biomass of disturbed and undisturbed progeny remarkably differed according to the disturbance history of plants and maternal nutrients (Fig. 3). Such ME could favor resprouting strategies in two contrasting environments: (1) environments with limited nutrients and regular, but less frequent disturbance events (disturbance does not occur in each generation or reproductive cycle) and/or (2) in highly productive environments with regular and common disturbance events (disturbance occurs in each generation or reproductive cycle).

The first scenario could take place, for instance, in woody and shrubby communities where the prevailing occurrence of resprouting over a seeding strategy has been documented under low nutrient availability (Bloom et al. 1985, Iwasa and Kubo 1997, Bellingham and Sparrow 2000, Cruz et al. 2002, García and Zamora 2003). Disturbances (e.g. fire) may regularly occur in certain periods after accumulation of dead biomass providing material for fire. The second scenario could resemble highly productive and regularly disturbed biotopes such as managed fields or urban communities occupied mainly by annuals or short lived perennials. There

are studies demonstrating that a resprouting strategy can be more successful than a seeding strategy on such biotopes (Denslow 1985, Klimešová and Martínková 2004, Latzel et al. 2008).

Between species differences

Although ME showed mostly a consistent role among species, we identified some species specific aspects of ME. The growth of progeny was altered by disturbance history in *P. lanceolata* but not in *P. media*. Similarly, maternal nutrients in relation to progeny's nutrients altered leaf length in *P. lanceolata* but not in *P. media*. Such dissimilarity in expression of ME between plantain species showed that the extent and importance of ME differs between closely related species, and thus contributes to ecological diversity among species (Sultan et al. 2009).

Conclusion

Our study outlined that transgenerational plasticity may alter the response of plants to disturbance affecting thus ecology of disturbed communities. Despite the experimental constraints of the study, we pointed out some possible aspects of maternal effects in sessile organisms that should be investigated in more detail in natural conditions and in other species. We conclude that if the observed ME are common in plants, such maternal effects could be applicable in agriculture or restoration ecology. For example, the varying ability of plants to compensate for biomass loss in relation to disturbance history of their mothers should be regarded in preparation of seeds for meadows or for restoration of disturbed communities. The effect of higher growth, photosynthesis and seed production of progeny due to nutrient poor maternal conditions in the first year of progeny life (Latzel et al. 2009) could be used to reach better yields in annual crops.

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References

Allen, R. M. et al. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. – *Am. Nat.* 171: 225–237.

Agrawal, A. A. et al. 1999. Transgenerational induction of defences in animals and plants. – *Nature* 401: 60–63.

Bellingham, P. J. and Sparrow, A. D. 2000. Resprouting as a life history strategy in woody plant communities. – *Oikos* 89: 409–416.

Bertin, A. et al. 2009. Trans-generational influence of human disturbances in Japanese quail: egg quality influences male social and sexual behaviour. – *Ethology* 115: 879–887.

Bird, A. 2007. Perceptions of epigenetics. – *Nature* 447: 396–398.

Bloom, A. J. et al. 1985. Resources limitation in plants – an economic analogy. – *Annu. Rev. Ecol. Evol. Syst.* 16: 363–392.

Bossdorf, O. et al. 2008. Epigenetics for ecologists. – *Ecol. Lett.* – 11: 106–115.

Bossdorf, O. et al. 2009. Genotype and maternal environment affect belowground interactions between *Arabidopsis thaliana* and its competitors. – *Oikos* 10: 1541–1551.

Breen, A. N. and Richards, J. H. 2008. Irrigation and fertilization effects on seed number, size, germination and seedling growth: implications for desert shrub establishment. – *Oecologia* 157: 13–19.

Buhk, C. et al. 2007. The challenge of plant regeneration after fire in the Mediterranean Basin: scientific gaps in our knowledge on plant strategies and evolution of traits. – *Plant Ecol.* 192: 1–19.

Cruz, A. et al. 2002. Resprouting in the Mediterranean-type shrub *Erica australis* affected by soil resource availability. – *J. Veg. Sci.* 13: 641–650.

Donohue, K. and Schmitt, J. 1998. Maternal environmental effects in plants: adaptive plasticity? – In: Mousseau, T. A. and Fox, C. W. (eds), *Maternal effects as adaptation*. Oxford Univ. Press, pp. 137–158.

Denslow, J. S. 1985. Disturbance-mediated coexistence of species. – In: Pickett, S. T. A. and White, P. S. (eds), *The ecology of natural disturbance and patch dynamics*. Academic Press, pp. 307–323.

Fox, C. W. et al. 1997. Egg size plasticity in a seed beetle: an adaptive maternal effect. – *Am. Nat.* 149: 149–163.

Galloway, L. F. 2005. Maternal effects provide phenotypic adaptation to local environmental conditions. – *New Phytol.* 166: 93–99.

Galloway, L. F. and Etterson, J. R. 2007. Transgenerational plasticity is adaptive in wild. – *Science* 318: 1134–1136.

García, D. and Zamora, R. 2003. Persistence and multiple demographic strategies in long-lived Mediterranean plants. – *J. Veg. Sci.* 14: 921–926.

Huhta, A. P. et al. 2000. A test of the compensatory continuum: fertilization increases and below-ground competition decreases the grazing tolerance of tall wormseed mustard (*Erysimum strictum*). – *Evol. Ecol.* 14: 353–372.

Huxman, T. E. et al. 2001. The effects of parental CO₂ and off-spring nutrient environment on initial growth and photosynthesis in an annual grass. – *Int. J. Plant Sci.* 162: 617–623.

Iwasa, Y. and Kubo, T. 1997. Optimal size of storage for recovery after unpredictable disturbances. – *Evol. Ecol.* 11: 41–65.

Jablonka, E. and Raz G. 2009. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. – *Q. Rev. Biol.* 84: 131–176.

Kaati, G. et al. 2002. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. – *Eur. J. Hum. Genet.* 10: 682–688.

Klimešová, J. and Martínková, J. 2004. Intermediate growth forms as a model for the study of plant clonality functioning: an example with root sprouters. – *Evol. Ecol.* 18: 669–681.

Klimešová, J. and Klimeš, L. 2006. CLO–PLA3 – a database of clonal growth architecture of Central–European plants. <www.clopla.butbn.cas.cz>.

Latzel, V. and Klimešová, J. 2009. Fitness of resprouters versus seeders in relation to nutrient availability in two *Plantago* species. – *Acta Oecol.* 35: 541–547.

Latzel, V. et al. 2008. Plant traits and regeneration of urban plant communities after disturbance: does the bud bank play any role? – *Appl. Veg. Sci.* 11: 387–394.

Latzel, V. et al. 2009. Nutrients and disturbance history in two *Plantago* species: maternal effects as a clue for observed dichotomy between resprouting and seeding strategies. – *Oikos* 118: 1669–1678.

Laugen, A. T. et al. 2005. Quantitative genetics of larval life-history traits in *Rana temporaria* in different environmental conditions. – *Genet. Res.* 86: 161–170.

- Mabry, C. M. and Wayne, P. W. 1997. Defoliation of the annual herb *Abutilon theophrasti*: mechanisms underlying reproductive compensation. – *Oecologia* 111: 225–232.
- Marshall, D. J. and Uller, T. 2007. When is a maternal effect ‘adaptive’? – *Oikos* 116: 1957–1963.
- Mashinski, J. and Whitham, T. G. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. – *Am. Nat.* 134: 1–19.
- Oosterheld, M. and McNaughton, S. J. 1991. Effect of stress and time for recovery on the amount of compensatory growth after grazing. – *Oecologia* 85: 305–313.
- Owen, D. and Wiegert, R. 1976. Do consumers maximize plant fitness. – *Oikos* 27: 488–492.
- Plaistow, S. J. and Benton, T. G. 2009. The influence of context-dependent maternal effects on population dynamics: an experimental test. – *Philos. Trans. R. Soc. B.* 364: 1049–1058.
- Plaistow, S. J. et al. 2007. How to put all your eggs in one basket: empirical patterns of offspring provisioning throughout a mother’s lifetime. – *Am. Nat.* 170: 520–529.
- Richards, E. J. 2006. Inherited epigenetic variation – revisiting soft inheritance. – *Nat. Rev. Genet.* 7: 395–401.
- Roach, D. A. and Wulff, R. D. 1987. Maternal effects in plants. – *Annu. Rev. Ecol. Syst.* 18: 209–235.
- Rossiter, M. C. 1996. Incidence and consequences of inherited environmental effects. – *Annu. Rev. Ecol. Syst.* 27: 451–476.
- Schutz, A. E. N. et al. 2009. Juggling carbon: allocation patterns of a dominant tree in a fire-prone savanna. – *Oecologia* 160: 235–246.
- Strauss, S. Y. and Agrawal, A. A. 1999. The ecology and evolution of plant tolerance to herbivory. – *Trends Ecol. Evol.* 14: 179–185.
- Sultan, S. E. 1996. Phenotypic plasticity for offspring traits in *Polygonum persicaria*. – *Ecology* 77: 1791–1807.
- Sultan, E. S. et al. 2009. Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. – *Ecology* 90: 1831–1839.
- Thomson, V. P. et al. 2003. Compensation for herbivory by *Cucumis sativus* through increased photosynthetic capacity and efficiency. – *Oecologia* 134: 167–175.
- Trumble, J. T. et al. 1993. Plant compensation for arthropod herbivory. – *Annu. Rev. Entomol.* 38: 93–119.
- Vanyushin, B. F. 2006. DNA methylation in plants. – *Curr. Topics Microbiol. Immunol.* 301: 67–122.
- Wagner, D. 2003. Chromatin regulation of plant development. – *Curr. Opin. Plant Biol.* 6: 20–28.
- Whitfield, D. M. et al. 1980. Carbon dioxide exchange in response to change of environment and to defoliation in a tobacco crop. – *Austr. J. Plant Physiol.* 7: 473–485.
- Wise, M. J. and Abrahamson, W. G. 2007. Effects of resource availability on tolerance of herbivory: a review and assessment of three opposing models. – *Am. Nat.* 169: 443–454.