Adaptive transgenerational plasticity in the perennial *Plantago lanceolata*

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Phenotypes of plants, and thus their ecology and evolution, can be affected by the environmental conditions experienced by their parents, a phenomenon called parental effects or transgenerational plasticity. However, whether such effects are just passive responses or represent a special type of adaptive plasticity remains controversial because of a lack of solid tests of their adaptive significance. Here, we investigated transgenerational effects of different nutrient environments on the productivity, carbon storage and flowering phenology of the perennial plant *Plantago lanceolata*, and whether these effects are influenced by seasonal variation in the maternal environment. We found that maternal environments significantly affected the offspring phenotype, and that plants consistently produced more biomass and had greater root carbohydrate storage if grown under the same environmental conditions as experienced by their mothers. The observed transgenerational effects were independent of the season in which seeds had matured. We therefore conclude that transgenerational effects on biomass and carbon storage in *P. lanceolata* are adaptive regardless of the season of seed maturation.

An intriguing question about maternal environmental effects is whether they represent adaptive mechanisms, i.e. they enhance plant growth and success in environments resembling maternal conditions (Galloway 2005, Galloway and Etterson 2007). Although transgenerational plasticity is frequently found in plants, and despite the obvious potential benefits of a ‘prepared phenotype’, rigorous demonstrations of ‘adaptive’ transgenerational plasticity are still surprisingly rare (Herman and Sultan 2011). The limited support for adaptive significance of transgenerational effects could either mean that adaptive effects are truly rare, or that appropriate experiments are difficult to conduct and/or the wrong traits have been measured (Marshall and Uller 2007, Herman and Sultan 2011).

As adaptive transgenerational plasticity requires some degree of predictability of environmental conditions across generations (Marshall and Uller 2007), it could be restricted to species with limited seed dispersal and dormancy, and to species growing in sufficiently stable environments. Moreover, as gene flow through pollen is usually greater than through seed (McCauley 1994), offspring of outcrossing plants should experience maternal rather than paternal environmental conditions, which means that one should expect adaptive significance particularly for maternal effects in outcrossing species (Galloway 2005).

Most previous studies that demonstrated adaptive transgenerational effects have been performed on annual species, with fitness estimated from seed production, seed mass, or seed germination (Case et al. 1996, Agrawal 2001, 2002, Galloway and Etterson 2007, Whittle et al. 2009; but see Lacey 1996 for an example with a perennial plant), or from biomass production (Sultan et al. 2009, Dyer et al. 2010). In polycarpic perennials, however, fitness estimates based on fecundity in one year can be inaccurate because of interannual variation in seed production, and because of potential tradeoffs among investment into current reproduction and future growth and reproduction (Reznick 1985, Stearns 1992). Moreover, fitness of perennials may also critically
depend on their life-span that can be strongly modified by seasonal weather variation (Roach 2003). Hence, other plant characteristics like biomass or storage could provide other important, or even more robust, estimates of overall success of polycarpic species. Plant size in particular is often correlated with competitive and long-term reproductive success (Watkinson and White 1985, Aarsen and Taylor 1992, Roach and Gampe 2004). Another important predictor of long-term growth and reproductive success of perennials might be the degree of stored carbohydrates (Garcia and Ehrlén 2002, Horibata et al. 2007). Carbohydrates stored belowground play also crucial role during rebuilding of above-ground photosynthetic tissue after overwintering. The amount of stored resources also influences the competitive ability as well as stress tolerance of plants (Smith 1969, Greub and Wedin 1971, Menke and Trlica 1983, Bloom et al. 1985, Heilmieer et al. 1986, Chapin et al. 1990, Chaparro et al. 1996). Thus, the total amounts of storage carbohydrates may provide estimates of the long-term success, and ultimately fitness, of polycarpic perennials across several seasons, and it should therefore be a valuable measure of plants success when testing for adaptive significance of transgenerational effects.

There are other factors which could potentially influence the expression of maternal effects and should therefore be taken into account in experimental studies. Since many perennials have long flowering periods this inevitably adds another dimension of maternal environmental variability, as environmental conditions during offspring formation can influence expression of transgenerational effects (Case et al. 1996, Lacey 1996, Lacey and Herr 2000) and thus also their adaptive potential. Transgenerational effects could also be to some degree genotype-specific if genotypes differ in their transgenerational plasticity (Alexander and Wulff 1985, Schmitt et al. 1992, Schmid and Dolt 1994, Andalo et al. 1998, Agrawal 2001, 2002, Riginos et al. 2007, Bossdorf et al. 2009).

Here, we conducted an experiment with the perennial herb *Plantago lanceolata*, in which we tested the adaptive significance of transgenerational effects in response to different maternal nutrient conditions. We evaluated these effects with regard to flowering phenology, biomass production and, for the first time, root carbohydrate storage. To test for potential seasonal influences on adaptive maternal effects our experiment included offspring generated in different seasons. Moreover, to get an idea of genotypic differences in maternal effects, we worked with three different genotypes. We tested the following hypothesis: offspring experiencing maternal nutrient conditions have a higher fitness in terms of biomass and carbon storage than offspring experiencing non-maternal nutrient conditions.

### Material and methods

#### Study system

*Plantago lanceolata* is an obligate outcrossing polycarpic perennial with short stems, leaf rosettes, and a taproot (which may be replaced by adventitious roots in older plants). Inflorescences occur in the form of spikes. The species is widespread in Europe, where it is found on a wide range of soil types and disturbed habitats. The life span of the species can be over 10 years (Cavers et al. 1980) but is often shorter in more stressful environments (Roach 2003). *Plantago lanceolata* regenerates vegetatively from roots after damage (Klimešová and Klimeš 2006), but it also produces a considerable number of seeds (about 600 seeds of 1–2 mg weight per plant) within each growing season (Latzel and Klimešová 2009). Seed production of *P. lanceolata* is positively correlated with the amount of root storage carbohydrates (Latzel and Klimešová unpubl.). The dispersal of seeds is very limited, usually to the close surroundings of maternal plants. We worked with three different maternal genotypes of *P. lanceolata*, which were collected from a natural population in Třeboň, Czech Republic (49°0′20.59″N, 14°46′16.56″E), with a minimum distance of 50 m between plants. We used the leaf transplantation technique of Budzianowska et al. (2004) to generate genetically identical replicates of each of these three plants.

#### Experimental design

We grew eight replicates of each of the three maternal genotypes in nutrient-poor soil, and another eight replicates of each genotype in nutrient-rich soil. In May 2009, all plants were individually planted in pots (15 × 15 × 20 cm) filled with a mixture of commercial potting substrate and sand in a 2:3 ratio. Nutrient-rich conditions were created by regular fertilisation with slow-release fertilizer (approximately 1.2 g N, 0.3 g P and 0.4 g K per plant per season). The nutrient-poor treatment consisted of just using the soil mixture without adding any fertilizer. To avoid frost damage over the winter and to ensure homogenous conditions among individuals, all pots were placed in shallow pits filled with sand, in completely randomised order. The experiment was conducted in a garden of the Institute.

In central Europe, *Plantago lanceolata* produces seeds from June until the end of September. In order to be able to examine seasonal effects of the time of seed maturation and also changing age of maternal plant on subsequent expression of maternal effects, we collected mature seeds bi-weekly during the 2010 growing season from all plants. Later, all seeds collected from the beginning of the season until 22 July 2010 were lumped together and classified as early-season offspring, while seeds collected after 22 July were classified as late-season. This classification was based on climatic data from the Třeboň meteorological station, located 3 km from the experimental garden, which showed two distinct temperature periods in the summer: during early summer (1 June–22 July) the average day temperature (24 h) was 19.5°C and maximal day (24 h) average temperature was 27°C (16 July 2010). In contrast, in late summer (23 July–22 Sept), the average day temperature was 15.4°C and maximal day average temperature was 22.8°C (15 August 2010). Altogether, our experimental design comprised 12 categories of seeds (three maternal genotypes × two nutrient levels × two seed maturation periods). As we did not control pollination and plants were grown in randomised order, we assumed all plants to be pollinated from the same pool of pollen donors.

The offspring generation was established in April 2011. Seeds from each of the 12 groups were planted in pots for
the same nutrient-poor and nutrient-rich treatments as in the previous generation. Approximately 5–10 seeds from each group were sown into each of 20 pots, with seedlings that emerged then thinned until one individual remained in each pot. Thus, the final design comprised three maternal genotypes × two maternal nutrient levels × two seed maturation seasons × two offspring nutrient levels × 20 individuals = 480 plants in total.

Measurements

For each plant, we recorded the number of days to first inflorescence spike emergence based on twice weekly observations. To assess total dry biomass production (i.e. including root mass), and to sample roots for total non-structural carbohydrate content analysis, five randomly chosen plants from each treatment combination were harvested on August 2011.

Roots were carefully washed and the root samples, taken from the main root at the time of harvest, were used for analysing the following non-structural carbohydrates: sorbitol, mannitol, galactose, glucose, fructose, sucrose and raffinose family oligosaccharides (RFOs). These saccharides are the main non-structural carbohydrates in *Plantago lanceolata* (Janeček et al. 2011). The glucose, fructose and sucrose contents were assessed using a high-performance anion exchange chromatography-pulsed amperometric detector (HPAE-PAD) with a Dionex ISC-3000 system. Carbohydrates were extracted into boiling ethanol, transferred into distilled water and separated on an analytical column. RFOs were calculated as the difference between ethanol-soluble carbohydrates (galactose, glucose, fructose and sucrose) before and after addition of α-galactosidase to the ethanol extract. Because the individual types of non-structural carbohydrates showed very similar patterns of variation to each other among genotypes and treatments, only the root total non-structural carbohydrates contend (TNC) was considered in the study. The values for TNC were calculated as the total concentration of all of measured non-structural carbohydrates multiplied by the dry root mass.

### Statistical analyses

The effects of maternal nutrients, maternal genotype, seed maturation season and offspring nutrient conditions on flowering phenology, biomass production and root TNC storage were analysed using a general linear model (GLM) with a four-way full factorial design. All four factors were considered fixed effects. Whenever a significant interaction was found, we performed post hoc tests to test whether differences among maternal nutrients or genotype, or seed maturation seasons, were significant within individual treatments. To meet the assumptions of homoscedasticity and normality, all measured variables were log transformed prior to analyses. All statistical analyses were performed using JMP statistical software (JMP 10, SAS Inst.).

### Results

Progeny were larger and had greater root TNC if grown in the nutrient-rich environment. Moreover, increased nutrient availability in the offspring generation advanced time to flowering by about three days on average (Table 1).

Maternal nutrient conditions affected time to flowering, but this effect was dependent on the offspring environment’s nutrient availability (interaction M × P, Table 1): While no effect of maternal nutrient conditions on time to flowering was apparent for offspring grown under nutrient-rich conditions, flowering was strongly affected by maternal nutrient conditions if offspring experienced nutrient-poor conditions. These offspring started flowering on average five days later if they were from mother plants grown in the same nutrient-poor conditions compared to offspring of mothers from the nutrient-rich environment (Fig. 1a).

Total biomass production was affected by both maternal and offspring environmental nutrient levels (interaction M × P, Table 1). When grown in nutrient-poor conditions, offspring of nutrient-poor environment maternal plants produced significantly more biomass than did offspring of mothers from nutrient-rich conditions (Fig. 1b).

### Table 1. Results of general linear models testing for the effects of plant genotype, maternal and offspring nutrient environment, seed maturation season, and their interactions on flowering phenology, biomass and total belowground non-structural carbohydrate (TNC) storage in *Plantago lanceolata*.

<table>
<thead>
<tr>
<th></th>
<th>Time to flowering</th>
<th>Total biomass</th>
<th>TNC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>SS</td>
<td>F-ratio</td>
</tr>
<tr>
<td>Progeny nutrients (P)</td>
<td>1</td>
<td>0.154</td>
<td>7.18</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>2</td>
<td>0.731</td>
<td>17.00</td>
</tr>
<tr>
<td>Maternal nutrients (M)</td>
<td>1</td>
<td>0.222</td>
<td>10.32</td>
</tr>
<tr>
<td>Season of seed maturation (S)</td>
<td>1</td>
<td>0.000</td>
<td>0.00</td>
</tr>
<tr>
<td>M × P</td>
<td>1</td>
<td>0.088</td>
<td>4.10</td>
</tr>
<tr>
<td>G × P</td>
<td>2</td>
<td>0.063</td>
<td>1.47</td>
</tr>
<tr>
<td>G × M</td>
<td>2</td>
<td>0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>P × S</td>
<td>1</td>
<td>0.011</td>
<td>0.52</td>
</tr>
<tr>
<td>G × S</td>
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<td>0.350</td>
<td>8.14</td>
</tr>
<tr>
<td>M × S</td>
<td>1</td>
<td>0.016</td>
<td>0.76</td>
</tr>
<tr>
<td>G × M × P</td>
<td>2</td>
<td>0.050</td>
<td>1.16</td>
</tr>
<tr>
<td>M × P × S</td>
<td>1</td>
<td>0.022</td>
<td>0.09</td>
</tr>
<tr>
<td>G × M × S</td>
<td>2</td>
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<tr>
<td>G × P × S</td>
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<td>0.018</td>
<td>0.43</td>
</tr>
<tr>
<td>G × M × P × S</td>
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<td>0.016</td>
<td>0.38</td>
</tr>
</tbody>
</table>
situation was reversed for plants in nutrient-rich conditions, where offspring from mother plants raised in the same nutrient-rich conditions tended to produce more biomass than the offspring of mothers grown in the nutrient-poor environment (Fig. 1b).

The root TNC in the offspring generation were also significantly affected both by maternal and offspring environmental nutrients (interaction M × P, Table 1). Offspring stored significantly more carbohydrates if they were grown in maternal environment nutrient conditions, and this was the case both if offspring grown in either the nutrient-poor or nutrient-rich environment (Fig. 1c).

Plants derived from different maternal genotypes differed in their flowering phenology, biomass, and root TNC storage, but responded similarly to increased nutrient availability (no significant G × P interaction, Table 1). Maternal genotypes differed in particular in their onset of flowering and in the degree to which the season of seed maturation affected flowering date (interaction G × S, Table 1). However, maternal genotype had overall no effect on the expression of maternal effects (Table 1), and the season of seed maturation had no effect on performance of the offspring generation.

Discussion

Our study provides solid evidence for transgenerational effects of different nutrient conditions on the phenology, biomass production and carbon storage of *Plantago lanceolata*. Our results also suggest that these maternal effects are fairly consistent across different parts of the growing season, and are likely adaptive.

Probably the most intriguing finding of our study is that maternal environments affect carbohydrate storage – a proxy for plant survival and long-term success, and thus also fitness – of the offspring in an adaptive manner. Storage plays an important role in the success of perennials, particularly in overcoming periods with limited resources, in reproduction, and in dealing with stresses such as disturbance or overwintering (Reynolds and Smith 1962, Smith 1969, Greub and Wedin 1971, Menke and Trlica 1983, Bloom et al. 1985, Heilmeier et al. 1986, Chapin et al. 1990, Chaparro et al. 1996). Accordingly, our study provides the first evidence that transgenerational effects can potentially influence the ecology of perennials by modifying their carbohydrate storage management across generations. Maternally-influenced storage strategies in plants could be, for instance, a mechanism underlying the observed differences in stress tolerance between plants of mothers grown in differently productive environments (Latzel et al. 2010). It is conceivable that part of the frequently observed variation in competitive ability, fecundity or stress tolerance in perennials plants is due to the different maternally-influenced storage strategies.

We also found that adaptive transgenerational plasticity to soil nutrients was similar for early-season and late-season offspring although the climatic conditions during seed development in the two offspring groups differed considerably. Thus, climatic fluctuations during offspring development may not affect adaptive maternal effects to other environmental factors in our system. Adaptive transgenerational effects on biomass and carbon storage were also consistent across maternal

![Figure 1](image-url)
genotypes. Hence, although genotypes may vary in their immediate environmental responses (i.e. phenotypic plasticity) and their ability to express transgenerational effects (Alexander and Wülf 1985, Schmitt et al. 1992, Schmid and Dolt 1994, Andalo et al. 1998, Agrawal 2001, 2002, Riginos et al. 2007, Bossdorf et al. 2009) adaptive maternal effects may be fairly robust across genotypes. However, our conclusion is of course based on only three genotypes and should therefore be considered rather preliminary. Clearly, a more robust test of genotype effects would require a broader survey of different genotypes from different populations. As our plants were open-pollinated it is also possible that genetic differences were reduced because offspring of different genotypes were pollinated by the same pollen.

Not all transgenerational effects in our study appeared to be adaptive. In a nutrient-poor environment, the offspring of mothers from nutrient-poor environments flowered later than the offspring from nutrient-rich environments. In a nutrient-rich environment, the two groups had similar phe-

In conclusion, our study provides strong experimental evidence for adaptive transgenerational effects in a common perennial plant. Nevertheless, our experiment certainly provides only a glimpse of the potential ecological and evolutionary implications of transgenerational effects in plants. To improve our understanding of these implications in natural ecosystems, it is highly desirable that future studies be carried out on other species in more natural conditions, as done for instance by Galloway and Etterson (2007) on Campanulastrum americanum, that more perennial species are studied, and that reciprocal designs are employed more frequently to test the adaptive significance. More generally, we want to know how important maternal environmental influences are, relative to other influences, for the phenotypic variation observed in natural populations, and under which conditions adaptive transgenerational mechanisms will evolve.

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References


