plant biology

Plant Biology ISSN 1435-8603

RESEARCH PAPER

Effects of parental light environment on growth and morphological responses of clonal offspring

B.-C. Dong[1], J . Meng[1] & F.-H. Yu[1,2]

1 School of Nature Conservation, Beijing Forestry University, Beijing, China

2 Institute of Wetland Ecology & Clone Ecology/Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou, China

Keywords

5-azacytidine; *Alternanthera philoxeroides*; clonal plant; DNA methylation; parental shading effect; vegetative offspring.

Correspondence

F.-H. Yu, School of Nature Conservation, Beijing Forestry University, Beijing 100083, China.

E-mail: feihaiyu@126.com

Editor

W. Adams

Received: 14 January 2019; Accepted: 28 April 2019

doi:10.1111/plb.13001

ABSTRACT

- Environments experienced by parent ramets of clonal plants can potentially influence fitness of clonal offspring ramets. Such clonal parental effects may result from heritable epigenetic changes, such as DNA methylation, which can be removed by application of DNA de-methylation agents such as 5-azacytidine.
- To test whether parental shading effects occur *via* clonal generation and whether DNA methylation plays a role in such effects, parent plants of the clonal herb *Alternanthera philoxeroides* were first subjected to two levels of light intensity (high *versus* low) crossed with two levels of DNA de-methylation (no or with de-methylation by application of 5-azacytidine), and then clonal offspring taken from each of these four types of parent plant were subjected to the same two light levels.
- Parental shading effects transmitted *via* clonal generation decreased growth and modified morphology of clonal offspring. Offspring responses were also influenced by DNA methylation level of parent plants. For clonal offspring growing under low light, parental shading effects on growth and morphology were always negative, irrespective of the parental de-methylation treatment. For clonal offspring growing under high light, parental shading effects on offspring growth and morphology were negative when the parents were not treated with 5-azacytidine, but neutral when they were treated with 5-azacytidine.
- Overall, parental shading effects on clonal offspring performance of *A. philoxeroides* were found, and DNA methylation is likely to be involved in such effects. However, parental shading effects contributed little to the tolerance of clonal offspring to shading.

INTRODUCTION

Light availability is an essential environmental factor influencing survival, growth and reproduction of plant species (Kilkenny & Galloway 2008; Lambers *et al.* 2008; Valladares & Niinemets 2008). Plants need to capture sufficient light to meet the photosynthetic demand for inorganic carbon fixation (Lambers *et al.* 2008; Valladares & Niinemets 2008). To cope with spatial and temporal variations in light, plants often display a broad spectrum of phenotypic plasticity (Alpert & Simms 2002; Valladares & Niinemets 2008; Nicotra *et al.* 2010). Under shade, for instance, to intercept light efficiently and thus to improve potential carbon gain, plants generally increase biomass allocation to aboveground organs (leaves and stems) and adjust leaf and stem morphology by *e.g.* producing thinner and larger leaves and longer internodes (Seidlova *et al.* 2009; Valladares *et al.* 2011; Dlugos *et al.* 2015; Huang *et al.* 2017). Therefore, phenotypic plasticity can be a key strategy promoting plant fitness under changing light conditions (Delagrange *et al.* 2004; Valladares *et al.* 2007).

The effects of light environments experienced by parent plants may persist across offspring generations (Galloway 2005; Galloway & Etterson 2007; Heger 2016). Such parental environmental effects are considered to be of great importance for offspring performance, *via* regulating their early survival and development, and subsequent growth (Galloway 2005; Galloway & Etterson 2007; Verhoeven & Preite 2014; Heger 2016). This is especially the case if parental effects allow offspring to pre-adapt to environments similar to those that parent plants have encountered *via* the persistence (or inheritance) of specific phenotypic traits that match parental conditions (Galloway & Etterson 2007; Herman & Sultan 2011; McIntyre & Strauss 2014). For instance, offspring of *Polygonum persicaria* produced by their parent plants growing under shade produced significantly higher leaf area and specific leaf area to optimise photosynthetic potential compared to offspring produced by their parent plants growing in sunlight (Baker *et al.* 2018). Also, such parent effects are context-dependent: they were highly significant for seedlings growing under shade, but generally not significant for seedling growing in sunlight (Baker *et al.* 2018).

One possible mechanism that drives parental effects is the modification of DNA methylation in plants, which regulates gene expression by the addition of a methyl group to nucleotides, without changes in the DNA sequence (Bossdorf *et al.* 2008; Ho & Burggren 2010; Herman & Sultan 2011; Verhoeven & Preite 2014). According to findings on the model species *Arabidopsis thaliana* and a few other wild species, a considerable proportion of environment-induced DNA methylation is heritable and independent of genetic variation, thereby triggering the adaptation of offspring to stressful environmental

conditions such as drought (González *et al.* 2016; Herman & Sultan 2016), nutrient deficiency (Bossdorf *et al.* 2010; Kou *et al.* 2011), salinity (Boyko *et al.* 2010; Bilichak *et al.* 2012) and shading (Tatra *et al.* 2000).

Parental effects can transmit not only *via* sexual generation (Bossdorf *et al.* 2010; González *et al.* 2016; Herman & Sultan 2016; Baker *et al.* 2018), but also *via* clonal generation (González *et al.* 2016, 2017, 2018; Dong *et al.* 2017, 2018; Wibowo *et al.* 2018). However, the underlying mechanism of parental effects has rarely been tested in clonal plant species (Latzel & Klimešová 2010). Clonal parental effects may also result from heritable epigenetic modifications such as DNA methylation, which can be removed by application of DNA demethylation agents such as 5-azacytidine (hereafter 5-azaC) and zebularine (Bossdorf *et al.* 2008; González *et al.* 2016, 2017). Thus, it is predicted that application of DNA demethylation agents can decrease parental effects on clonal offspring performance if DNA methylation is involved in such effects.

Using a well-studied clonal species, *Alternanthera philoxeroides*, we conducted an experiment to examine the parental effects of light environment on growth, morphology and biomass allocation of clonal offspring. We also examined the potential role of DNA methylation in parental effects by employing the de-methylating agent 5-azaC. Specifically, we tested the following hypotheses. (i) Parental shading effects can be adaptive for *A. philoxeroides*, which can pre-adapt the offspring ramets produced by the parent plants under shade to similar shading conditions. One specific prediction is that the growth performance of offspring ramets of *A. philoxeroides* produced by shaded parents is better than that of offspring ramets produced by parents under sunlight when the offspring ramets grow in shade. Similarly, the growth performance of offspring ramets of *A. philoxeroides* produced by parents under sunlight is predicted to be better than the growth performance of offspring ramets produced by shaded parents when the offspring ramets grow under sunlight. (ii) Parental shading effects of *A. philoxeroides* on the performance of the offspring generation can be mediated by DNA methylation. Our specific prediction is that parental shading effects of *A. philoxeroides* can be removed or weakened by application of the DNA de-methylation agent (*i.e.* 5-azaC) to the parent plants. Thus, parental effects are likely to occur in offspring ramets produced by parent plants not treated with 5-azaC, but unlikely to occur in offspring ramets produced by parent plants treated with 5-azaC.

MATERIAL AND METHODS

Plant species

Alternanthera philoxeroides (Mart.) Griseb., native to South America, is a creeping perennial herb of *Amaranthaceae* (Julien *et al.* 1995; Holm *et al.* 1997). The species is considered a highly invasive weed in many countries including China, Australia and the USA (Julien *et al.* 1995; Wang *et al.* 2009). In China, *A. philoxeroides* rarely produces viable seed, but instead forms offspring *via* stem and/or root fragments (Dong *et al.* 2010, 2012). This species is widespread across habitats ranging from terrestrial to aquatic (Sainty *et al.* 1998; Pan *et al.* 2006), and it frequently experiences abiotic stress such as flooding (Schooler *et al.* 2010; Luo *et al.* 2014) and herbivory (Wei *et al.* 2016; Dong *et al.* 2017). Severe ecological and environmental problems are caused by the invasion of *A. philoxeroides*. For example, aquatic populations can block waterways and restrict the survival and development of fish, and terrestrial populations can colonise pastoral and agricultural lands, displacing native plant species (Sainty *et al.* 1998; Ma & Wang 2005).

Recent studies have shown that epigenetic variation is abundant in populations of *A. philoxeroides*, despite genetic variation being extremely low in China (Gao *et al.* 2010; Shi *et al.* 2018, 2019). Also, in the recent decade this species has been frequently used as a model clonal plant species (*e.g.* Wang *et al.* 2009; Yu *et al.* 2009; Dong *et al.* 2010, 2011, 2012, 2017, 2018) to examine ecological questions. Thus, here we also used *A. philoxeroides* as a model to test the role of epigenetics in parent shading effects. *A. philoxeroides* plants were collected from three separate populations in a riparian agricultural area in May 2011 in Taizhou, Zhejiang Province, China (28.87°N, 121.01°E). The collected plants were then mixed and cultivated in a greenhouse at the Forest Science Co., Ltd. of Beijing Forestry University, Beijing, China.

Experimental design

The overall design of the experiment included two vegetative generations of *A. philoxeroides*. For simplicity, we defined the first vegetative generation as the parental generation and the subsequent vegetative generation as the offspring generation. Parent plants were first subjected to two levels of light (high *versus* low) crossed with two levels of DNA methylation (no or with application of the DNA demethylation agent, 5-azaC). The offspring taken from each of the four types of parent plant were then subjected to the same two light levels (high *versus* low).

On 25 July 2012, 320 similar-sized stem fragments, each consisting of one node with 2-cm proximal and distal internodes and two opposite leaves, were cut off the stock plants. The clonal fragments were individually planted in plastic pots (14-cm diameter and 12-cm deep) filled with a 1:1 (v:v) mixture of quartz sand (0.5–1.0 mm particle size) and peat (Pindstrup Seedling; Pindstrup Mosebrug, Pindstrup, Denmark), plus 1 $g \cdot l^{-1}$ slow-release fertiliser (16 N:9 P:12 K:2 Mg; Osmocote Standard; Scotts, Marysville, OH, USA). The sand–soil mixture itself contained about 2.17 mg $N \cdot l^{-1}$, 1.07 mg $P \cdot l^{-1}$, 3.56 mg $K \cdot l^{-1}$.

On 13 August 2012, 96 plants that were approximately 10-cm long were selected for the experiment and treated as the parental generation. For light treatments of the parental generation, half of the parental plants (48) were grown under high light (full light in the greenhouse) and the other half (48) were grown under low light, receiving 63% of full light. The low-light treatment was achieved by covering the target plants with black, neutral shading net, which did not change the red to far-red light ratio. Within the initial 6 days after the parental generation light treatments began, half of the plants (24) at each light level were treated with 100 $\mu mol \cdot l^{-1}$ solution of 5-azaC (Sigma, St. Louis, MO, USA). The concentration of 5-azaC used here was considered to be appropriate to inhibit DNA methylation in plant species but did not cause strong and direct toxicity to plant performance (*e.g.* Tatra *et al.* 2000; González *et al.* 2016). The other half (24) was treated with distilled water as a control treatment. The leaf surface and stem nodes of each treated plant were sprayed with 20 µl 5-azaC solution or 20 µl distilled water once every 2 h (from 09:00 to 17:00 h). In total, each of four combined parental generation

light × 5-azaC treatments had 24 replicates. The parental generation treatments lasted for 3 weeks, from 13 August to 3 September 2012. The photosynthetically active radiation measured at noon during the entire experiment averaged 136.9 μmol photons $m^{-2}{\cdot}s^{-1}$.

On 3 and 4 September 2012, eight randomly chosen replicates (*i.e.* 32 plants in total for the four treatments) were harvested to measure growth (total mass, leaf mass, stem mass, root mass, number of nodes, number of leaves and stem length), biomass allocation (root:shoot ratio) and morphological traits [area of the fourth youngest leaf on the main stem, mass of the fourth youngest leaf, mean length of the four youngest internodes of the main stem, mean mass of the four youngest internodes of the main stem, specific leaf area (SLA = area of the fourth youngest leaf/mass of the fourth youngest leaf; $cm{\cdot}g^{-1}$) and specific internode length (SIL = mean length of the four youngest internodes/mean mass of the four youngest internodes; $cm{\cdot}g^{-1}$)]. To measure mass, plant parts were dried at 70 °C for 48 h then weighed. Leaf area was measured using WinFOLIA pro 2004a (Regent Instruments, Quebec, Canada).

The remaining 16 replicates (64 plants in total for the four treatments) were used to test the effects of light levels and 5-azaC treatment in the parental generation on the performance of clonal offspring. One new stem fragment was taken from each of the remaining parent plants and grown in a pot as described above. Each stem fragment consisted of only the fifth youngest ramet node along the main stem, which had two opposite leaves and two internodes (proximal and distal) of half length. The 16 new stem fragments taken from each of the four types of parent plant were then randomly assigned to either of the two light levels experienced by plants of the parent generation. Therefore, there were eight treatments in the offspring experiment and eight replicates for each offspring treatment. The offspring treatments started on 5 September 2012 and ended on 24 and 25 October 2012, lasting for 7 weeks. Growth, allocation and morphological traits were measured as described above.

During the whole experiments (13 August to 25 October 2012), pots were randomly arranged and periodically repositioned to minimise possible effects of the microenvironment in the greenhouse. Tap water was supplied daily to maintain soil moisture. The air temperature and relative humidity in the greenhouse were 21.6 ± 0.4 °C and 77.2 ± 0.7% (mean ± 1SE), respectively, as measured with HOBO Temp/RH loggers (Onset Computer, Bourne, MA, USA).

Data analysis

Two-way ANOVAS were used to test the effects of light levels (high *versus* low) and DNA de-methylation (control *versus* 5-azaC) on growth (total mass, leaf mass, stem mass, root mass, number of nodes, number of leaves and stem length), allocation (root:shoot ratio) and morphology (area of fourth youngest leaf, mass of fourth youngest leaf, mean internode length, mean internode mass, SLA and SIL) of parent plants. Three-way ANOVAS were used to test the effects of parental light levels (high *versus* low), parental DNA de-methylation (control *versus* 5-azaC) and offspring light levels (high *versus* low) on growth, allocation and morphological traits of clonal offspring. Data were transformed when needed to natural logarithm to meet the assumptions of homoscedasticity and normality (for details see Tables 1 and 2). All analyses were conducted using SPSS 22.0 (SPSS, Chicago, IL, USA).

RESULTS

Effects of light and 5-azaC application on parent plants

For parent plants of *A. philoxeroides*, the growth measures and biomass allocation were all significantly affected by light levels, whereas only number of nodes, number of leaves and total stem length were negatively affected by the 5-azaC treatment (Table 1). All growth traits (total mass, leaf mass, stem mass, root mass, number of nodes, number of leaves, stem length) and the root:shoot ratio were significantly lower under low light than under high light (Table 1, Fig. 1). Number of nodes, number of leaves and stem length were lower in the 5-azaC treatment than in the control treatment (Fig. 1e–g).

Among the six morphological traits measured, only the area of the fourth youngest leaf was significantly affected by light levels, and SLA was affected by both light level and the 5-azaC treatment (Table 1). The area of the fourth leaf and SLA were significantly higher under low light than under high light, and SLA was also higher in the 5-azaC treatment than in the control treatment (Fig. 2a and c). There were no significant interactions between light levels and 5-azaC treatments on any of the growth, biomass allocation or morphological traits (Table 1).

Parental and offspring generation effects on clonal offspring

There were significant or marginally significant ($P < 0.1$) three-way interaction effects of parental light levels, parental 5-azaC

Table 1. ANOVA results for effects of light level (high *versus* low) and de-methylation agent treatment (control *versus* 5-azaC) on growth, biomass allocation and morphological traits of parent plants of *Alternanthera philoxeroides*.

	light (L)		agent (A)		L × A	
effect	$F_{1,28}$	P	$F_{1,28}$	P	$F_{1,28}$	P
growth traits						
total mass[a]	**15.1**	**0.001**	3.9	0.058	0.3	0.566
leaf mass[a]	**12.1**	**0.002**	4.1	0.051	1.1	0.305
stem mass[a]	**12.6**	**0.001**	4.0	0.054	<0.1	0.765
root mass[a]	**21.9**	**<0.001**	0.4	0.520	<0.1	0.870
number of nodes[a]	**11.7**	**0.002**	**7.1**	**0.013**	0.3	0.596
number of leaves	**21.5**	**<0.001**	**7.3**	**0.012**	1.6	0.218
stem length	**5.0**	**0.034**	**5.9**	**0.022**	0.8	0.387
allocation						
root:shoot ratio	**5.8**	**0.023**	2.2	0.147	0.2	0.633
morphological traits						
4th leaf area	**5.1**	**0.032**	0.3	0.561	1.7	0.197
4th leaf mass[a]	2.3	0.142	1.9	0.185	0.7	0.412
specific leaf area	**63.3**	**<0.001**	**4.8**	**0.037**	0.3	0.577
mean internode length	<0.1	0.902	0.1	0.738	<0.1	0.982
mean internode mass	1.7	0.201	1.5	0.237	0.3	0.577
specific internode length	4.0	0.054	1.8	0.188	0.3	0.615

Degrees of freedom (*df*), *F* and *P* values are given. Values for $P < 0.05$ are in bold.

[a]Natural log transformation.

Table 2. ANOVA results for effects of light level (high *versus* low) and de-methylation agent treatment (control *versus* 5-azaC) in parental generation, and light level (high *versus* low) in offspring generation on growth, biomass allocation and morphological traits of clonal offspring of *Alternanthera philoxeroides*.

	parental (Pa)		agent (A)		offspring (Off)		Pa × A		Pa × Off		A × Off		Pa × A × Off	
effects	$F_{1,48}$	P	$F_{1,48}$	P	$F_{1,48}$	P	$F_{1,48}$	P	$F_{1,48}$	P	$F_{1,48}$	P	$F_{1,48}$	P
growth traits														
total mass[a]	**31.7**	**<0.001**	0.4	0.525	**176.9**	**<0.001**	0.1	0.751	0.9	0.338	1.0	0.324	**4.2**	**0.045**
Leaf mass[a]	**28.3**	**<0.001**	0.3	0.574	**136.6**	**<0.001**	<0.1	0.795	0.5	0.484	1.5	0.232	**4.2**	**0.045**
stem mass[a]	**33.6**	**<0.001**	0.8	0.380	**201.6**	**<0.001**	<0.1	0.787	0.5	0.486	1.3	0.268	**4.5**	**0.040**
root mass[a]	**23.4**	**<0.001**	<0.1	0.947	**130.3**	**<0.001**	0.1	0.735	**4.4**	**0.042**	<0.1	0.763	2.9	0.093
number of nodes[a]	**16.6**	**<0.001**	1.5	0.228	**212.3**	**<0.001**	<0.1	0.902	0.3	0.605	2.0	0.168	**4.1**	**0.048**
number of leaves[a]	**15.7**	**<0.001**	2.4	0.127	**164.8**	**<0.001**	0.1	0.710	0.3	0.607	0.2	0.622	3.8	0.057
stem length[a]	**21.0**	**<0.001**	1.2	0.278	**187.4**	**<0.001**	<0.1	0.974	0.6	0.425	3.1	0.083	**6.0**	**0.018**
allocation														
root:shoot ratio	0.1	0.708	1.2	0.284	**6.8**	**0.012**	0.2	0.635	**9.5**	**0.003**	2.7	0.107	<0.1	0.769
morphological traits														
4th leaf area	**23.8**	**<0.001**	<0.1	0.888	**56.1**	**<0.001**	0.7	0.401	**7.1**	**0.011**	0.4	0.531	**4.9**	**0.031**
4th leaf mass	**20.3**	**<0.001**	0.2	0.650	**88.3**	**<0.001**	1.5	0.229	2.6	0.117	<0.1	0.970	**4.2**	**0.046**
specific leaf area[a]	**7.8**	**0.008**	0.2	0.687	**51.3**	**<0.001**	<0.1	0.766	3.5	0.069	<0.1	0.926	<0.1	0.763
mean internode length	**11.4**	**0.001**	<0.1	0.952	**37.4**	**<0.001**	0.3	0.618	**5.8**	**0.02**	0.1	0.725	**4.7**	**0.035**
mean internode mass	**10.6**	**0.002**	0.9	0.361	**148.7**	**<0.001**	**6.3**	**0.016**	<0.1	0.773	1.7	0.203	**9.9**	**0.003**
specific internode length[a]	**17.7**	**<0.001**	0.2	0.689	**205.7**	**<0.001**	1.6	0.218	0.9	0.342	2.5	0.117	**5.6**	**0.022**

Degrees of freedom (*df*), *F* and *P* values are given. Values for $P < 0.05$ are in bold.
[a]Natural log transformation.

treatments and offspring light levels on all growth measures and on five of the six morphological traits (SLA being the exception) of the offspring (Table 2). When the offspring grew under high light, parental shading effects were significantly negative on all growth measures and four of the six morphological traits (fourth leaf area, fourth leaf mass, fourth internode length, fourth internode mass) of the offspring taken from parents not treated with 5-azaC, but neutral on those of offspring taken from parents treated with 5-azaC (Figs 3 and 4). When the offspring grew under low light, parental shading effects were significantly negative on growth and morphology of the offspring, irrespective of the parental 5-azaC treatments (Figs 3 and 4). When the offspring grew under high light, parental shading effects were significantly positive on SIL of offspring taken from the parents not treated with 5-azaC, but neutral on SIL of offspring taken from the parents treated with 5-azaC (Fig. 4f). When the offspring were under low light, parental shading effects were always positive on SIL of the offspring, irrespective of parental 5-azaC treatments (Fig. 4f).

There was a two-way interaction effect of parental and offspring light levels on root:shoot ratio of the offspring (Table 2). When the offspring grew under high light, offspring taken from the unshaded parents had lower root:shoot ratio than offspring taken from the shaded parents (Fig. 3h). The opposite pattern for parental shading effects was detected under low light (Fig. 3h). There were only main effects of parental and offspring light levels on SLA of the offspring (Table 2). The offspring taken from the shaded parents had higher SLA than those taken from unshaded parents, and offspring growing under low light had higher SLA than those growing under high light (Fig. 4c).

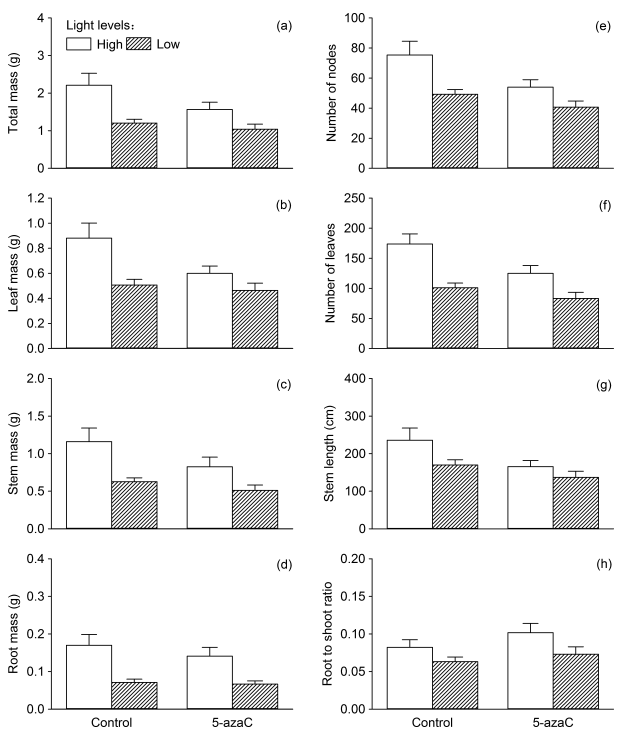

Fig. 1. Effects of light level (high *versus* low) and de-methylation agent treatment (control *versus* 5-azaC) on growth (a–g) and biomass allocation (h) of parent plants of *Alternanthera philoxeroides*. Bars indicate mean ± 1 SE.

DISCUSSION

Effects of light and de-methylation on parent plants

The growth performance of parent *A. philoxeroides* plants was reduced when they were grown under the shaded conditions, as shown by lower biomass accumulation, ramet production and creeping stem expansion. However, in response to the

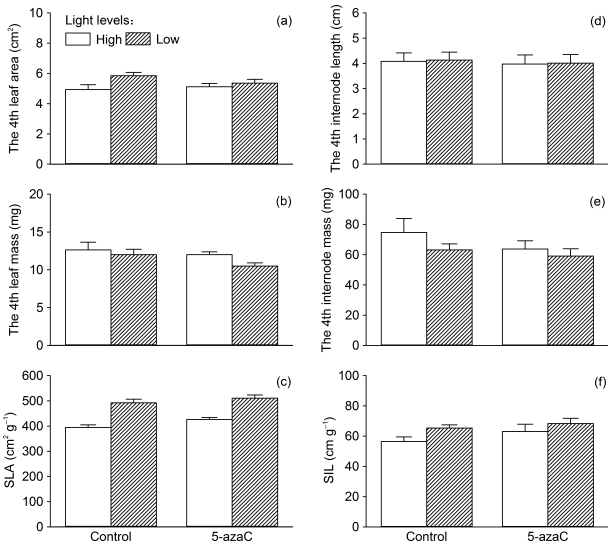

Fig. 2. Effects of light level (high *versus* low) and de-methylation agent treatment (control *versus* 5-azaC) on morphological traits (a-f) of parent plants of *Alternanthera philoxeroides*. Bars indicate mean ± 1 SE.

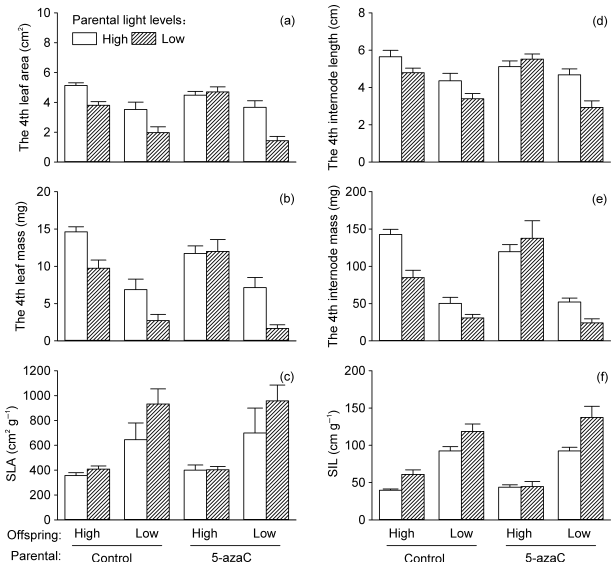

Fig. 4. Effects of light level (high *versus* low) and de-methylation agent treatment (control *versus* 5-azaC) in parental generation, and light level (high *versus* low) in offspring generation on morphological traits (a-f) of clonal offspring of *Alternanthera philoxeroides*. Bars indicate mean ± 1 SE.

shading, parent plants of *A. philoxeroides* utilised at least two strategies to improve light harvesting and potential carbon gain, namely, increasing biomass allocation to shoots and producing thinner, larger leaves. These potentially adaptive strategies of *A. philoxeroides* are similar to the shading-induced responses of many other clonal plants and can act as a buffer against the reduced capacity for photosynthesis (Weijschedé *et al.* 2006; Valladares & Niinemets 2008; Baker *et al.* 2018; Wang *et al.* 2018).

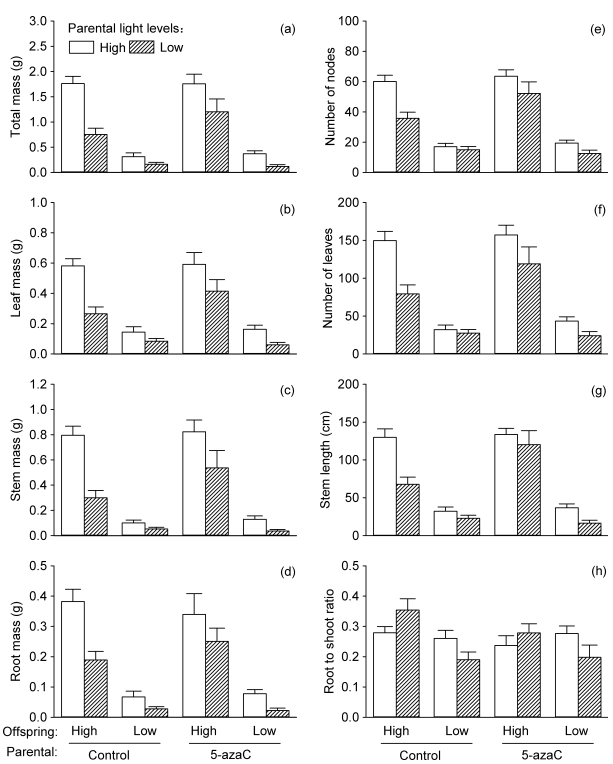

Fig. 3. Effects of light level (high *versus* low) and de-methylation agent treatment (control *versus* 5-azaC) in parental generation, and light level (high *versus* low) in offspring generation on growth (a–g) and biomass allocation (h) of clonal offspring of *Alternanthera philoxeroides*. Bars indicate mean ± 1 SE.

We employed 5-azaC, a de-methylation agent (Bossdorf *et al.* 2010; González *et al.* 2016, 2017), to reduce DNA methylation levels of parent plants. While the application of 5-azaC had no significant effect on biomass, root:shoot ratio or the majority of morphological traits (excluding SLA) of parent plants, it negatively affected the development of aboveground organs, including ramet number, leaf number and creeping stem length. The restriction to aboveground clonal spread and leaf production may be mainly attributed to the potential toxicity of 5-azaC, because the chemically induced DNA demethylation in parent plants was often followed by generalised/non-directed gene expression that is considered maladaptive for plant development (Tatra *et al.* 2000).

Parental and ongoing effects of shading on offspring plants

Previous studies have reported that parental shading effects could be adaptive for the shaded offspring of sexually produced species such as *P. persicaria* and *Claytonia perfoliata* (McIntyre & Strauss 2014; Baker *et al.* 2018). However, our results did not support our first hypothesis, *i.e.* parental shading effects could contribute to the pre-adaptation of clonal offspring produced by shaded parents to similar shading conditions. In the present study, parental shading effects severely inhibited the early growth and development of clonal offspring produced by shaded parents, and induced potentially maladaptive changes in leaf and stem morphological traits (*e.g.* decrease in the mean leaf and stem size as indicated in Fig. 4). Moreover, the changes in DNA methylation status of parent plants did not mediate

parental effects on the shaded offspring of *A. philoxeroides*. Therefore, we speculate that, only across one or a few generations, clonal reproduction may not allow vegetative offspring of *A. philoxeroides* to obtain an adaptive shading-induced phenotype from parent plants in response to the similar stressful environment. In other words, the majority of adaptive morphological traits of parent plants (*e.g.* larger leaf area and higher SLA under shading) may not be heritable by next clonal generations.

DNA de-methylation counteracted the negative parental shading effects on offspring growing under the unshaded condition, so that chemically de-methylated offspring of shaded parents developed similar growth and morphological features as the offspring of unshaded parents. These results were not fully consistent with the second hypothesis, and imply that the process of DNA methylation may be maladaptive for clonal offspring of *A. philoxeroides*, since it contained the negative consequence caused by parental shading (Boyko *et al.* 2010; Baker *et al.* 2018). Similarly, DNA methylation was also found to impede the occurrence of adaptive parental effects on offspring development, *e.g.* under drought (Herman & Sultan 2016) or salt stress (Boyko *et al.* 2010; Boyko & Kovalchuk 2011). These results together suggest that the effects of parental stressful environments and the modification of DNA methylation in such environments may not always be adaptive (Baker *et al.* 2018). This is because DNA methylation generally reduces transcriptional activity, and the ecological consequence caused by DNA methylation is often unpredictable in the next generations (Baker *et al.* 2018).

Apart from the modification of DNA methylation, parental shading effects may be partly attributed to changes in quality of propagule provisioning induced by parental environments (Herman & Sultan 2011; Dong *et al.* 2018). In the present study, shaded parent plants produced smaller offspring ramets compared to unshaded parent plants (ramets weight average of 26.5 mg under high light *versus* 23.5 mg under low light), indicating a possible lower investment in storage resources containing carbohydrate compounds and N for the early growth of clonal offspring. The decrease in propagule size further resulted in a prolonged regeneration time of clonal offspring (average regeneration time of 11.9 days under high light *versus* 13.8 days under low light) and delayed individual development (see also Song *et al.* 2013 for similar results in other species). However, in some other sexually produced species, such as *P. persicaria*, parental shading effects could inhibit the accumulation of seed provisioning but could did not influence seedling performance (Sultan 1996; Lundgren & Sultan 2005). Therefore, the variation in parental effects triggered by propagule provisioning between clonal and sexually produced plants should be taken into account in future studies.

CONCLUSIONS

Parental shading effects can persist between vegetative generations of *A. philoxeroides*, negatively influencing the responses of clonal offspring to shade conditions. Such parental effects in clonal plants were partly attributed to variation in the quality of propagule provisioning and to changes in the DNA methylation status of parent plants. Parental shading effects are maladaptive for clonal offspring of *A. philoxeroides*, possibly because the offspring cannot inherit any potentially adaptive changes from their parent plants. Our work suggests that only across one clonal generation, parental shading effects may not offer a potential means of rapid adaptation for *A. philoxeroides* under predictable shaded environments.

ACKNOWLEDGEMENTS

We are grateful to Zhi-Wen Ma for assistance with the experiment, and two reviewers for their valuable comments. This work was supported by National Key Research and Development Program of China (2016YFC1201101), the National Natural Science Foundation of China (no. 31670428, 31500331) and Fundamental Research Funds for the Central Universities (2015ZCQ-BH-01).

REFERENCES

Alpert P., Simms E.L. (2002) The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evolutionary Ecology*, **16**, 285–297.

Baker B.H., Berg L.J., Sultan S.E. (2018) Context-dependent developmental effects of parental shade versus sun are mediated by DNA methylation. *Frontiers in Plant Science*, **9**, 1251.

Bilichak A., Ilnystkyy Y., Hollunder J., Kovalchuk I. (2012) The progeny of *Arabidopsis thaliana* plants exposed to salt exhibit changes in DNA methylation, histone modifications and gene expression. *PLoS ONE*, **7**, e30515.

Bossdorf O., Richards C.L., Pigliucci M. (2008) Epigenetics for ecologists. *Ecology Letters*, **11**, 106–115.

Bossdorf O., Arcuri D., Richards C.L., Pigliucci M. (2010) Experimental alteration of DNA methylation affects the phenotypic plasticity of ecologically relevant traits in *Arabidopsis thaliana*. *Evolutionary Ecology*, **24**, 541–553.

Boyko A., Kovalchuk I. (2011) Genome instability and epigenetic modification – heritable responses to environmental stress? *Current Opinion in Plant Biology*, **14**, 260–266.

Boyko A., Blevins T., Yao Y., Golubov A., Bilichak A., Ilnytskyy Y., Hollunder J., Meins F., Kovalchuk I. (2010) Transgenerational adaptation of *Arabidopsis* to stress requires DNA methylation and the function of Dicer-like proteins. *PLoS ONE*, **5**, e9514.

Delagrange S., Messier C., Lechowicz M.J., Dizengremel P. (2004) Physiological, morphological and allocational plasticity in understory deciduous trees: importance of plant size and light availability. *Tree Physiology*, **24**, 775–784.

Dlugos D.M., Collins H., Bartelme E.M., Drenovsky R.E. (2015) The non-native plant *Rosa multiflora* expresses shade avoidance traits under low light availability. *American Journal of Botany*, **102**, 1323–1331.

Dong B.-C., Yu G.-L., Guo W., Zhang M.-X., Dong M., Yu F.-H. (2010) How internode length, position and presence of leaves affect survival and growth of *Alternanthera philoxeroides* after fragmentation? *Evolutionary Ecology*, **24**, 1447–1461.

Dong B.-C., Liu R.-H., Zhang Q., Li H.-L., Zhang M.-X., Lei G.-C., Yu F.-H. (2011) Burial depth and stolon internode length independently affect survival of small clonal fragments. *PLoS ONE*, **6**, e23942.

Dong B.-C., Alpert P., Guo W., Yu F.-H. (2012) Effects of fragmentation on the survival and growth of the invasive, clonal plant *Alternanthera philoxeroides*. *Biological Invasions*, **14**, 1101–1110.

Dong B.-C., Fu T., Luo F.-L., Yu F.-H. (2017) Herbivory-induced maternal effects on growth and defense traits in the clonal species *Alternanthera philoxeroides*. *Science of the Total Environment*, **605–606**, 114–123.

Dong B.-C., van Kleunen M., Yu F.-H. (2018) Context-dependent parental effects on clonal offspring performance. *Frontiers in Plant Science*, **9**, 1824.

Galloway L.F. (2005) Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist*, **166**, 93–100.

Galloway L.F., Etterson J.R. (2007) Transgenerational plasticity is adaptive in the wild. *Science*, **318**, 1134.

Gao L.-X., Geng Y.-P., Li B., Chen J.-K., Yang J. (2010) Genome-wide DNA methylation alterations of *Alternanthera philoxeroides* in natural and manipulated habitats: implications for epigenetic regulation of rapid responses to environmental fluctuation and phenotypic variation. *Plant, Cell and Environment*, **33**, 1820–1827.

González A.P.R., Chrtek J., Dobrev P.I., Dumalasová V., Fehrer J., Mráz P., Latzel V. (2016) Stress-induced memory alters growth of clonal offspring of white clover (*Trifolium repens*). *American Journal of Botany*, **103**, 1567–1574.

González A.P.R., Dumalasová V., Rosenthal J., Skuhrovec J., Latzel V. (2017) The role of transgenerational effects in adaptation of clonal offspring of white clover (*Trifolium repens*) to drought and herbivory. *Evolutionary Ecology*, **31**, 345–361.

González A.P.R., Preite V., Verhoeven K.J.F., Latzel V. (2018) Transgenerational effects and epigenetic memory in the clonal plant *Trifolium repens*. *Frontiers in Plant Science*, **9**, 11.

Heger T. (2016) Light availability experienced in the field affects ability of following generations to respond to shading in an annual grassland plant. *Journal of Ecology*, **104**, 1432–1440.

Herman J.J., Sultan S.E. (2011) Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Frontiers in Plant Science*, **2**, 102.

Herman J.J., Sultan S.E. (2016) DNA methylation mediates genetic variation for adaptive transgenerational plasticity. *Proceedings of the Royal Society of London, B: Biological Sciences*, **283**, 20160988.

Ho D.H., Burggren W.W. (2010) Epigenetics and transgenerational transfer: a physiological perspective. *Journal of Experimental Biology*, **213**, 3–16.

Holm L.G., Doll J., Holm E., Pancho J.V., Herberger J.P. (1997) *World weeds: natural histories and distribution*. John Wiley and Sons, New York, USA, pp 37–44.

Huang Q.-Q., Shen Y.-D., Li X.-X., Fan Z.-W., Li S.-L., Liu Y. (2017) Performance of the invasive *Eupatorium catarium* and *Ageratum conyzoides* in comparison with a common native plant under varying levels of light and moisture. *Weed Biology and Management*, **17**, 112–121.

Julien M.H., Skarratt B., Maywald G.F. (1995) Potential geographical distribution of alligator weed and its biological control by *Agasicles hygrophila*. *Journal of Aquatic Plant Management*, **33**, 55–60.

Kilkenny F.F., Galloway L.F. (2008) Reproductive success in varying light environments: direct and indirect effects of light on plants and pollinators. *Oecologia*, **155**, 247–255.

Kou H.-P., Li Y., Song X.-X., Ou X.-F., Xing S.-C., Ma J., Von W.-D., Liu B. (2011) Heritable alteration in DNA methylation induced by nitrogen-deficiency stress accompanies enhanced tolerance by progenies to the stress in rice (*Oryza sativa* L.). *Journal of Plant Physiology*, **168**, 1685.

Lambers H., Chapin F.S. III, Pons T. (2008) *Plant physiological ecology*. Springer, New York, USA.

Latzel V., Klimešová J. (2010) Transgenerational plasticity in clonal plants. *Evolutionary Ecology*, **24**, 1537–1543.

Lundgren M.R., Sultan S.E. (2005) Seedling expression of cross-generational plasticity depends on reproductive architecture. *American Journal of Botany*, **92**, 377–381.

Luo F.-L., Chen Y., Huang L., Wang A., Zhang M.-X., Yu F.-H. (2014) Shifting effects of physiological integration on performance of a clonal plant during submergence and de-submergence. *Annals of Botany*, **113**, 1265–1274.

Ma R.-Y., Wang R. (2005) Invasive mechanism and biological control of alligatorweed, *Alternanthera philoxeroides* (Amaranthaceae), in China. *Chinese Journal of Applied and Environmental Biology*, **11**, 246–250.

McIntyre P.J., Strauss S.Y. (2014) Phenotypic and transgenerational plasticity promote local adaptation to sun and shade environments. *Evolutionary Ecology*, **28**, 229–246.

Nicotra A.B., Atkin O.K., Bonser S.P., Davidson A.M., Finnegan E., Mathesius U., Poot P., Purugganan M.D., Richards C., Valladares F. (2010) Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, **15**, 684–692.

Pan X.-Y., Geng Y.-P., Zhang W.-J., Li B., Chen J.-K. (2006) The influence of abiotic stress and phenotypic plasticity on the distribution of invasive *Alternanthera philoxeroides* along a riparian zone. *Acta Oecologica*, **30**, 333–341.

Sainty G., McCorkelle G., Julien M. (1998) Control and spread of alligator weed *Alternanthera philoxeroides* (Mart.) Griseb., in Australia: lessons for other regions. *Wetlands Ecology and Management*, **5**, 195–201.

Schooler S.S., Cook T., Prichard G., Yeates A.G. (2010) Disturbance-mediated competition: the interacting roles of inundation regime and mechanical and herbicidal control in determining native and invasive plant abundance. *Biological Invasions*, **12**, 3289–3298.

Seidlova L., Verlinden M., Gloser J., Milbau A., Nijs I. (2009) Which plant traits promote growth in the low-light regimes of vegetation gaps? *Plant Ecology*, **200**, 303–318.

Shi W., Chen X.-J., Gao L.-X., Xu C.-Y., Qu X.-K., Bossdorf O., Yang J., Geng Y.-P. (2019) Transient stability of epigenetic population differentiation in a clonal invader. *Frontiers in Plant Science*, **9**, 1851. https://doi.org/10.3389/fpls.2018.01851

Shi W., Hu X., Chen X.-J., Ou X.-K., Yang J., Geng Y.-P. (2018) Increased population epigenetic diversity of the clonal invasive species *Alternanthera philoxeroides* in response to salinity stress. *Genes & Genetic Systems*, **96**, 259–269. https://doi.org/10.1266/ggs.1218-00039

Song Y.-B., Yu F.-H., Li J.-M., Keser L.H., Fischer M., Dong M., van Kleunen M. (2013) Plant invasiveness is not linked to the capacity of regeneration from small fragments: an experimental test with 39 stoloniferous species. *Biological Invasions*, **15**, 1367–1376.

Sultan S. (1996) Phenotypic plasticity for offspring traits in *Polygonum persicaria*. *Ecology*, **77**, 1791–1807.

Tatra G.S., Miranda J., Chinnappa C.C., Reid D.M. (2000) Effect of light quality and 5-azacytidine on genomic methylation and stem elongation in two ecotypes of *Stellaria longipes*. *Physiologia Plantarum*, **109**, 313–321.

Valladares F., Niinemets U. (2008) Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, & Systematics*, **39**, 237–257.

Valladares F., Gianoli E., Gomez J.M. (2007) Ecological limits to plant phenotypic plasticity. *New Phytologist*, **176**, 749–763.

Valladares F., Gianoli E., Saldana A. (2011) Climbing plants in a temperate rainforest understorey: searching for high light or coping with deep shade? *Annals of Botany*, **108**, 231–239.

Verhoeven K.J., Preite V. (2014) Epigenetic variation in asexually reproducing organisms. *Evolution*, **68**, 644–655.

Wang N., Yu F.-H., Li P.-X., He W.-M., Liu J., Yu G.-L., Song Y.-B., Dong M. (2009) Clonal integration supports the expansion from terrestrial to aquatic environments of the amphibious stoloniferous herb *Alternanthera philoxeroides*. *Plant Biology*, **11**, 483–489.

Wang M.-Z., Bu X.-Q., Li L., Dong B.-C., Li H.-L., Yu F.-H. (2018) Constraints on the evolution of phenotypic plasticity in the clonal plant *Hydrocotyle vulgaris*. *Journal of Evolutionary Biology*, **31**, 1006–1017.

Wei H., He M.-Y., Lu X.-M., Ding J.-Q. (2016) Differences in interactions of aboveground and belowground herbivores on the invasive plant *Alternanthera philoxeroides* and native host *A. sessilis*. *Biological Invasions*, **18**, 3437–3447.

Weijschedé J., Martínková J., de Kroon H., Huber H. (2006) Shade avoidance in *Trifolium repens*: costs and benefits of plasticity in petiole length and leaf size. *New Phytologist*, **172**, 655–666.

Wibowo A., Becker C., Durr J., Price J., Spaepen S., Hilton S., Putra H., Papareddy R., Saintain Q., Harvey S., Bending G.D., Schulze-Lefert P., Weigel D., Gutierrez-Marcos J. (2018) Partial maintenance of organ-specific epigenetic marks during plant asexual reproduction leads to heritable phenotypic variation. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, E9145–E9152.

Yu F.-H., Wang N., Alpert P., He W.-M., Dong M. (2009) Physiological integration in an introduced, invasive plant increases its spread into experimental communities and modifies their structure. *American Journal of Botany*, **96**, 1983–1989.