Adaptive plasticity in response to light and nutrient availability in the clonal plant *Duchesnea indica*

Mo-Zhu Wang[1,2,3], Hong-Li Li[2], Chun-Xiang Liu[2], Bi-Cheng Dong[2], and Fei-Hai Yu[1,2*]

[1] Institute of Wetland Ecology & Clone Ecology; Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou 318000, China

[2] School of Ecology and Nature Conservation, Beijing Forestry University, Beijing 100083, China

[3] State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

[*]Correspondence: Fei-Hai Yu

E-mail: feihaiyu@126.com

This article has been accepted For publication And undergone full peer review but has Not been through the copyediting, typesetting, pagination And proofreading process, which may lead to differences between this Version And the Version of Record. Please cite this article as doi: 10.1093/jpe/rtab116.

Abstract

Aims

Phenotypic plasticity enables plants to buffer against environmental stresses and match their phenotypes to local conditions. However, consistent conclusive evidence for adaptive plasticity has only been obtained for a few traits. More studies on a wider variety of plant functional traits and environmental factors are still needed to further understand the adaptive significance of plasticity.

Methods

We grew 21 genotypes of the stoloniferous clonal plant *Duchesnea indica* under different light and nutrient conditions, and used selection gradient analyses to test the adaptive value (benefits) of morphological and physiological plasticity responding to variation in light and nutrient availability.

Important Findings

Plants grown in shade exhibited lower values for fitness measures (fruit number, ramet number, and biomass), shortened thinner internode length, and decreased adult leaf chlorophyll content, but higher petiole length, specific leaf area, and old leaf chlorophyll content, than plants grown without shade. Plants grown in the low nutrient condition had shorter petiole length, thicker and smaller leaf area, lower chlorophyll content, but higher fruit number and root: shoot ratio than plants grown under the high nutrient condition. Selection gradient analyses revealed that plasticity of petiole length and old leaf chlorophyll content in response to light variation was adaptive, and plasticity of old and adult leaf chlorophyll content in response to nutrient variation was adaptive. Therefore, the adaptive value of plasticity in different traits depends on the specific ecological context. Our findings contribute to understanding the adaptive significance of phenotypic plasticity of clonal plants in response to environmental variation.

Keywords: adaptive plasticity, clonal plants, *Duchesnea indica*, stoloniferous plant, phenotypes

Introduction

Phenotypic plasticity is defined as the ability of a genotype to produce different phenotypes in different environments (Sultan 1995; Pigliucci 2005). By altering morphology, physiology, development, and/or life history, phenotypic plasticity enables plants to buffer against environmental stresses and match their phenotypes to local conditions (Weijschedé *et al*. 2006; van Kleunen *et al*. 2007; Nicotra *et al*. 2015; Wang *et al.* 2018; Lampei 2019). Therefore, phenotypic plasticity is often assumed to be adaptive, although this is not necessarily true (Schmitt *et al*. 1999; van Kleunen and Fischer 2001; Caruso *et al*. 2006; Acasuso-Rivero *et al*. 2019).

In fact, plastic responses of plants to different environments are likely to be neutral or even maladaptive (also referred as non-adaptive; Nicotra *et al*. 2015). The prerequisites for adaptive plasticity are differences in selective pressures among environments as well as a selective advantage of the induced phenotype in the inductive environment (Dorn *et al*. 2000; van Kleunen and Fischer 2001; Weinig *et al*. 2004). Adaptive plasticity promotes the expression of an optimal phenotype in each given environment (Wang *et al*. 2016). However, the induced phenotype is sometimes further away from the local optimum, responding in the opposite direction as what is favored by selection in that derived environment, consequently leading to decreased mean fitness across environments, and such plasticity is considered as maladaptive (Ghalambor *et al*. 2007; Scheiner 2013). In addition, neutral plasticity in a trait having no effect on plant fitness could be attributed to lack of selection either for or against variation accumulated through processes such as mutation or selection on other functionally related traits (Alpert and Simms 2002).

Some studies have empirically tested the adaptive value of phenotypic plasticity, commonly using the phenotypic or genetic selection analysis that reveals the strength and

direction of selection (Lande and Arnold 1983; Avramov *et al*. 2007; Wang *et al*. 2018; Arnold *et al.* 2019b). However, conclusive evidence for adaptive plasticity has only been obtained for a few traits (van Kleunen and Fischer 2005; Huang *et al*., 2015; Engqvist *et al*., 2016; Acasuso-Rivero *et al*. 2019; Arnold *et al.* 2019a). More studies for a wider variety of plant functional traits and environmental factors are still needed to further understand the adaptive significance of plasticity (Dorn *et al*. 2000; Palacio-Lopez *et al*. 2015; Acasuso-Rivero *et al*. 2019).

Light and mineral nutrients are two of the most essential environmental factors with substantial spatial and temporal variation, eliciting ecologically important plant responses, including specific adjustments in all aspects of their phenotype - growth, morphology, and physiology (Franklin and Whitelam 2005; Avramov *et al*. 2007). Morphological and physiological traits are two important groups of functional traits that impact plant fitness (Roiloa and Hutchings 2013; Masarovičová *et al*. 2015). Morphological changes in petioles, internodes and leaves are often cited as crucial for plants in capturing light and obtaining nutrients (Picotte *et al*. 2007; Li *et al*. 2018). Photosynthesis is one of the most important physiological activities in plants, as it controls carbon assimilation and therefore primary productivity (Wang *et al*. 2020). Chlorophyll, the principal pigment accounting for absorption of solar radiation to drive reactions of photosynthesis, not only determines plant photosynthetic capacity, but also is a key factor indicating nutrient status of plants (Silla *et al*. 2010; Wang *et al*. 2020).

Clonal growth in plants is characterized by vegetative reproduction of ramets (asexual individuals) that remain physically connected via stolons, rhizomes or roots for a variable period of time (Xu *et al*. 2012; Dong *et al*. 2019; Portela *et al*., 2019). Via clonal growth, many clonal plants can form large networks colonizing a considerable area and therefore have a greater likelihood of encountering environmental heterogeneity (Roiloa *et al*., 2014;

Portela *et al*., 2020). Clonal functional traits such as foraging behavior, clonal integration and division of labor are expected to benefit performance of clonal plants in the face of temporal and spatial environmental variation, as these properties are conducive to resource exploitation, stress buffering, internal resource exchange, and risk spreading (e.g. van Kleunen and Fischer 2001; Wang *et al*. 2017; Lin *et al*. 2018; Gao *et al*. 2020). Moreover, clonal plants are good model systems to study adaptive value of plasticity because genotypes of clonal plants can be easily replicated by vegetative growth (clonal growth). In other words, the genetic background of individual plants (ramets) used for different treatments can be strictly controlled to be the same, so that we can make sure that any differences between treatments are due to differences in environmental conditions (i.e. phenotypic plasticity), but not genetic variation (Wang *et al*. 2018).

While the adaptive value of plasticity has been assessed in a number of clonal and non-clonal plant species for a number of traits (e.g. Weijschedé *et al*. 2006; Maherali *et al*. 2010; Engqvist *et al*., 2016; Wang *et al*. 2018; Acasuso-Rivero *et al*. 2019), few studies have investigated adaptive plasticity in morphological and physiological traits simultaneously, particularly in stoloniferous clonal plants. Stoloniferous clonal plants consisting of multiple, genetically identical individuals (ramets) interconnected through aboveground lateral extended stems (stolons) often possess stronger dispersal ability, facilitating their wide spread in various heterogeneous habitats (Herben and Klimešová 2019; Adomako *et al*. 2020).

Here, we grew 21 genotypes of the stoloniferous clonal plant *Duchesnea indica* under different light and nutrient conditions (control vs. shade/low nutrient availability), and used selection gradient analyses to investigate adaptive plasticity in their morphological and physiological traits. Specifically, we aim to answer the following questions. (1) What are the phenotypic responses of *D. indica* to different light and nutrient conditions? (2) Is there variation among genotypes in their phenotypic plasticity? (3) Is the plasticity in response to

light or nutrient availability adaptive? We predicted that the adaptive value of plasticity in different traits is environment-specific.

Material and methods

Plant species

Duchesnea indica (Andr.) Focke (Rosaceae) is a perennial rosette herb, and occurs in various habitats in Asia, including hillside, river bank, roadside, and grassland areas (Wang *et al*. 2012). In natural habitats, the species often experiences spatial and temporal variation in light and nutrient availability. Each leaf is composed of a slender petiole with three leaflets. The species can reproduce clonally (vegetatively) by producing the stolon along which each node has the potential to produce roots and leaves, forming a ramet (Supplementary Fig. S1; Wang *et al*. 2012). *D. indica* also reproduces sexually by producing red fleshy fruits; it flowers from June to August and fruits from August to October (Flora of China Editorial Committee 2003; Wang *et al*. 2012).

Experimental material

Between August 2011 and April 2012, ramets of *D. indica* were collected from 33 populations across China and then vegetatively propagated in a greenhouse in Beijing (Liu *et al*. 2016). The genotypes of the collected ramets were identified based on analysis of microsatellite markers (Liu *et al*. 2016). In this study, 21 genotypes belonging to 15 populations were used (Supplementary Fig. S2). On July 15, 2013, 15 ramets originating from each of the 21 genotypes (totaling 315 ramets) were disconnected from their genets and selected for the experiment described below. All these ramets were at the same

developmental stage and thus similar in size, i.e. with four to five leaves, and some roots. Each ramet was planted in a pot (17 cm in diameter and 10 cm in height) filled with a 1:1 (v:v) mixture of river sand and peat. Since before the start of the experiment plants had been cultivated under the same greenhouse conditions across several generations after the field collection, potential maternal effects on different genotypes should be minimal.

Experimental design

The 15 ramets of each genotype were randomly assigned to three treatments, and each treatment had five replicates (ramets). The three treatments were (1) control, (2) shade, and (3) low nutrient availability. The control was shared by both the shade and the low nutrient treatment. The experiment was conducted in an open area in Bajia in Beijing. In the control treatment, the ramet received 80% sunlight by covering with a black, neutral shading net, and the soil in the pot was evenly mixed with 2 g L^{-1} slow release fertilizer (20% N, 20% P, 20% K, Peters Professional, Scotts-Sierra Horticultural Products Co., Marysville, Ohio, USA). In the shade treatment, ramets received only 50% of the light in the control environment, with the same soil and fertilizer mixture as that used in the control treatment. In the low nutrient treatment, each ramet received the same amount of sunlight as that in the control treatment, and the soil was supplemented with only 0.5 g L^{-1} fertilizer.

The experiment started on July 15, 2013 and ended on October 24, 2013. Tap water was supplied regularly to keep the soil moist. During the experiment, all initial (mother) ramets produced stolons and offspring ramets. The offspring ramets were placed in the same light conditions as their mother (initial) ramets. We did not add additional pots (with soil) for the offspring ramets. Thus, along each stolon, only the first offspring ramet closest to the mother

ramet might root in the pot where the mother ramet grew. No other offspring ramets rooted during the experiment.

Harvest and measurements

For each plant (the initial mother ramet plus all of its offspring ramets and stolons), fruits and ramets were counted and two primary stolons of similar developmental stages were selected. Biomass, ramet number and fruit number were used as fitness proxies to reflect survival, growth and reproduction of *D. indica* individuals (Tchokponhoué *et al*., 2019). Along each selected stolon, the lengths of the third, fourth, and fifth internode and the length of the mature petioles of the ramets on the third, fourth, and fifth node were measured. The length of three randomly selected mature petioles of each mother ramet was also measured. For plants with abundant leaves, ten mature leaves were randomly selected, and for plants with fewer leaves, five were selected. Areas (WinFOLIA, Pro2004a, Regent Instruments, Québec, Canada) and biomass of the selected leaves of each plant were determined after drying in an oven at 70°C for 48 h. Each plant was then separated into laminae, petioles, stolons, and roots, and biomass of each part was measured after drying at 70°C for 48 h. For each plant, mean petiole length of the mother ramet, mean petiole length of the offspring ramet, mean stolon internode length, specific stolon internode length (stolon length/stolon biomass), mean leaf area, specific leaf area (leaf area/leaf biomass), and root: shoot ratio (root biomass/shoot biomass) were used as morphological measures.

As physiological measures, the chlorophyll content of leaves at various developmental stages was determined before harvesting based on the averages of old (on the fourth node), adult (on the intermediate node), and young leaf (on the last fourth node) chlorophyll contents across two selected stolons. At each node, we selected only the most mature leaf (i.e.

the first developed leaf on that node) to detect chlorophyll content. Due to the fact that stolons extend from the initial (mother) ramets, we can identify that the selected leaves were in different developmental stages, and the relatively younger one was on the last fourth node. The content of chlorophyll was measured using the SPAD-502 Chlorophyll Meter Model (Konica Minolta, Tokyo, Japan).

Statistical analyses

Treatment effects

MANOVA was used to assess the effects of treatment (control, shade, and low nutrient availability; fixed effect), genotype (random effect), and treatment × genotype (random effect) on the overall response of *D. indica*. In the MANOVA model, the independent variables were all traits related to fitness (fruit number, ramet number, and biomass), morphology (mother ramet petiole length, offspring ramet petiole length, stolon internode length, specific stolon internode length, mean leaf area, specific leaf area, and root: shoot ratio), and physiology (chlorophyll content of old, adult, and young leaves) of *D. indica*. Following the MANOVA model, the treatment effect was further separated into the shade effect (control vs. shade) and nutrient effect (control vs. low nutrient availability) by two planned contrasts, and the treatment × genotype effect was also separated into the shade × genotype effect [(control vs. shade) × genotype] and nutrient × genotype effect [(control vs. low nutrient availability) × genotype] by two planned contrasts (Sokal and Rohlf, 1981). Following MANOVA, the results of ANOVA for each variable were also obtained. Before analyses, ramet number and mother ramet petiole length were square-root transformed, and the old leaf chlorophyll content and adult leaf chlorophyll content were transformed by the

inverse square root to improve homoscedasticity. Evidence of plasticity was indicated by a significant treatment effect and/or treatment × genotype interaction (Dorn *et al*., 2000).

Calculation of plasticity

Plasticity of a trait for a genotype responding to variation in light or nutrient availability ($\bar{P}_{ij}$) was calculated according to the formula of Valladares *et al*. (2000) as follows:

$$\bar{P}_{ij} = \frac{|\bar{Z}_{ij,1} - \bar{Z}_{ij,2}|}{\text{Max}\{\bar{Z}_{ij,1}, \bar{Z}_{ij,2}\}} \qquad (1)$$

where $\bar{Z}_{ij,1}$ is the mean value of trait *i* for genotype *j* across replicates in the control treatment, and $\bar{Z}_{ij,2}$ is the mean value of trait *i* for genotype *j* across the replicates in the shade or low nutrient treatment.

Adaptive value of plasticity

Two complementary regression approaches were used to test the adaptive value of plasticity to variation in light or nutrient availability. First, an across-environment genotypic selection analysis was conducted based on the equation of van Kleunen and Fisher (2001):

$$\bar{W}_j = \gamma_i + \alpha_i \bar{Z}_{ij} + \beta_i \bar{P}_{ij} \qquad (2)$$

where $\bar{W}_j$ is the relativized mean fitness of genotype (*j*) over two environments (control and shade, or control and low nutrient availability), $\bar{Z}_{ij}$ is the standardized mean value of trait *i* for genotype *j* across the corresponding two treatments, $\bar{P}ij$ is the standardized plasticity of trait *i* for genotype *j* in response to the treatment (shade or low nutrient availability), γ_i is the constant term of the regression equation for trait *i*, and α_i and β_i are the two partial regression

coefficients of the equation for trait *i*. Thus, α_i and β_i indicate the relationship of a fitness measure with the mean value and plasticity of trait *i*, respectively. A significant positive value of β_i indicates that plasticity of trait *i* is adaptive (i.e. more plastic genotypes have a higher fitness across the two conditions), a significant negative value of β_i indicates that plasticity of trait *i* is maladaptive (i.e. more plastic genotypes have a lower fitness across the two conditions), and a non-significant value of β_i indicates that plasticity of trait *i* is neutral (i.e. similar fitness between plastic and fixed genotypes across the two conditions). A significant positive or negative value of α_i suggests that the mean value of trait *i* is positively or negatively related to the fitness measure. Each genotypic mean fitness across treatments was relativized by dividing by the grand mean fitness of all genotypes across treatments, and each independent variable was standardized to mean = 0 and variance = 1 (Caruso *et al*. 2006). Regression coefficients are expressed as standardized values to allow comparisons among coefficients.

Second, a within-environment phenotypic selection analysis was used to calculate selection differentials by regressing each individual plant's relativized fitness on its standardized traits in each environment (Caruso *et al*. 2006). Relative fitness within each environment was estimated as a fitness measure of an individual divided by the mean fitness in that environment. Regression models were run separately for each combination of environment and trait. A plastic response in the same direction as selection within an environment is considered adaptive, and a response in the opposite direction of selection is maladaptive. Plasticity is classified as neutral when no significant selection acts on that trait in the relevant treatment (Dorn *et al*. 2000; Caruso *et al*. 2006; Maherali *et al*. 2010). If selection exists in one environment, a genotype × treatment interaction acts on the plant without a treatment effect, and genotypes respond differently in direction (i.e. some genotypes respond in the same direction as selection and others respond in the opposite

direction), then we consider the plastic response to be both adaptive and maladaptive (Dorn *et al*. 2000; Maherali *et al*. 2010). If selection was detected in a pair of environments (control vs. shade or control vs. low nutrient availability), ANCOVA was used to test differences in selection differentials between these two environments, with the standardized trait value as the covariate, treatment as a main effect, and relative fitness as the dependent variable. A significant treatment × trait interaction indicates that the selection pattern differs between environments (Bell and Galloway 2008).

As the sign and magnitude of a regression coefficient (for across-environment genotypic selection analysis) or selection differential (for within-environment phenotypic selection analysis) describe the direction and strength of linear selection, further indicating different types of plasticity (i.e. adaptive, maladaptive, or neutral plasticity), we used one-tailed tests to examine the significance. To control Type I error rates, a sequential Bonferroni procedure was used to correct *p*-values for multiple comparisons (Rice 1989). All analyses were implemented in SPSS 19.0 (SPSS, Chicago, IL, USA).

Results

Effects of treatment and genotypic variation

There were highly significant overall effects of treatment (MANOVA result: Wilk's λ = 0.060, $F_{26, 336} = 39.68$, $P < 0.001$), genotype (Wilk's $\lambda = 0.015$, $F_{260, 1874} = 3.35$, $P < 0.001$), and their interaction (Wilk's $\lambda = 0.025$, $F_{520, 2131} = 1.42$, $P < 0.001$) on the traits of *D. indica* (Table 1). Plants grown in the shade treatment exhibited lower values for fitness measures (fruit number, ramet number, and biomass), internode length, and adult leaf chlorophyll content, but higher petiole length of the mother ramet and offspring ramet, specific internode

length, specific leaf area, and old leaf chlorophyll content, than plants grown in the control treatment (Table 1; Fig. 1). Variation among genotypes was detected for all traits except specific leaf area (Table 1). A shade-genotype interaction was detected for ramet number, petiole length of the mother ramet, mean leaf area, old leaf chlorophyll content, and young leaf chlorophyll content (Table 1), indicating that plastic responses of these traits to light variation differed among genotypes.

Compared to the control, plants grown in the low nutrient condition had lower petiole length, mean leaf area, specific leaf area, and chlorophyll content, but higher fruit number and root: shoot ratio (Table 1; Fig. 1; Supplementary Fig. S3). A significant interaction of nutrient availability and genotype was detected for biomass, petiole length of the mother ramet, and internode length (Table 1).

Adaptive value of plasticity to light variation

In cross-environment analyses, we detected a significant negative regression coefficient for the plasticity of young leaf chlorophyll content (Table 2a), suggesting that genotypes with greater plasticity in the young leaf chlorophyll content had a lower fitness across treatments and that this plasticity is maladaptive. Within-environment analyses indicated that there was significant selection for the longer mother ramet petiole (for biomass, selection was stronger under shade; ANCOVA, $F_{1, 192} = 5.437$, $P = 0.021$) and offspring ramet petiole (for biomass, selection was similar between control and shade; $F_{1, 175} = 1.642$, $P = 0.202$) within the shade treatment and for lower old leaf chlorophyll content in the control condition (Table 3). Given that petioles elongated under shade, while chlorophyll in old leaves decreased under normal light (Fig. 1d, e and j), plasticity of these three traits was adaptive. In addition, selection favored longer internode, lower specific internode length (for biomass, selection was similar

between control and shade; ANCOVA, $F_{1, 185} = 1.383$, $P = 0.241$), and higher adult leaf chlorophyll content under shade, in contrast to plant responses in which the internode length and adult leaf chlorophyll content decreased and specific internode length increased under shade (Table 3; Fig. 1f, g and k), indicating maladaptive plasticity of these traits. Selection favored larger leaf area within the shade treatment (for biomass, selection was stronger under shade; ANCOVA, $F_{1, 172} = 3.984$, $P = 0.048$, Table 3). However, we detected a significant shade × genotype interaction without a treatment effect for this trait (Table 1). The plastic response of leaf area to light variation differed among genotypes; some genotypes exhibited an increase of leaf area under shade and others exhibited a decrease (mixed-direction plastic responses, Fig. 1h). Plasticity of leaf area was therefore both adaptive and maladaptive.

Adaptive value of plasticity to nutrient variation

In cross-environment analyses, significant regression coefficients for plasticity were not obtained for any traits (Table 2b). Within-environment analyses detected significant selection for lower chlorophyll of old and adult leaves under low nutrient availability, in the same direction as the plastic responses of these two traits (Table 3; Fig. 1j and k). Thus, decreased chlorophyll of old and adult leaves under the low nutrient treatment were likely adaptive. Selection favored the longer mother ramet petiole (for biomass, selection was similar between control and low nutrient conditions; ANCOVA, $F_{1, 195} = 0.264$, $P = 0.608$) and offspring ramet petiole (for biomass, selection was similar between control and low nutrient conditions; ANCOVA, $F_{1, 183} = 0.006$, $P = 0.941$), larger leaf area (for biomass, selection was similar between control and low nutrient conditions; ANCOVA, $F_{1, 181} = 198$, $P = 0.657$), and reduced root: shoot ratio under low nutrient availability, in contrast to plant responses, including a decrease of petiole length and leaf area, and an increase of root: shoot ratio (Table

3; Fig. 1d, e and h; Supplementary Fig. S3), indicating maladaptive plasticity of these traits. Selection favored longer internode length in the low nutrient treatment (Table 3). We observed a significant nutrient × genotype interaction for this trait, with some genotypes exhibited an increase of internode length and others exhibiting a decrease (Table 1; Fig. 1f). Thus, plasticity of internode length was in mixed directions and thereby both adaptive and maladaptive.

Discussion

Adaptive value of plasticity to light variation

Petiole length and specific leaf area of *D. indica* increased under shade, congruent with previous studies of common shade avoidance responses in plants, such as the elongation of petioles and vertical stem internodes to improve light foraging and increases in leaf area per unit mass (i.e. specific leaf area) to expand photon-harvesting surfaces (Dorn *et al*. 2000; Steinger *et al*. 2003; Weijschedé *et al*. 2006; Bell and Galloway 2008). The increase of petiole length responding to shading was adaptive, similar to observations in some other species (e.g. *Geranium carolinianum*; Bell and Galloway 2008). By vertical elongation, the plant can place leaves at a higher level to enhance light capture and ameliorate the reduced availability of light, further conferring its fitness advantage (Weijschedé *et al*. 2006; Huber *et al*. 2011). However, increased specific leaf area under shade is neutral plasticity without an impact on individual fitness. One possible explanation is that strong selection toward the optimal reaction norm has depleted genetic variation around the trait optimum, leading to little opportunity to detect potential selection, even though the observed plastic response is actually adaptive (Dorn *et al*. 2000).

Shade-induced horizontal stem elongation is a well-documented example of adaptive plasticity as it helps plants escape from photosynthesis limitation caused by shading and thereby enhances plant fitness (Alpert and Simms 2002). In this study, shortened and thinner internodes towards the phalanx growth form (i.e. forming closely packed clumping ramets) under shade were maladaptive, potentially because the limited carbon investment to internodes did not increase light capture when plants cannot overtop the foliage canopy (Dorn *et al*. 2000; Xue *et al*. 2018, 2020). Therefore, we inferred that horizontal light foraging of *D. indica* was inhibited and that it mainly depended on vertical elongation to maximize lifetime light interception.

Many clonal plants can form large connected clonal networks, with ramets of the same clone expressing a range of phenotypic responses to environmental changes (Wang *et al.* 2017; Gao *et al.* 2020). We found that increased old leaf chlorophyll content in response to shading was adaptive, while the decrease of adult leaf chlorophyll content was maladaptive. Also, genotypes with greater plasticity of young leaf chlorophyll content experienced a fitness disadvantage across environments over genotypes with a fixed trait expression. The difference in adaptive significance of physiological responses at different growth stages may be caused by clonal integration and division of labor (Roiloa and Hutchings 2013; Dong *et al*. 2019). It is reported that physiological integration of clonal plants has effects on light reactions of the photosynthetic processes (e.g. Roiloa *et al*. 2014). With connected stolons, physiological integration among ramets allows transport of resources, such as photosynthates, water and nutrients, from established (older) ramets to developing (younger) ramets (Roiloa and Hutchings 2013; Dong *et al*. 2019). Such physiological integration could also facilitate to develop a division of labor in clonal plants (Roiloa *et al*. 2007). In this sense, our results also suggest that old leaves tend to specialize for abundance (light capture) by increasing chlorophyll content and posterior reciprocal transport of the captured resource to the

connected younger ramet, while adult and young ramets that received resources would promote escape from unfavorable conditions into new habitats (Roiloa and Hutchings 2013; Roiloa *et al*. 2014).

Adaptive value of plasticity to nutrient variation

Under low nutrient availability, plants often allocate more resources to belowground organs (indicated by higher root: shoot ratio for low nutrient availability than for control) to facilitate nutrient absorption and buffer fitness reduction (as indicated by constant ramet number and total biomass in our study; Zhang *et al*. 2007; Littschwager *et al*. 2010; Roiloa and Hutchings 2013). Expenditures of the limited aboveground nutrient into fruit production may provide a way of escaping from the low nutrient condition to better environments by seed dispersal (Jacquemyn *et al*. 2006). Due to deficiency of aboveground resource allocation, petiole length, leaf area, and specific leaf area decreased, subsequently leading to reduced light capture ability and lower leaf chlorophyll content. Decreases in petiole length and leaf area under the low nutrient condition were maladaptive. It might because that allocation of limited resources to structural or defensive compounds, rather than to photosynthetic components, results in reduced photosynthesis that cannot compensate for the decrease in fitness caused by nutrient limitation (Sage and Pearcy 1987; Funk *et al*. 2007; Littschwager *et al*. 2010). It is reported that decreased specific leaf area was favored by selection under low nutrient availability to prolong leaf life spans and maximize nutrient retention (Bonser *et al*. 2010). However, we detected no selection acting on plasticity of this trait.

Decreased chlorophyll content in old, adult and young leaves under low nutrient availability suggest that nutrient deficiency accelerates leaf senescence at the whole plant

level. For old and adult ramets, such response is adaptive, in line with the notion that nutrient withdrawal from less effective leaves during senescence is advantageous under nutrient shortage stress (Munné-Bosch & Alegre 2004; Sandner and Matthies 2018). Chlorophyll content is an indicator of nitrogen content (Caruso *et al*. 2006). Funk *et al*. (2007) detected a positive relationship between photosynthetic nitrogen-use efficiency and leaf nitrogen, and found that lower photosynthetic nitrogen-use efficiency in response to low nutrient availability was adaptive. However, the decreased young leaf chlorophyll content under the low nutrient condition is neutral plasticity, which may suggest that stoloniferous clonal plants start to "move" by giving up the elderly ramets to search for new habitats.

As foraging behavior of stoloniferous clonal plants, longer and thicker spacer, elongated petiole, and larger leaf area were selected for under unfavorable conditions (shade or low nutrient availability). However, we only detected adaptive foraging traits in petiole length responding to shading, suggesting that factors like light and nutrient availability do not trigger a predictable adaptive foraging pattern across all plant traits. Besides biomass, trait plasticity was more closely related to asexual reproduction implications (i.e. ramet number) for light availability, while to sexual reproduction (i.e. fruit number) for nutrient availability variation. Such results suggest that there might be a trade-off between asexual and sexual reproduction under different environmental factors, with light-capture modules affecting asexual reproduction, whereas fruit number reflecting nutrient storage. Therefore, we conclude that the adaptive value of plasticity in different traits depends on the specific ecological context.

The common form of adaptive response with the same direction favored by directional selection but below the local adaptive peak could increase the frequency of beneficial alleles, further promoting local adaptation and serving as a critical bridge to adaptive evolution (Ghalambor *et al*. 2015; Wagner and Mitchell-Olds 2018). Moreover, adaptive trait plasticity

may enhance the ecological amplitude of clonal species when encountering a broad range of habitats (Wang and Hu *et al*. 2016). Nevertheless, to date, there is still limited empirical evidence demonstrating the evolutionary and ecological consequences of adaptive plasticity in natural populations (Fischer *et al*. 2016; Grenier *et al*. 2016).

Conclusions

We revealed possible key morphological and physiological traits in which plasticity (e.g. elongated petiole length and increased old leaf chlorophyll content in response to shade, and decreased adult and old leaf chlorophyll content in response to low nutrient availability) could contribute to adaptation of *D. indica* to light and nutrient availability variation. Such understanding of adaptive phenotypic plasticity could be indicative of future adaptive potential in stoloniferous clonal plants to colonize variable environments. However, considering plant responses to isolated environmental cues in this study may limit our understanding of the adaptive value of plasticity under natural conditions, where environmental change is often multifactorial (Lampei 2019). Moreover, at the whole organism level, a new environment could simultaneously induce different types of plasticity (adaptive, maladaptive or neutral) in a suite of traits, but the consequences of such mosaic responses for evolution on ecological time-scales remains largely unexplored (Ghalambor *et al*. 2007).

Funding

This work was supported by the National Natural Science Foundation of China (31870610, 31761123001) and the Ten Thousand Talent Program of Zhejiang Province (2018R52016) and the Joint Fund of Zhejiang Provincial Natural Science Foundation (LTZ20C030001). The authors declare no conflict of interests.

Acknowledgments

The authors thank Prof. Ming Yue, Dr. Fang-Li Luo, and many students for help with plant collection.

References

Acasuso-Rivero C, Murren CJ, Schlichting CD, *et al.* (2019) Adaptive phenotypic plasticity for life-history and less fitness-related traits. *P Roy Soc B: Biol Sci* **286**: 1904.

Adomako MO, Alpert P, Du DL, *et al*. (2020) Effects of clonal integration, nutrients and cadmium on growth of the aquatic macrophyte *Pistia stratiotes*. *J Plant Ecol* **13:** 765-772.

Alpert P, Simms EL (2002) The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evol Ecol* **16**: 285-297.

Arnold PA, Kruuk LEB, Nicotra AB (2019a) How to analyse plant phenotypic plasticity in response to a changing climate. *New Phytol* **222:** 1235-1241

Arnold PA, Nicotra AB, Kruuk LEB (2019b) Sparse evidence for selection on phenotypic plasticity in response to temperature. *Phil Trans R Soc B* **374:** 20180185

Avramov S, Pemac D, Tucić B (2007) Phenotypic plasticity in response to an irradiance gradient in *Iris pumila*: adaptive value and evolutionary constraints. *Plant Ecol* **190**: 275-290.

Bell DL, Galloway LF (2008) Population differentiation for plasticity to light in an annual herb: adaptation and cost. *Am J Bot* **95**: 59-65.

Bonser SP, Ladd B, Monro K, *et al.* (2010) The adaptive value of functional and life-history traits across fertility treatments in an annual plant. *Ann Bot-London* **106**: 979-988.

Caruso CM, Maherali H, Sherrard M (2006) Plasticity of physiology in *Lobelia*: testing for adaptation and constraint. *Evolution* **60**: 980-990.

Dong BC, Zhang LM, Li KY, *et al*. (2019) Effects of clonal integration and nitrogen supply on responses of a clonal plant to short-term herbivory. *J Plant Ecol* **12:** 624-635.

Dorn LA, Pyle EH, Schmitt J (2000) Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptive value and costs. *Evolution* **54**: 1982-1994.

Flora of China Editorial Committee (2003) Flora of China. Science Press and Missouri Botanical Garden Press, Beijing, China.

Fischer EK, Ghalambor CK, Hoke KL (2016) Can a network approach resolve how adaptive vs nonadaptive plasticity impacts evolutionary trajectories? *Integ Comp Biol* **56**: 877-888.

Franklin KA, Whitelam GC (2005) Phytochromes and shade-avoidance responses in plants. *Ann Bot-London* **96**: 169-175.

Funk JL, Jones CG, Lerdau MT (2007) Leaf- and shoot-level plasticity in response to different nutrient and water availabilities. *Tree Physiol* **27**: 1731-1739.

Gao FL, Alpert P, Yu FH (2020) Parasitism induces negative effects of physiological integration in a clonal plant. *New Phytol* **229:** 585-592.

Ghalambor CK, McKay JK, Carroll SP Reznick DN (2007) Adaptive versus nonadaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol* **21**: 394-407.

Ghalambor CK, Hoke KL, Ruell EW, *et al.* (2015) Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* **525**: 372-375.

Grenier S, Barre P, Litrico I (2016) Phenotypic plasticity and selection: nonexclusive mechanisms of adaptation. *Scientifica* **2016**: 1-9.

Hans Jacquemyn, Rein Brys, Olivier Honnay, *et al*. (2006) Sexual reproduction, clonal diversity and genetic differentiation in patchily distributed populations of the temperate forest herb *Paris quadrifolia* (Trilliaceae). *Oecologia* **147**: 434-444.

Herben T, Klimešová J (2019) Evolution of clonal growth forms in angiosperms. *New Phytol* **225**: 999-1010.

Huber H, von Wettberg EJ, Aguilera A, *et al.* (2011) Testing mechanisms and context dependence of costs of plastic shade avoidance responses in *Impatiens capensis* (Balsaminaceae). *Am J Bot* **98**: 1602-1612.

Lampei C (2019) Multiple simultaneous treatments change plant response from adaptive parental effects to within‐generation plasticity, in *Arabidopsis thaliana. Oikos* **128**: 368-379.

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* **37**: 1210-1226.

Li KN, Chen JS, Wei Q, *et al.* (2018) Effects of transgenerational plasticity on morphological and physiological properties of stoloniferous herb *Centella asiatica* subjected to high/low light. *Front Plant Sci* **9**: 1640.

Lin HF, Alpert P, Zhang Q, *et al.* (2018) Facilitation of amphibious habit by physiological integration in the clonal, perennial, climbing herb *Ipomoea aquatica*. *Sci Total Environ* **618**: 262-268.

Littschwager J, Lauerer M, Blagodatskaya E, *et al.* (2010) Nitrogen uptake and utilisation as a competition factor between invasive *Duchesnea indica* and native *Fragaria vesca*. *Plant Soil* **331**: 105-114.

Liu CX, Li JM, Li HL, *et al.* (2016) Genetic diversity and structure of a stoloniferous clonal plant *Duchesnea indica*. *Jiangsu J Agr Sci* **44**: 215-220.

Maherali H, Caruso CM, Sherrard ME (2010) Adaptive value and costs of physiological plasticity to soil moisture limitation in recombinant inbred lines of *Avena barbata*. *Am Nat* **175**: 211-224.

Masarovičová E, Májeková M, Vykouková I (2015) Functional traits and plasticity of plants in ecological research and education. *Chem Didact Ecol Metrol* **20**: 59-65.

Nicotra AB, Segal DL, Hoyle GL, *et al.* (2015) Adaptive plasticity and epigenetic variation in response to warming in an Alpine plant. *Ecol Evol* **5**: 634-647.

Palacio-Lopez K, Beckage B, Scheiner S, *et al.* (2015) The ubiquity of phenotypic plasticity in plants: a synthesis. *Ecol Evol* **5**: 3389-3400.

Picotte JJ, Rosenthal DM, Rhode JM, *et al.* (2007) Plastic responses to temporal variation in moisture availability: consequences for water use efficiency and plant performance. *Oecologia* **153**: 821-832.

Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? *Trends Ecol Evol* **20**: 481-486.

Portela R, Dong BC, Yu FH, *et al*. (2019) Effects of physiological integration on defense strategy against herbivory by the clonal invader *Alternanthera philoxeroides*. *J Plant Ecol* **12**: 662-672.

Portela R, Dong BC, Yu FH, *et al*. (2020) Trans-generational effects in the clonal invader *Alternanthera philoxeroides*. *J Plant Ecol* **13**: 122-129.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution* **43**: 223-225.

Roiloa SR, Alpert P, Tharayil N, *et al*. (2007) Greater capacity for division of labour in clones of *Fragaria Chiloensis* from patchier habitats. *J Ecol* **95:** 397-405.

Roiloa SR, Hutchings MJ (2013) The effects of physiological integration on biomass partitioning in plant modules: an experimental study with the stoloniferous herb *Glechoma hederacea*. *Plant Ecol* **214**: 521-530.

Roiloa SR, Rodriguez-Echeverria S, Lopez-Otero A, *et al.* (2014) Adaptive plasticity to heterogeneous environments increases capacity for division of labor in the clonal invader *Carpobrotus edulis* (Aizoaceae). *Am J Bot* **101**: 1301-1308.

Sage RF, Pearcy RW (1987) The nitrogen use efficiency of C3 and C4 plants. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiol* **84**: 959-963.

Scheiner SM (2013) The genetics of phenotypic plasticity. XII. Temporal and spatial heterogeneity. *Ecol Evol* **3**: 4596-4609.

Schmitt J, Dudley SA, Pigliucci M (1999) Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade-avoidance responses in plants. *Am Nat* **154**: S43-S54.

Silla F, González-Gil A, González-Molina ME, *et al.* (2010) Estimation of chlorophyll in Quercus leaves using a portable chlorophyll meter: effects of species and leaf age. *Annals of Forest Science* **67**: 108.

Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. Freeman, New York

Steinger T, Roy BA, Stanton ML (2003) Evolution in stressful environments II: adaptive value and costs of plasticity in response to low light in *Sinapis arvensis*. *J Evol Biol* **16**: 313-323.

Sultan SE (1995) Phenotypic plasticity and plant adaptation. *Acta Bot Neerl* **44**: 363-383.

Tchokponhoué DA, N'Danikou S, Houéto JS, *et al.* (2019) Shade and nutrient-mediated phenotypic plasticity in the miracle plant *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell. *Scientific Reports* **9:** 5135.

Valladares F, Wright SJ, Lasso E, *et al.* (2000) Plastic phenotypic response to light of 16 congeneric shrubs from a panamanian rainforest. *Ecology* **7**: 1925-1936.

van Kleunen M, Fischer M (2001) Adaptive evolution of plastic foraging responses in a clonal plant. *Ecology* **82**: 3309-3319.

van Kleunen M, Fischer M (2005) Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytol* **166**: 49-60.

van Kleunen M, Fischer M (2007) Progress in the detection of costs of phenotypic plasticity in plants. *New Phytol* **176**: 727-730.

van Kleunen M, Lenssen JPM, Fischer M, *et al.* (2007) Selection on phenotypic plasticity of morphological traits in response to flooding and competition in the clonal shore plant *Ranunculus reptans*. *J Evol Biol* **20**: 2126-2137.

Wagner MR, Mitchell-Olds T (2018) Plasticity of plant defense and its evolutionary implications in wild populations of *Boechera stricta*. *Evolution* **72**: 1034-1049.

Wang MZ, Bu XQ, Li L, *et al.* (2018) Constraints on the evolution of phenotypic plasticity in the clonal plant *Hydrocotyle vulgaris*. *J Evol Biol* **31:** 1006-1017.

Wang P, Lei JP, Li MH, *et al.* (2012) Spatial heterogeneity in light supply affects intraspecific competition of a stoloniferous clonal plant. *PLoS ONE* **7**: e39105.

Wang SQ, Li Y, Ju WM, *et al.* (2020) Estimation of leaf photosynthetic capacity from leaf chlorophyll content and leaf age in a subtropical evergreen coniferous plantation. *Journal of geophysical research: Biogeosciences* **125**: e2019JG005020.

Wang YJ, Müller-Schärer H, van Kleunen M, *et al.* (2017) Invasive alien plants benefit more from clonal integration in heterogeneous environments than natives. *New Phytol* **216**: 1072-1078.

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

Weinig C, Gravuer KA, Kane NC, *et al.* (2004) Testing adaptive plasticity to UV: costs and benefits of stem elongation and light-induced phenolics. *Evolution* **58**: 2645-2656.

Weinig C, Johnston J, German ZM, *et al.* (2006) Local and global costs of adaptive plasticity to density in *Arabidopsis thaliana*. *Am Nat* **167**: 826-836.

Xu L, Yu FH, van Drunen E, *et al.* (2012) Trampling, defoliation and physiological integration affect growth, morphological and mechanical properties of a root-suckering clonal tree. *Ann Bot-London* **109:** 1001-1008.

Xue W, Huang L, Yu FH, *et al.* (2018) Intraspecific aggregation and soil heterogeneity: competitive interactions of two clonal plants with contrasting spatial architecture. *Plant Soil* **425**: 231-240.

Xue W, Huang L, Yu FH (2020) Importance of starting points in heterogeneous environments: interactions between two clonal plants with contrasting spatial architectures. *J Plant Ecol* **13**: 323-330.

Zhang SM, Yu FH, Dong M (2007) Nutrient availability affects biomass allocation to stolons in *Potentilla reptans* var. *sericophylla*. *Chinese J Plant Ecol* **31**: 652-657.

Table 1 Summary of MANOVA and ANOVA results for effects of treatments, genotypes, and their interaction on (**a**) the overall response and (**b**) the response of each trait in *Duchesnea indica*. The treatment effect was further separated into the shade effect (S) and the low nutrient effect (LN) by two planned contrasts, and the treatment × genotype effect was further separated into the S × G effect and the LN × G effect by two planned contrasts.

Trait	Treatment (T)			Genotype	T × G		
	Overall	S	LN	(G)	Overall	S × G	LN × G
(a) MANOVA							
All	**39.7*****	**23.4*****	**48.2*****	**3.3*****	**1.4*****	**1.3*****	**1.3****
(b) ANOVA							
Fitness trait							
Fruit number	**32.3*****	**23.7*****	**12.5*****	**6.1*****	1.4	0.7	1.4
Ramet number	**54.1*****	**77.2*****	1.1	**8.1*****	**1.9****	**2.4*****	1.5
Biomass	**49.0*****	**74.4*****	0.2	**7.7*****	**1.8****	1.5	**2.2****
Morphological trait							
Petiole length of mother ramet	**52.1*****	**28.5*****	**29.1*****	**9.3*****	**1.9****	**1.6***	**2.0****
Petiole length of offspring ramet	**71.4*****	**62.2*****	**20.2*****	**7.1*****	**1.5***	1.3	1.1
Stolon internode length	**13.9*****	**23.8*****	0.2	**4.5*****	1.4	0.9	**1.8***
Specific stolon internode length	**45.4*****	**65.8*****	0.7	**3.7*****	1.1	1.6	0.6
Mean leaf area	**41.2*****	2.1	**54.4*****	**8.6*****	**1.6***	**1.9***	1.3
Specific leaf area	**31.2*****	**31.4*****	**6.1***	0.7	0.8	0.8	0.5
Root: shoot ratio	**7.0*****	0.003	**11.1*****	**4.6*****	0.7	0.000	1.2
Physiological trait							
Old leaf chlorophyll content	**213.6*****	**27.4*****	**241.6*****	**2.5*****	**1.5***	**1.6***	1.5

Adult leaf chlorophyll content	**230.5*****	**7.0****	**409.6*****	**3.1*****	**1.6***	1.5	1.0
Young leaf chlorophyll content	**11.7*****	0.4	**19.5*****	**1.9***	**1.9****	**1.9***	1.4

F-values and significance levels are reported ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). In MANOVA analysis, df_{num}, df_{denom} = 26, 336 for overall T; df_{num}, df_{denom} = 13, 168 for S and LN; df_{num}, df_{denom} = 520, 2131 for overall T × G; df_{num}, df_{denom} = 260, 1874 for G, S × G, and LN × G. In ANOVA analysis, df_{num}, df_{denom} = 2, 180 for T; df_{num}, df_{denom} = 1, 180 for S and LN; df_{num}, df_{denom} = 40, 180 for T × G; df_{num}, df_{denom} = 20, 180 for G, S × G, and LN × G.

Table 2 Cross-environment analysis of adaptive plasticity for the nine morphological and physiological traits of *Duchesnea indica* in response to variation in (**a**) light and (**b**) nutrient availability. Regression coefficients (β) for the plasticity term are shown (n = 21), and a significant positive value indicates that plasticity is adaptive

Trait	(a) Variation in light availability			(b) Variation in nutrient availability		
	Fruit number	Ramet number	Biomass	Fruit number	Ramet number	Biomass
Morphological trait						
Petiole length of mother ramet	0.087	0.197	0.102	0.127	0.008	-0.111
Petiole length of offspring ramet	-0.046	0.051	-0.001	0.070	-0.010	-0.068
Internode length	-0.310	-0.427	-0.257	-0.439	-0.391	-0.458
Specific internode length	-0.070	-0.217	0.119	-0.096	-0.285	-0.257
Mean leaf area	-0.168	-0.108	-0.152	-0.060	0.114	-0.038
Specific leaf area	-0.210	-0.137	-0.037	0.290	0.123	0.098
Root: shoot ratio	0.205	-0.027	-0.033	0.078	-0.402	-0.171

Physiological trait						
Old leaf chlorophyll content	0.219	-0.060	0.095	0.301	0.425	0.322
Adult leaf chlorophyll content	-0.521	-0.212	-0.265	0.389	0.672	0.684
Young leaf chlorophyll content	**-0.598****	**-0.508****	**-0.577****	-0.217	0.237	0.080

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Significant values of β are shown in bold after Bonferroni correction.

Table 3 Within-environment analysis of selection differentials for the nine morphological and physiological traits of *Duchesnea indica* under the three treatments. Selection differentials were calculated by regressing each individual plant's relative fitness on its standardized traits in each environment (n = 81-102)

Trait	Fruit number			Ramet number			Biomass		
	Control	Shade	LN	Control	Shade	LN	Control	Shade	LN
Morphological trait									
Petiole length of mother ramet	0.09	**0.37*****	**0.30****	0.10	**0.29****	0.17	**0.32*****	**0.54*****	**0.37*****
Petiole length of offspring ramet	0.18	0.21	**0.34*****	0.21	**0.37*****	0.18	**0.52*****	**0.57*****	**0.50*****
Internode length	0.01	0.20	**0.25****	0.10	**0.58*****	0.06	0.24	**0.65*****	**0.25****
Specific internode length	-0.02	-0.18	**-0.40*****	-0.09	**-0.33*****	-0.21	**-0.47*****	**-0.52*****	**-0.57*****
Mean leaf area	0.08	**0.37*****	**0.25****	0.25	**0.40*****	0.22	**0.50*****	**0.62*****	**0.54*****
Specific leaf area	0.06	-0.04	-0.11	0.13	0.08	-0.08	0.05	0.13	-0.19
Root: shoot ratio	-0.17	-0.19	**-0.25****	-0.07	-0.08	0.04	-0.18	-0.07	0.005
Physiological trait									
Old leaf chlorophyll content	**-0.29****	0.05	**-0.40*****	-0.18	-0.02	-0.21	-0.04	0.01	**-0.35*****

Adult leaf chlorophyll content	-0.03	**0.29****	**-0.32*****	0.02	**0.26****	-0.09	0.16	**0.35*****	-0.19
Young leaf chlorophyll content	0.04	0.002	-0.10	0.14	-0.09	0.03	0.16	0.06	0.002

LN – low nutrient availability. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Significant values for selection differentials are shown in bold after Bonferroni corrections.

Figure 1: Responses of the 12 traits of *Duchesnea indica* to light and nutrient variation. Treatment codes: CK – control; S – shade; LN – low nutrient availability. Lines represent the trait values for each of the 21 genotypes, and filled circles stand for the mean trait values across the 21 genotypes. The significance levels for the differences in the mean values between treatments: $^{n.s.}$ $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Figure 1