

Transgenerational plasticity of *Elymus nutans* was regulated by multiple factors and not directly related to within-generational plasticity

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Abstract

Background and Aims

Transgenerational plasticity (TGP) have been proven as significant driver of organisms to cope with environmental rapid changes. However, our understanding of key aspects of TGP is limited, especially its influencing factors and relationship to within-generation plasticity (WGP).

Methods

Firstly, we constructed different maternal and offspring environmental backgrounds for five populations of *E. nutans* through two years of common garden experiments and three years of reciprocal sowing experiments. Subsequently, we examined the influencing factors of TGP of leaf traits and its relationship to WGP.

Results

Maternal environment exerted influences on leaf traits of *E. nutans* for a minimum of three growing seasons. Progeny plant from low altitude maternal environment had larger and thicker leaves than those from high elevation maternal environment. Morphological traits exhibited higher TGP in high elevation progeny environment, while nutritional traits were higher in low elevation progeny environment. TGP diminished in the third growing season. Remarkably, no clear correlation was identified between TGP and WGP.

Conclusions

Our results revealed the dependence of TGP on trait type, progeny environment, population, and growing season and provided the first evidence that TGP are not correlated with WGP, reflecting the complexity of environmental conditions interactions experienced by the maternal and progeny generations. These findings underscore the importance of accounting for the maternal environment when assessing and utilizing *E. nutans*. It is advisable to opt for seed sources from environments with similar elevations for the cross regional using of seed sources in ecological restoration.

Introduction

Understanding how organisms will respond to environmental changes is a major and urgent ecological and evolutionary research theme, which may depend upon our understanding of transgenerational plasticity (TGP), especially against rapid environmental changes caused by climate change and human activities (Donelan et al. 2020; Vivas et al. 2019; Wang et al. 2022). Defined as the influence exerted by environments experienced by previous generations on phenotypes of subsequent generations

independent of genetic contributions (Schmid and Dolt 1994; Van Hinsberg 1998), TGP plays a crucial role in shaping phenotypic traits and could buffer populations against the immediate effects of climate change and provide time for genetic adaptation (Miao et al. 1991b; Walter et al. 2016; Williams et al. 2017).

Both parents can alter the phenotype of their offspring through a range of non-genetic or epigenetic processes and importantly, these processes can be influenced by the environment, thus provide a mechanism by which the parental environment can influence the performance of offspring (Donelson et al. 2018; Vivas et al. 2019). However, these effects have been given various names, such as TGP, non-genetic inheritance, parental effects or maternal effects, carry-over effects, which causing some confusion, especially between TGP, within-generational plasticity (WGP), and developmental plasticity (Donelson et al. 2018; Guillaume et al. 2015; Sturiale and Bailey 2023). For instance, seed size, the most frequently reported trait influenced by diverse maternal environments, which also undergo maternal environment during their formation stage, thus, it is difficult to attribute this part variation to TGP or WGP. Therefore, conducting research on adult traits through multiple generations to mitigate the impact of seed stage developmental plasticity is crucial to determine the duration of transgenerational effects persist and its relationship with WGP (Donelson et al. 2018).

Although environmental changes in nature always affect multiple generations of almost all organisms, the detection of TGP is not always straightforward due to its various influencing factors (Dechaine et al. 2015; González-Rodríguez et al. 2011). TGP could be affected by the magnitude of maternal and progeny environments, genotypes (Groot et al. 2017; Kuijper and Hoyle 2015; Qi et al. 2020), and was reported to diminish over ontogeny (Miao et al. 1991a). Furthermore, its direction and magnitudes varied widely across taxa, where adaptive transgenerational effects are more frequently observed for taxa with short lifespan and limited dispersal (Donelson et al. 2018; Yin et al. 2022). This dependence on multiple factors and temporal constraints, leading researches on TGP exhibited significant heterogeneity (Walter et al. 2016). Taking the duration of the transgenerational effect as an example, although most are found in the seed and seedling stages and diminished in later growth stages, transgenerational effect was also reported can affect adult traits and persist for multiple growing seasons (Vivas et al. 2019; Walter et al. 2016). However, previous studies have mostly focused on a single factor, lack of the comprehensive understanding of the interconnected influence of various factors, limiting our predictive capacity to comprehend and predict TGP.

Making consistent conclusions about the TGP for species is made more difficult by the fact that all traits do not show the same sensitivity (Donelson et al. 2018). In animal researches, a prevailing notion is that morphological traits are more susceptible to maternal environment than other traits (Moore et al. 2019), but it is not clear whether this applies to plants and its underlying mechanisms. In nature, to maximize fitness, different traits may exhibit a 'hierarchy of plasticity', where certain traits, such as physiological traits, demonstrate greater WGP compared to reproductive traits (Arnold et al. 2022; Sadras and Slafer 2012). This raises the question of whether the differences in WGP of traits account for its distinct TGP. Plant phenotype was determined by the interplay between environmental information from previous and

present generations (Galloway and Etterson 2007; Kraft et al. 2015; Uller 2008), therefore, the relationship between TGP and WGP seems inevitable, but its relationship still blurred and indistinct (Donelson et al. 2018). Addressing this question require investigations into differential responses of multiple traits with distinct WGP to maternal environments.

Elevation, as a complex environmental factor, is accompanied by significant variations in multiple abiotic factors such as atmospheric pressure, temperature, solar radiation, and precipitation, which leading to strongly selection pressure on plants traits (Read et al. 2014). Plants' responses to elevation gradient can reflect climate change realistically over longer time scales, prompting extensive research that can provides valuable insights into their acclimation and resilience capacities in the face of climate change (Midolo et al. 2019). For instance, a global scale meta-analysis revealing that leaf mass per area, nitrogen concentration per unit of area, nitrogen concentration per unit mass significantly increased with increasing elevation (Midolo et al. 2019). However, the question of whether the impact of elevation on plant traits is heritable to offspring remains unanswered.

Elymus nutans, a perennial herbaceous plant in Poaceae family, is predominant in alpine meadows on the Qinghai-Tibet Plateau, primarily distributed within an elevation range of approximately 2500–4000 meters (Fu et al. 2016). Due to its high adaptability, nutritional value, high yield potential, and resistance to various abiotic stresses, such as low temperature and large diurnal temperature changes, it is frequently employed in ecological restoration and artificial grassland construction. Additionally, it plays a crucial role as a pasture species for enhancing livestock production and ground cover (Qi et al. 2020).

In this study, we utilized *E. nutans* as a biological model to examine the comprehensive effects of trait types, offspring environment, population, and growing season on TGP in a five-year field experiment. Two main hypotheses were expected: (1) the different altitude environments experienced by the parent plant can affect the leaf traits of *E. nutans* but is regulated by multiple factors, (2) this transgenerational plasticity is related to within-generational plasticity.

Materials and methods

Seed collection

Freshly matured seeds from five populations of *E. nutans* were gathered at the eastern edge of the Qinghai Tibet Plateau in northwest China along an elevation gradient from 2750 m–3530 m during dispersal in September 2016 (Fig. 1). Inflorescences containing ripe seeds were obtained from several hundred individual plants at each collection sites and transported to the laboratory. Subsequently, the seeds were meticulously separated and air-dried at room temperature for one week (RH 20–35%, 18–25 °C). The desiccated seeds were then stored at -20 °C until employed in experiments. Supplementary Data Table S2 provides the basic climate and soil nutrient status of five collection sites.

Field experiments

Common garden and reciprocal sowing experiments were conducted to establish different maternal and progeny environmental backgrounds, as depicted in the schematic diagram of the experimental process (Fig. 2). Field experiments were conducted within two weeks of seed collection.

Common garden experiments

In October 2016, seeds of 5 populations were sown in the Hezuo common garden (102.496° E, 35.097° N, 2930 m a.s.l, collection site of population B) and Maqu common garden (101.871° E, 33.676° N, 3530 m a.s.l, collection site of population E), respectively. Hezuo common garden experience a mean annual temperature and mean annual precipitation of 2.61 °C and 577 mm, respectively, while Maqu recorded 1.54 °C and 747 mm. Further, Hezuo common garden exhibited superior soil nutrients, while Maqu exhibited better water conditions. Therefore, this experiment established two maternal environments with significant elevation differences (i.e., low elevation Hezuo, abbreviated as LE and high elevation Maqu, HE). The common garden experiments were implemented using a completely randomized block design, with each common garden was divided into 20 blocks. The distance between blocks is 100 cm, and each block containing 1 replicate for each population, separating by wooden frames (20 × 20 cm) by 50 cm. In each frame, 10 seeds were sown to ensure seedling emergence. The seedlings are thinned 10 days after emergence to retain one individual plant for each replicate. Each replicate was retaining individual plants without further intervention except for the daily manual removal of weeds.

Reciprocal sowing experiments

In October 2018, freshly harvested seeds from individual plants in Hezuo and Maqu common gardens were reciprocally sown between the two common gardens. The reciprocal sowing experiment employed a random block design, with five blocks, each containing 10 individual plants for each population with same maternal environment. The sowing method were same in reciprocal sowing sites, totaling 1000 individual plants were established in reciprocal sowing experiments (2 progeny environments × 2 maternal environments × 5 populations × 50 replicates). This experiment established two progeny environments (i.e., progeny environment Hezuo, abbreviated as 'H' and progeny environment Maqu, 'M'). Supplementary Data Figure S1 provides mean maximum, mean minimum, and mean monthly temperature of two progeny environments for 2020 and 2021.

Trait measurements

In 2019, all plants were generally small, precluding sample collection. In August 2020 and 2021, 10 individuals with consistent growth were chosen from each population with same maternal environment at two progeny environments. Five fully unfolded flag leaves per individual were selected for measurements. The relative chlorophyll content (RCC) was measured by chlorophyll meter (SPAD-502, Minolta, Japan) in the field. Then the leaves were transferred to the laboratory in self-sealing bags containing distilled water and scanned with a scanner (Epson v700, Konica Minolta, Japan). The leaf area (LA), leaf length (LL) and leaf width (LW) were obtained by analyzing the images through Winseedle.2019 software. The leaf thickness (LT) was measured by avoiding the main vein with a spiral

micrometer. Additionally, the scanned leaves were placed in the oven and dried to constant weight at 80°C for 48 h. The leaf dry weight (LDW) was weighed and recorded (Precision: 0.0001 g). The leaf mass per area (LMA) was calculated as the ratio of mass to area. The leaf volume was calculated as the product of LA and LT, the leaf tissue density (LTD) was calculated as the ratio of mass to volume (Craine and Towne 2010). Average value of five flag leaves were calculate to obtain the trait value of an individual.

For each selected individual, we also sampled the green and senesced leaves in mid-August 2020 at two reciprocal growing sites. All leaf samples for chemical analysis were oven-dried at 80°C for 48 hr to a constant weight and then subjected to bead-beating using a Retsch 400MM beating mill (Verder Shanghai Instruments and Equipment Co., Ltd., China). Then the samples were sieved through a 0.5 mm wire mesh for chemical analysis. Total N and P contents in samples were determined by adding 1.65 g of catalyst (K₂SO₄ vs. CuSO₄ at a ratio of 10:1) and 10 ml of concentrated sulfuric acid to a 0.2 g leaf sub-sample, digested for 1.5 hr at 420°C and analyzed by flow injection analysis, using a FIAstar 5000 Analyser (FOSS Analytical, Denmark) to calculate leaf nitrogen concentration (LNC), leaf phosphorus concentration (LPC), nitrogen resorption efficiency (NRE) and phosphorus resorption efficiency (PRE). All adopted leaf traits and their categories, units, and abbreviations are shown in Supplementary Data Table S1.

Nutrient resorption efficiency

The nutrient resorption efficiency (NuRE), defined as the proportion of the mature leaf nutrient pool that was resorbed, was calculated as:

$$\text{NuRE (\%)} = (\text{Ngr} - \text{Nsen}) / \text{Ngr} \times 100\% \quad (1)$$

where Ngr was the nutrient concentration of N or P in the green leaves; Nsen was the nutrient concentration of N or P in the senesced leaves (Killingbeck 1996).

Coefficient of variation and simplified relative distances plasticity index

To quantify the magnitude of WGP of different traits in response to different progeny and maternal environments, we calculated coefficient of variation (CV) and simplified relative distances plasticity index (RDPI_S) for each trait. One value was calculated for each population with same progeny and maternal environment. Specifically, CV was calculated as follows:

$$\text{CV} = \text{SD}_{ji} / \sqrt{\text{mean}_{ji}}$$

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where SD_{ji} was standard deviation of trait *j* measured in environment *i*, and mean_{ji} the grand mean of trait *j* measured in environment *i*, as described elsewhere (Bloor and Grubb, 2004).

RDPI_S was calculated as follows:
$$\text{RDPI}_S = \frac{|\text{mean}_{ji} - \text{mean}_{jk}|}{\text{mean}_{ji} + \text{mean}_{jk}}$$

(3)

where mean_{ji} was the grand mean value of trait j measured in environment i , and mean_{jk} was the grand mean value of trait j measured in environment k (Godoy et al. 2012; Valladares et al. 2006).

Soil sampling

Three soil samples were collected from each of the 5 seed collection sites in 2016. Each sample comprised five soil cores (0–10 cm depth) using a soil auger (7.5 cm inner diameter). All soil samples were placed in individual plastic bags and immediately stored in a portable refrigerator before they were transported to the laboratory. Once in the laboratory, all soil samples were subdivided into two subsamples. One subsample was air-dried at room temperature for analyzing available phosphorus (AP), which was extracted in 50 ml 0.5 M NaHCO₃ solution (pH = 8.5) from 5 g dry soil subsample, and then measured calorimetrically using the molybdate-ascorbic acid method UV-1601, Shimadzu Inc.). And another one was stored at 4 °C until analysis for NO₃⁻-N (NON) and NH₄⁺-N (NHN). 10 g fresh soil was extracted in 50 ml 2 M KCl solution and then analyzed by colorimetry (UV-1601, Shimadzu Inc.).

Climate data

Mean annual temperature (MAT) and mean annual precipitation (MAP) from 2000 to 2020 were selected to represent the climatic differences of 5 seed collection sites. The data of MAT and MAP from Loess Plateau SubCenter, National Earth System Science Data Center, National Science & Technology Infrastructure of China (<http://loess.geodata.cn>) (Peng et al. 2019). The wetness index to some extent reflects the moisture selection intensity experienced by the leaves (Rufty et al. 1981).

Wetness index (WI) was calculated as follows:

$$\text{WI} = \text{MAP} / (\text{MAT} + 10) \quad (4)$$

Statistical analysis

All experimental data were checked for normality and log-transformed when necessary. First, in a sample that pooled all populations, we compared the differences in leaf traits between different maternal and progeny environments using independent sample t-tests. The transgenerational plasticity is estimated as effect sizes of maternal environment extracted from Linear mixed models (LMEs), which were performed on different leaf traits in each progeny environments with populations and individuals as random effects using the lmerTest package of R version 4.1.3 (Kuznetsova et al. 2017). Parameter estimates from each model were used to estimate the effect size of maternal environment. All continuous variables were log-transformed and then standardized to facilitate the direct comparison of the relative importance of each variable.

Second, we calculated the maternal environmental effect size for each population separately using LMEs with individuals as random effects. And then, using LMEs to investigate the effect of growing season, trait type, progeny environment and their interactions on effect size of maternal environment with populations as random effects. The significance of fixed effects was tested with Chi-square tests and type I errors, using function Anova in the car package, and the significance of random effects was tested using function ranova in the package lmerTest (Kuznetsova et al. 2017).

Finally, by comparing CV and RDPI_S of each trait covering all populations, we investigated magnitude and hierarchy of phenotypic plasticity. Further, we calculated CV and RDPI_S for each population separately, and then we investigated the effect transgenerational plasticity, trait type, and their interactions on within-generational plasticity of leaf traits with populations as random effects.

Results

Transgenerational effect on leaf traits

The leaf traits of *E. nutans* were affected by maternal environment, with most traits of progeny plant from low altitude maternal environment (LE) surpassing those from high altitude maternal environment (HE). In 2020, within the Hezuo progeny environment, except for LTD, all traits from LE showed an elevation of 0.88–11.09% compared to those from HE. Within the Maqu progeny environment, excluding LTD, LNC, and PRE, other traits from LE demonstrated an increase of 2.85–22.29% compared to those from HE. Moving to 2021, except for LW, the traits from LE were 0.22–16.45% higher than those from HE. Similarly, within the Maqu progeny environment, excluding LTD, traits from LE exhibited an increase of 1.88–19.92% compared to those from HE (Fig. 3).

Influence factors of Transgenerational plasticity

TGP differed significantly among traits types ($P < 0.001$; Table 1). For example, within the Maqu progeny environment, LTD of plants from LE showed a decrease 4.2% compared to that from HE in 2020, but LDW demonstrated an increase of 22.29%. Although the main effects of progeny environment were not statistically significant, its interaction with trait types significantly influenced TGP in 2020 ($P < 0.001$; Table 1). Regarding morphological traits, except for LMA and LW, which were not significantly affected by maternal environments in both progeny environments, all other traits were significantly affected in Maqu progeny environment in 2020; Conversely, for nutritional traits, most traits are only significantly affected in Hezuo progeny environment (Fig. 4).

Furthermore, TGP diminished in 2021, with only LL, LT and LDW were significantly affected by maternal environment effect in Maqu progeny environment (Fig. 4). From 2020 to 2021, within the Hezuo progeny environment, half of the traits exhibited reduced susceptibility to maternal environment, such as RCC and LW. In the Maqu progeny environment, apart from LT, TGP weakened for all other traits, suggesting that

the decline of TGP was influenced by traits types and progeny environment (Supplementary Data Fig. S2). The impact of population on TGP varied depending on the growing seasons (Table 1).

Table 1
Effect of trait type, progeny environment, and their interactions on transgenerational plasticity using Linear mixed models (LMEs). The asterisk refers to the interaction effect between variables before and after the asterisk

Year		Source	Transgenerational plasticity		
			Chisq	Df	<i>P</i>
2020					
	Fixed effects	Trait type (<i>TT</i>)	38.880	11	< 0.001
		Progeny environment (<i>PE</i>)	0.176	1	0.676
		<i>TT * PE</i>	32.751	11	< 0.001
	Random effects	Population (<i>POP</i>)	3.366	1	0.067
2021					
	Fixed effects	Trait type (<i>TT</i>)	4.287	7	0.746
		Progeny environment (<i>PE</i>)	0.693	1	0.405
		<i>TT * PE</i>	5.523	7	0.596
	Random effects	Population (<i>POP</i>)	35.253	1	< 0.001

Magnitude and hierarchy of WGP of different leaf traits

The trait type significantly influenced both CV and RDPI_S across both growing seasons, while maternal environment exhibited significant effects only in 2021. The interaction between trait type and maternal environment did not attain statistical significance (Table 2). Collectively, the magnitude of WGP was proved trait-dependent and susceptible to maternal environmental effect. Consistent rankings of WGP emerged from two growing seasons of both CV and RDPI_S results, with LDW and LA displaying the highest plasticity, while NRE, PRE, and RCC exhibiting the least plasticity (Fig. 5).

Table 2

Effect of maternal environment, trait type, and their interactions on coefficient of variation (CV) and simplified relative distances plasticity index (RDPI_S) of different leaf traits using Linear mixed models (LME_S). The asterisk refers to the interaction effect between variables before and after the asterisk

Year	Source	CV			RDPI _S		
		Chisq	Df	<i>P</i>	Chisq	Df	<i>P</i>
2020							
	Maternal environment (<i>ME</i>)	0.101	1	0.751	0.240	1	0.624
	Trait type (<i>TT</i>)	376.171	11	< 0.001	130.118	11	< 0.001
	<i>ME</i> * <i>TT</i>	19.637	11	0.051	19.291	11	0.056
2021							
	<i>ME</i>	4.181	1	0.041	3.866	1	0.049
	<i>TT</i>	162.503	7	< 0.001	138.869	7	< 0.001
	<i>ME</i> * <i>TT</i>	3.549	7	0.830	4.586	7	0.710

Relationship between transgenerational plasticity and within-generational plasticity

No significant relationship was observed between TGP and CV or RDPI_S across both progeny environments and growing seasons (Fig. 6).

Discussion

The impact of maternal environment on leaf traits of *E. nutans*

Our results indicate that the different elevation environments experienced by the maternal parent can affect the leaf traits of the *E. nutans* offspring, and this effect can last for multiple growth seasons. Offspring from high elevation maternal environments have small and short leaves, which is consistent with previous results (Liu et al. 2020; Midolo et al. 2019). However, contrary to previous findings, their leaf thickness is not thicker compared to low altitude maternal environments. This discrepancy may be due to differences in the combination of abiotic factors. In our study, high elevation environment was accompanied by higher precipitation in addition to lower temperatures, which may have altered the response of leaf thickness to increasing elevation.

Factors influencing transgenerational plasticity: trait types, progeny environments and growing seasons

The extent and duration of TGP varied with trait types. Traits such as LW, LMA, and PRE were less susceptible to maternal environment in both progeny environments and growing seasons compared to other traits, like LA and LT (Fig. 4). This difference in transgenerational plasticity among traits is consistent with the finding of a meta-analysis (Yin et al. 2022), which may be due to differences in the rate at which various traits changes can occur or the environmental thresholds that initiate plasticity of various traits (Donelson et al. 2018; Sadras and Slafer 2012). Therefore, the same maternal environment may exert different selection pressures on different traits, resulting in different foundation of transgenerational plasticity. For instance, offspring of *Polygonum persicaria* from limited nutrient plants allocate more to root growth, making the root length less sensitive to the maternal environment compared to seedling biomass (Sultan 1996).

Unlike in animal research, our results did not support the prevailing notion that morphological traits are more susceptible to transgenerational effects (Moore et al. 2019), by revealing the dependence of TGP on progeny environments alongside traits. This dependence is consistent with previous research (Herman and Sultan 2011; Uller et al. 2013; Yin et al. 2022). For instance, Stratton (1989) illustrated that differences in seedling size of *Erigeron annuus* attributable to transgenerational effect rapidly diminished under noncompetitive conditions, in contrast, under competitive conditions, transgenerational effect persisted (Stratton 1989). Both theoretical and empirical research have proposed that TGP could be favored by an appropriate level of selection pressure, yet could be obscured by excessively severe selection pressure (Kuijper and Hoyle 2015; Miao et al. 1991a). Likewise, in our study, although no significant main effect of progeny environment was observed, its interactions with trait type markedly influenced the extent and duration of TGP (Table 1). Furthermore, our findings reveal an interesting phenomenon: morphological traits showed higher TGP in the Maqu progeny environment, whereas nutritional traits were higher in the Hezuo progeny environment (Fig. 3). Indeed, these two progeny environments have different environmental conditions, with the Hezuo progeny environment having better nutrient and poorer water conditions, while the Maqu progeny environment has opposite conditions (Supplementary Data Table S2). This suggested that different traits not only differ in environmental thresholds, but may also in major limiting factor for regulating environmental response. For instance, offspring of *Polygonum persicaria* grown under low light allocated more resources to shoot growth, and offspring of limited nutrient plants allocated more to root growth than individuals with nutrient rich parents (Sultan 1996).

Moreover, our results provided new evidence for the decline of TGP (Fig. 4). TGP is rooted in the interplay of environments between different generations, and with the ontogeny progresses, the offspring are gradually influenced by their own environment, leading to TGP being 'overwritten' by WGP (Donelson et al. 2018; Moore et al. 2019). However, there are exceptions in the duration of TGP. For instance, maternal nutrient pulses effect on *Plantago major* (Miao et al. 1991a) and maternal salinity stress effect on

Atriplex centralasiatica (Wang et al. 2022), both persisted for three generations. In line with these exceptions, our results found sustained transgenerational effects on leaf traits of *E. nutans* for at least three growing seasons, highlighting the long-term of TGP of leaf traits, especially in perennial plants. Further, our study indicated that the population also exerted influence on TGP, which was consistent with the results of previous studies on *Arabidopsis thaliana* (Groot et al. 2017), *Vicia sativa* (Li et al. 2017) and *Drosophila mojavensis* (Diaz et al. 2021). This genotypic variation of TGP could be explained by local climates of different genotypes, especially by its environmental predictability (Diaz et al. 2021; Yin et al. 2022).

Hierarchy of phenotypic plasticity in leaf traits of E. nutans and its relationship with transgenerational plasticity

In nature, traits more directly related to fitness should show stronger selection leading to genetic differentiation, in contrast, traits less directly related to fitness would instead display higher plasticity, to buffer short term environmental perturbations and ultimately maintain fitness homeostasis (Villellas et al. 2021). Therefore, different traits of plants exhibit 'hierarchy of plasticity' to maximize fitness (Sadras and Slafer 2012). Our study introduced novel evidence supporting this concept, revealing a consistent hierarchy of plasticity in leaf traits of *E. nutans*, with LDW at the highest end of plasticity, and NRE at the lowest end (Fig. 5). Additionally, our experiment affirmed that while CV and RDPI₅ are mainly determined by trait types, maternal environment could affect phenotypic plasticity of leaf traits (Table 2), underscoring that WGP is subject to maternal environment pressures (Han and Lincoln 1997; Leung et al. 2020).

Establishing the relationship between TGP and WGP is of great significance for us to understand and predict the response of organisms to environmental changes (Donelson et al. 2018; Galloway and Etterson 2007; Guillaume et al. 2015). It is unreasonable to presume that an individual's capacity to transmit maternal effects to its offspring will not be influenced by its own capacity for WGP (Donelson et al. 2018). Through constructing an evolutionary model, Kuijper et al. (2015) have found that maternal effects were favored when traits experience very strong selection and for which plasticity is severely constrained, which seems to imply a negative correlation between TGP and WGP. However, our results reveal no clear correlation between TGP and WGP (Fig. 6). This unexpected result underscored the multifaceted factors influencing different plasticity. In terms of TGP alone, multiple factors beyond trait type, progeny environment, population, and growing season, it also includes previously reported factors, such as maternal environment (Miao et al. 1991a), all affected TGP. Different factors interact with the current generation environment to determine the phenotype, especially the interplay of environmental information time and intensity between different generations, which will lead to ever-changing relationship between TGP and WGP (Moore et al. 2019). An obvious example is that TGP are more easily detected during early life stages, while as the ontogeny progresses, the environmental effects of the progeny become stronger, and the TGP may be covered. Nevertheless, WGP was proved to be a poor predictor for TGP.

Conclusions

Our study conducted a pioneering exploration into the TGP of leaf traits of *E. nutans*. As we have hypothesized, elevation environments experienced by the maternal parent significantly affected leaf traits of *E. nutans* and persisted for at least three growing seasons. Furthermore, TGP was regulated by various factors, including trait types, progeny environments, growth seasons and populations. Importantly, we provided the first evidence that TGP are not correlated with WGP, reflecting the complexity of environmental conditions interactions experienced by the maternal and progeny generations. Our findings underscore the importance of considering the maternal environment when evaluating and utilizing *E. nutans*, especially for the cross regional using of different seed sources in ecological restoration. It is advisable to opt for seed sources from environments with similar elevations.

Declarations

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

Xiaowen Hu: Methodology, Supervision, Writing - review & editing; Cunzhi Jia: Writing - review & editing, Data curation, Roles/Writing - original draft; Dali Chen: Data curation, Supervision; Xiaohua Zhao: Formal analysis; Xiuzhen Fu: Visualization. all authors contributed to the manuscript.

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Figures

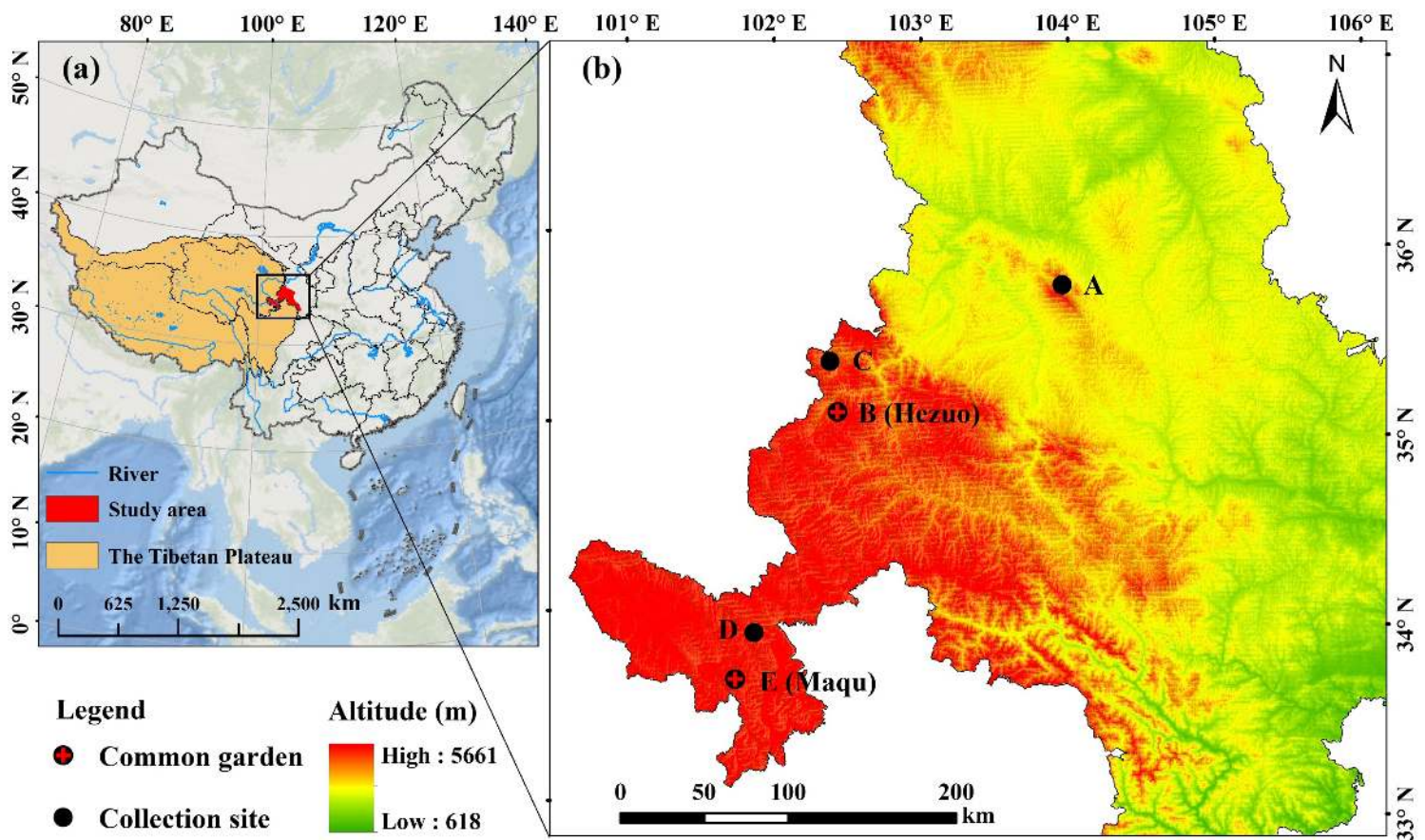


Figure 1

Seed collection sites in 2016. The black dots represent the collection sites, and the black dots with the red cross represent the common gardens. (a) shows the relative positional relationship between the

sampling area and the Qinghai Tibet Plateau and China. (b) shows the positional distribution of five sampling sites

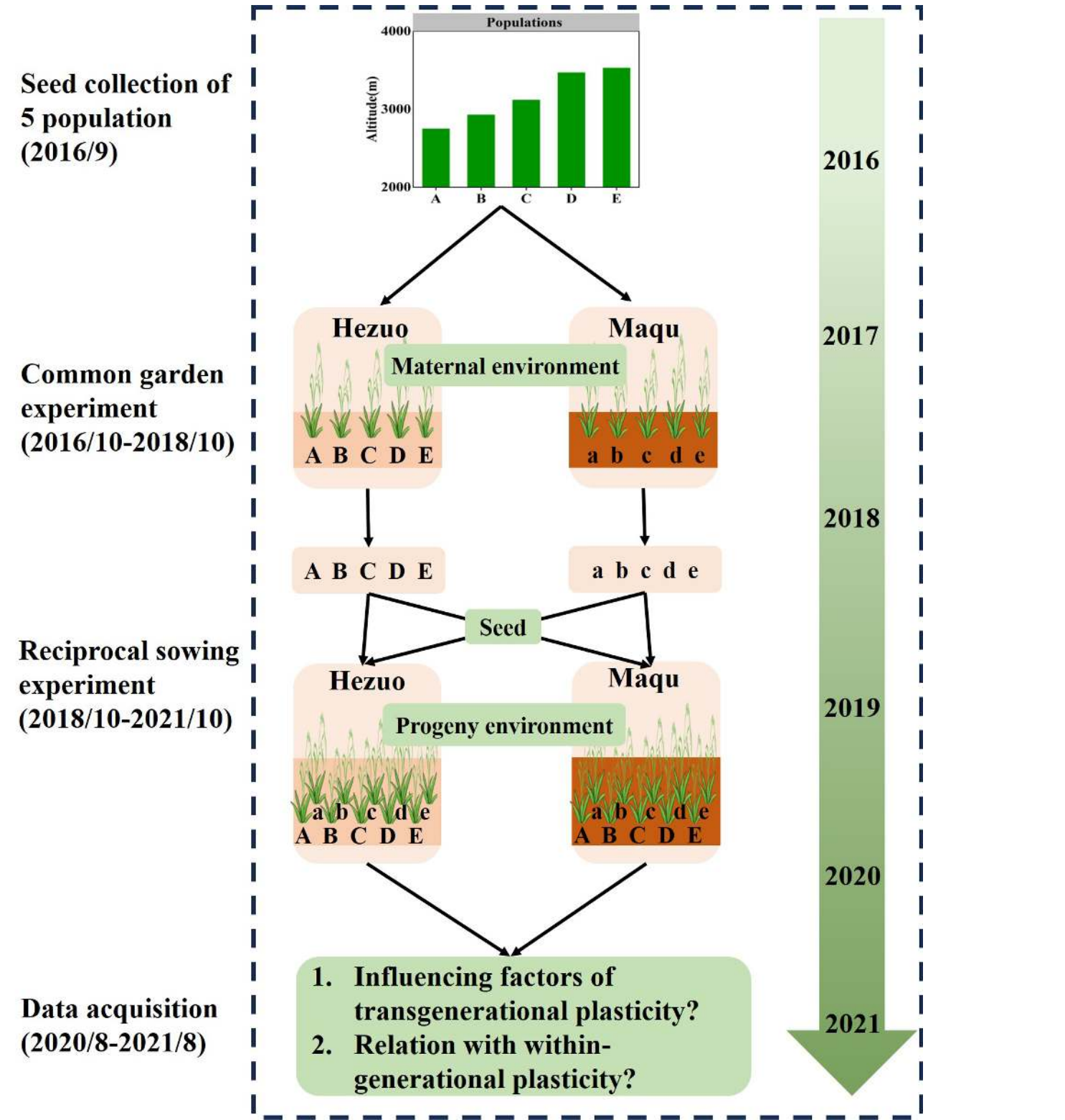


Figure 2

Schematic diagram of the experimental process. In Sep. 2016, seeds of five populations of *E. nutans* were collected along the altitude and were cultivated for two years separately (from Oct. 2016 to Oct. 2018). Then, in Sep. 2018, seeds of five populations with different maternal environmental backgrounds

were collected and sowed back to two common gardens reciprocally, and cultivate for three years (from Oct. 2018 to Oct. 2021) to constructed different offspring environmental backgrounds.

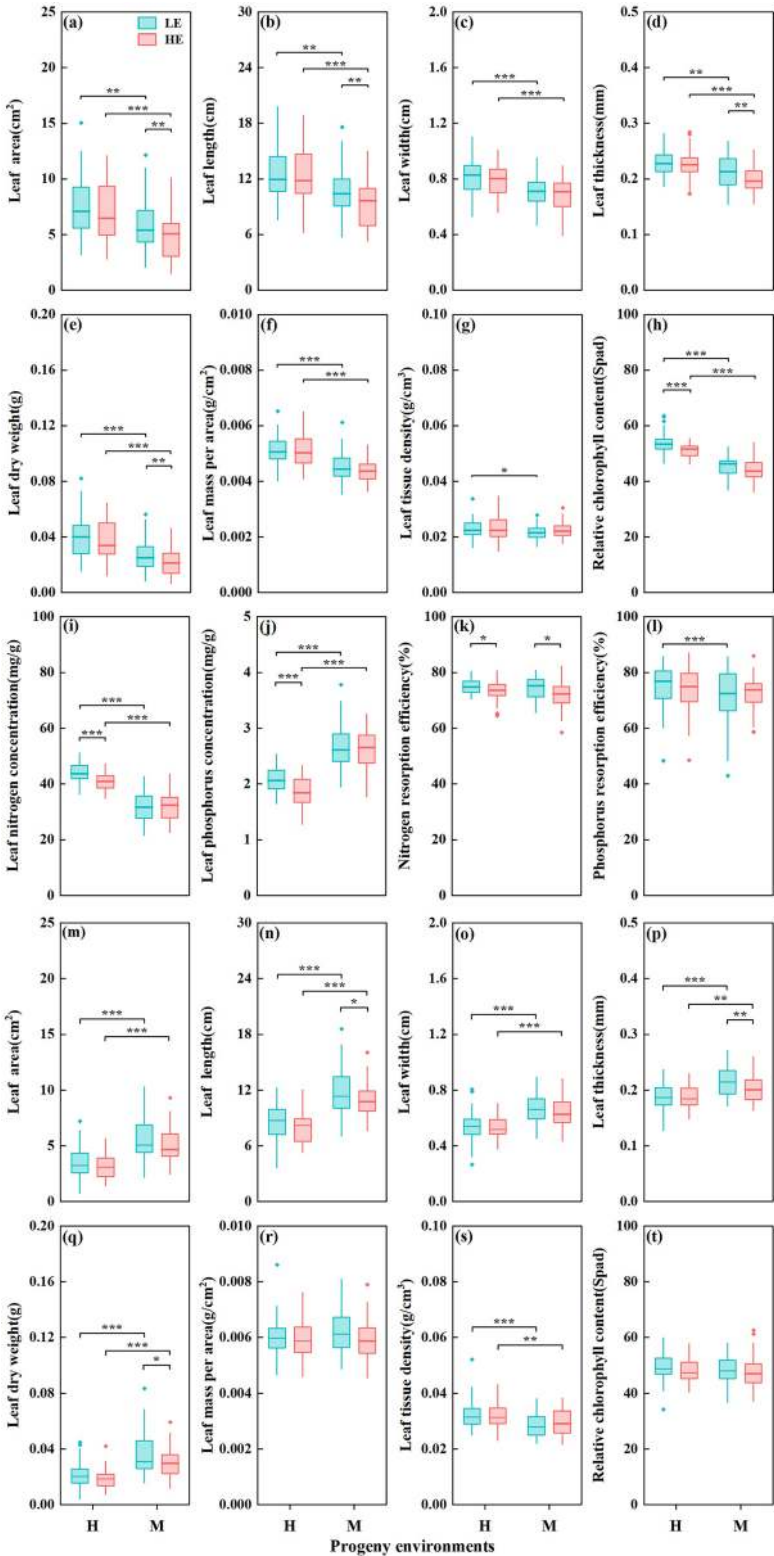


Figure 3

Effect of progeny environment and maternal environment on different leaf traits of *E. nutans*. **a-i**, traits in 2020, **m-t**, trait in 2021. Asterisks indicate significant pairwise difference. ‘LE’ and ‘HE’ represent low and

high elevation maternal environments, respectively, capital letters 'H' and 'M' represent low and high elevation progeny environments, respectively. Significance: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$

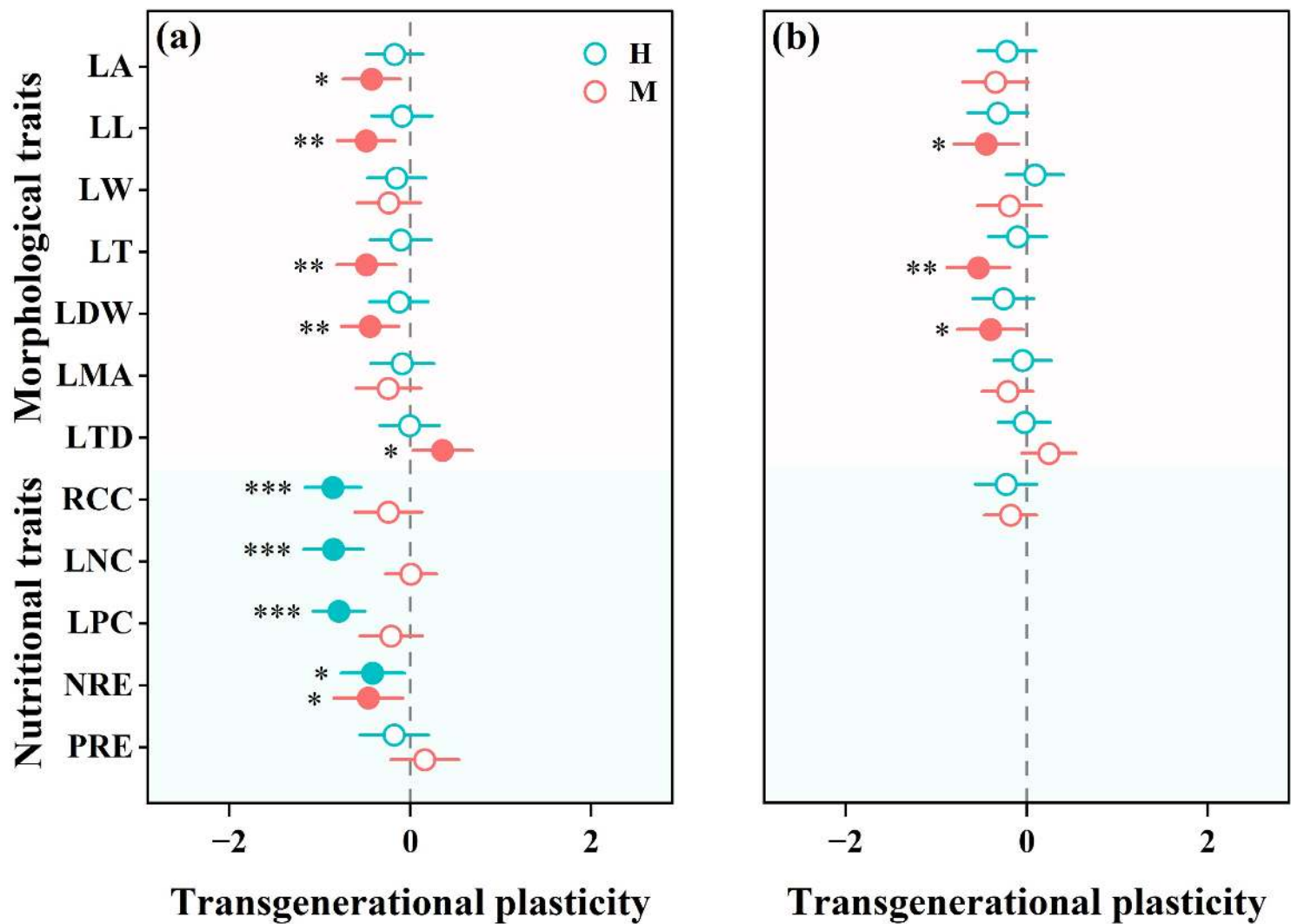


Figure 4

Transgenerational plasticity of each leaf trait of *Elymus nutans* in two progeny environments in 2020 (a) and 2021(b). Capital letter 'H' and 'M' represents two progeny environments. Parameter estimates from Linear mixed models (LMEs) fitted to estimate transgenerational plasticity of each leaf trait. Solid points indicate parameter estimates that are significantly different from zero (dashed lines) and bars indicate 95% confidence intervals. Significance: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$

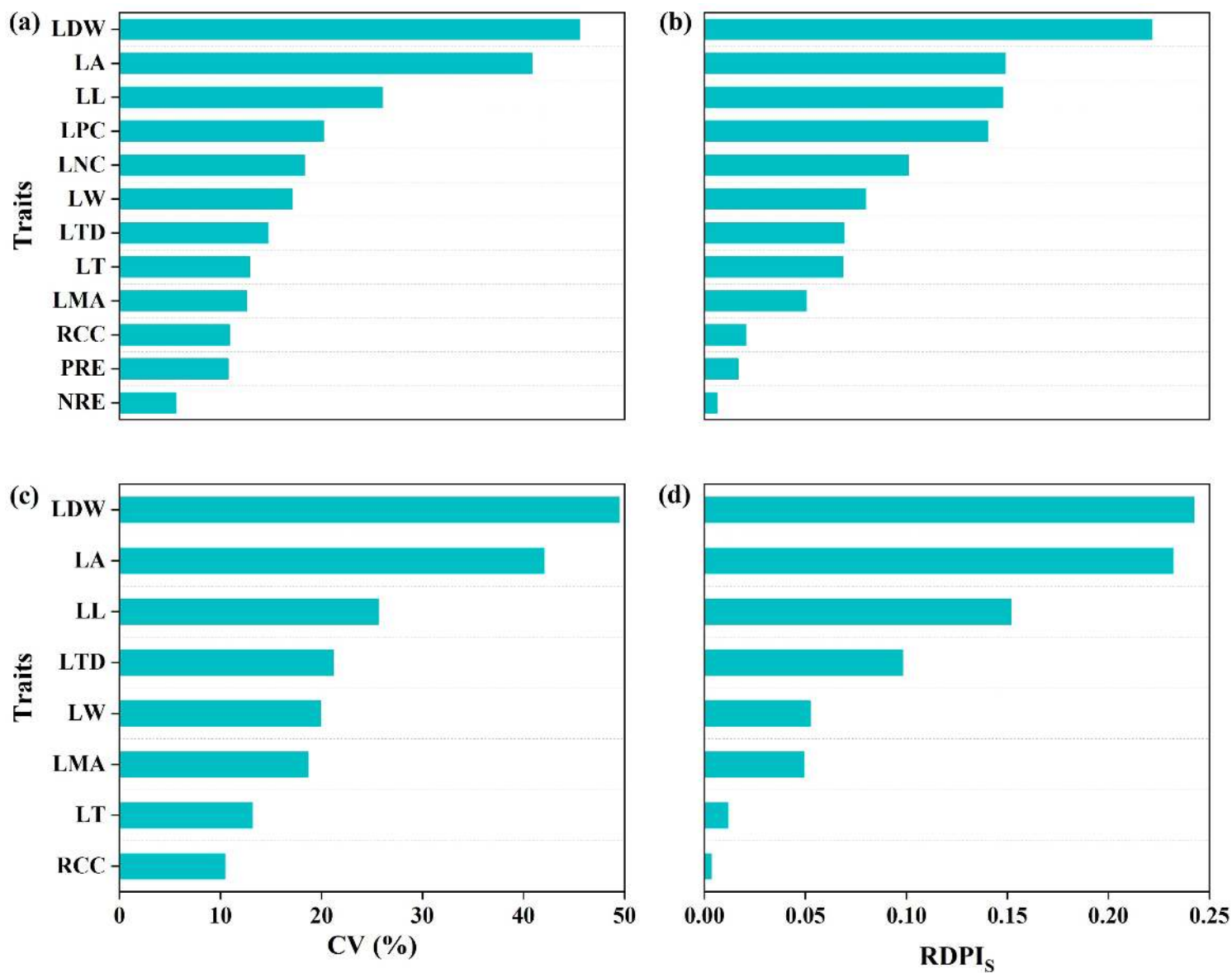


Figure 5

Coefficient of variation (CV) and simplified relative distances plasticity index (RDPI_s) of different leaf traits of *Elymus nutans*. (a) and (b) are CV and RDPI_s in 2020; (c) and (d) are CV and RDPI_s in 2021

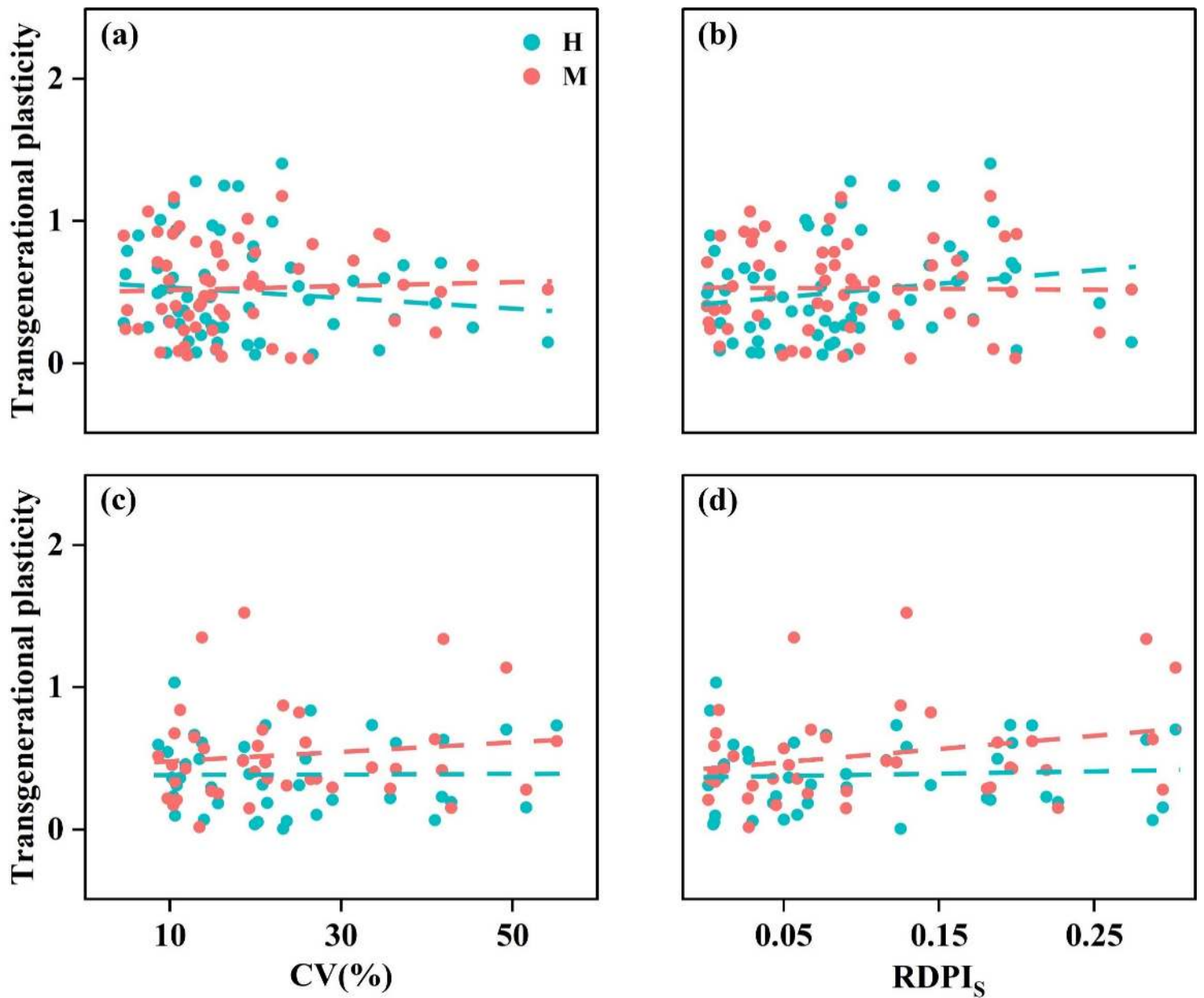


Figure 6

The relationship between transgenerational plasticity and within-generational plasticity of different *Elymus nutans* leaf traits across all populations under two progeny environments. **(a)** and **(b)** are relationship between transgenerational plasticity and CV and RDPI_S in 2020; **(c)** and **(d)** are relationship between transgenerational plasticity and CV and RDPI_S in 2021. The dashed line indicates insignificant correlation. Capital letters 'H' and 'M' represents two progeny environments

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