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Comparison of phenotypic plasticity in *Bistorta vivipara* in topographically rough and flat landscapes

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ABSTRACT

Topographic variation may create selective pressures on plants relevant to the scenarios of the biotic effects of climate change. Plants respond to these combined effects of topographic variation and climate change with plasticity and/or selection on varied genotypes. We conducted a greenhouse experiment to investigate if plants of *Bistorta vivipara* from topographically rough landscapes show more variation than plants from nearby flat landscapes when grown in constant moisture levels, and whether this variation is caused by a greater selection for plasticity in plants from the rough landscapes. Bulbils of the species were collected from 16 plots in five matched pairs of topographically rough and flat landscapes. These were grown in the greenhouse under 3 moisture treatments for 8 weeks. Principal Components Analyses revealed that five out of the eight morphological traits evaluated: - approximate mean leaf area, approximate total leaf area, number of leaves, root : shoot ratio and leaf shape, covaried closely. Total plant biomass, approximate specific leaf area and approximate leaf area ratio turned out to be less correlated with the other traits. Plants from the topographically flat landscapes exhibited a relatively greater phenotypic variation in the eight morphological traits than plants from the rough landscapes. However, phenotypic plasticity in response to moisture was weakly higher for the approximate specific leaf area, plant biomass and the closely correlated traits (e.g., root: shoot ratio) in plants from the rough landscapes than plants from the flat landscapes. Overall, results suggest that plants in heterogeneous landscapes are more plastic and therefore plants from such landscapes will cope better with climate change.

Key words: Topographic variation; Soil moisture; Phenotypic plasticity; Phenotypic variation; Buffering; Climate change; *Bistorta vivipara*.

INTRODUCTION

Mountains are known for their variation in topography (Körner, 2003). Topographic influences on environmental conditions, such as wind (differences between windward and leeward conditions), solar energy (shading and exposure effects) and hydrology (variation in drainage and snow accumulation) create selective pressures on plants. These selective pressures may be relevant to climate change impacts affecting plant physiological processes such as photosynthesis, growth and reproduction (Henry & Molau, 1997). The structure, composition and function of arctic and alpine ecosystems have been identified to undergo tremendous transformations by climate change impacts (Emanuel et al., 1985; Chapin & Körner, 1995). These impacts may further amount to other secondary environmental alterations which can affect plants. For instance, increased precipitation may change the timing of snow melt in arctic and alpine ecosystems, where species distribution and community composition are strongly affected by the duration of snow cover (Stanton et al., 1994; Walker et al., 1994). Thus, alpine ecosystems with their steep slopes, varying topography (Körner, 2003) and rather short growing season (Horandl, 2011) may be hit heavily by climate change impacts.

In the presence of such varying abiotic environments caused by the interplay between topography and climate change, species may migrate to track favourable niches (Davis & Shaw, 2001; Jump & Peñuelas, 2005) or persist and adapt. Sessile organisms such as plants will not always be able to track optimal habitats in a changing world. Therefore, continued existence may depend strongly on the abilities to adapt quickly to new environmental conditions. By altering their phenology, physiology and morphology, plants can respond effectively to these varying conditions (Sultan, 2003). Adaptive changes as response to changing environmental conditions may be achieved with plasticity and/or selection on varied genotypes (Matesanz et al., 2010). In particular, theory predicts that high phenotypic plasticity should be a major advantage in changing environments (Bradshaw & Hardwick, 1989), and rapid acclimations may enable survival in unpredictable environments (Schlichting, 1989).

Phenotypic plasticity specifically describes the property of a genotype to express different phenotypes in response to different environmental conditions (Bradshaw, 1965; Schlichting,

1986). Plants, especially those inhabiting environments with greater heterogeneity can use high plasticity as an adaptive strategy to cope with the varying environmental conditions (Bradshaw, 1965; Moran, 1992; Scheiner, 1993; Pigliucci, 2001; DeWitt & Scheiner, 2004). This suggests that varying conditions created by the interplay between topography and climate change may as well enhance the adaptive capacity of plants. Thus, topographic influence on environmental conditions may provide some buffering effects against future environmental changes in plants. Hence, environmental changes predicted to affect plants may have lesser impact in alpine ecosystems where landscapes of varying topography abound (Körner, 2003).

However, plasticity has a cost and there will always be trade-offs between different traits. This is because a build up of functional traits in response to a given environmental cue on one hand may affect performance and reproductive success of plants on the other hand (Sultan, 2001). A study by Reich et al. (2003) showed that a plant's ability to acquire and allocate resources is significantly affected by the variation in stem, root and leaf biomass. Therefore plants may be "selective" and not necessarily plastic to every change imposed by the environment.

Soil moisture is an important component of plant environment, one that is both vital to plant function and highly variable (Sultan & Bazzaz, 1993). In comparison to temperature and light that have been directly considered as agents of selective change within populations (Bradshaw and Hardwick 1989), variability in soil moisture is, in most habitats, primarily temporal and short-term in nature (Sultan & Bazzaz, 1993). Plastic response studies which have documented how environmental heterogeneity enhance plasticity and adaptive capacity of plants, used water as the environmental factor (Sultan & Bazzaz, 1993; Gianoli & González-Tuber, 2005; Molina-Montenegro et al., 2010). Plants from heterogeneous environments which experience limited conditions of moisture have often been identified to produce fewer, smaller, thicker and more pubescent leaves. These plants also produced smaller shoots and larger roots reflecting an increase in biomass allocation to water uptake (Sultan & Bazzaz, 1993; Gianoli & González-Tuber, 2005). In contrast to these observations, three populations of *Polygonum persicaria* of which two occupied variably dry sites and one from a consistently moist site demonstrated similar patterns of functional plasticity in response to soil moisture (Heschel et al., 2004). Thus, in some instances, plasticity may be equally demonstrated by individuals from environments with different heterogeneity.

Several other studies have documented plastic responses to moisture variation in plants (e.g. Bell & Sultan, 1999; Gordon et al., 1999; Fitter & Hay, 2002; Llorens et al., 2003). In all these studies, however, comparisons in plastic responses were made between plants from different sites (i.e., on a broader scale). Knowledge of how plastic responses differ between individuals occurring on the same site (smaller scale) with different terrain characteristics is limited.

When one walks a few meters in mountainous areas, individuals of the same species tend to show greater phenotypic variation. Opedal et al. (prep.) found that *Bistorta vivipara* from topographically rough landscapes tended to show greater phenotypic variation in plant height, approximate leaf area and number of bulbils than those in nearby flat landscapes. However, he could not know if the phenotypic variation observed was solely caused by the variation in the environment or if the plants in the topographically rough landscape have been selected to be more plastic. This served as a motivation for the present study where we tested if plants from topographically rough landscapes show more variation than plants from nearby flat landscapes when grown in constant moisture levels. This would indicate that there has been selection for more variation (ecotypic or plastic) in the topographically rough landscape. We thereafter tested if such variation is caused by the plants being selected to be more plastic in the topographical rough landscapes than those in comparable but flat landscapes.

Considering the effects of moisture on plants, its effects when it interacts with topography, and its tight association with *B. vivipara*, we hypothesized high plasticity in response to varying soil moisture by plants from the rough landscapes. Also, we expected plants from rough landscapes to demonstrate greater fitness which we used total plant biomass as its proxy (Gianoli & González-Tuber, 2005). We assumed that these plants have become tolerant to pronounced moisture variations and can perform better even in soil with limited moisture. Therefore these plants originating from the rough landscapes will overall have higher biomass when subjected to different moisture levels compared to plants from flatter landscapes. Further, we expected plants especially those from the rough landscapes to demonstrate greater variation, and responsiveness to moisture in specific leaf area, leaf area ratio and root : shoot ratio which are directly involved with water collection and conservation (Sultan & Bazzaz, 1993). We were of the view that greater moisture variations will have selected for greater phenotypic variation in these traits in plants originating from such

environments. In general, the study attempted to provide answers to the following research questions.

- 1) Do plants from topographically rough landscapes show more phenotypic variation than those from flat landscapes?
- 2) Are plants from topographically rough landscapes more plastic to soil moisture than those from flat landscapes?
- 3) Which morphological traits show high or low responsiveness to landscape heterogeneity and varying moisture conditions?

MATERIALS AND METHODS

Study species

Bistorta vivipara L. (Polygonaceae) is a perennial herbaceous plant distributed in a wide range of habitats from the high arctic fellfields to the closed plant communities in alpine meadows of the Northern Hemisphere (Callaghan & Emanuelsson, 1985; Wookey et al., 1994). Variable numbers of flowers are born on the reproductive spike, however, sexual reproduction occurs at a low extent due to absence of viable seeds (Callaghan, 1973; Petersen, 1981; Soyrintki, 1989; Bauert, 1993). The species reproduces asexually through the production of bulbils which are vegetative axillary buds borne within inflorescences (Diggle, 1997). *B. vivipara* grows actively during spring and summer producing new individuals by frequent bulbil production (Engell, 1973). Trade-off exists between the number of bulbils and the flowers in a reproductive spike as the two traits are negatively correlated (Law, Cook & Manlove, 1983; Bauert, 1993). Emergence and maturity of leaves is observed immediately after snow melt in early to mid June (Diggle, 1997).

A study conducted in Greenland by Petersen (1981) found soil moisture regime as a vital factor for the establishment of the species, and that competition for light had a decreasing effect on the establishment of the species. Wookey et al. (1994) in a study at Svalbard established that addition of nutrient significantly increased both growth and reproduction while an increase in temperature only increased the reproductive output. Addition of water was found to have no effect on the performance of the species.

Study site and data

The experimental material for the study was obtained from Finse Mountains in the Hardangervidda plateau of Ulvik in the alpine vegetation of southern Norway (centre at N 60° 36.23', E 7° 33.40'). The site has its centre of about 1430 meters above sea level, higher than the 1000m climatic treeline at Finse (Dahl, 1986). The total area of the site including the plots measures 5 x 105 m² with the vegetation in low to middle alpine tundra in rocky outcrops. The Norwegian Meteorological Institute (2012) documents the mean summer precipitation and temperature for Finse as 89mm and 6.3°C respectively during the normal period (June - August, 1961-1990).

In July, 2012, ten 40m x 40m land areas were selected as five matched pairs of topographically rough and flat landscape types during the summer periods. These landscapes

were selected such that each pair consisting of a rough and a flat landscape was situated on the same site (Figure 1). These landscapes which were mostly south-facing were marked out such that intra-pair distances were shorter than inter-pair distances (Figure 1). Each 40m x 40m landscape was further divided into sixteen 10m x 10m area and 16 random plots were allocated in them using a stratified coordinate system (Figure 2). *Bistorta vivipara* had a good representation on all the selected landscapes.

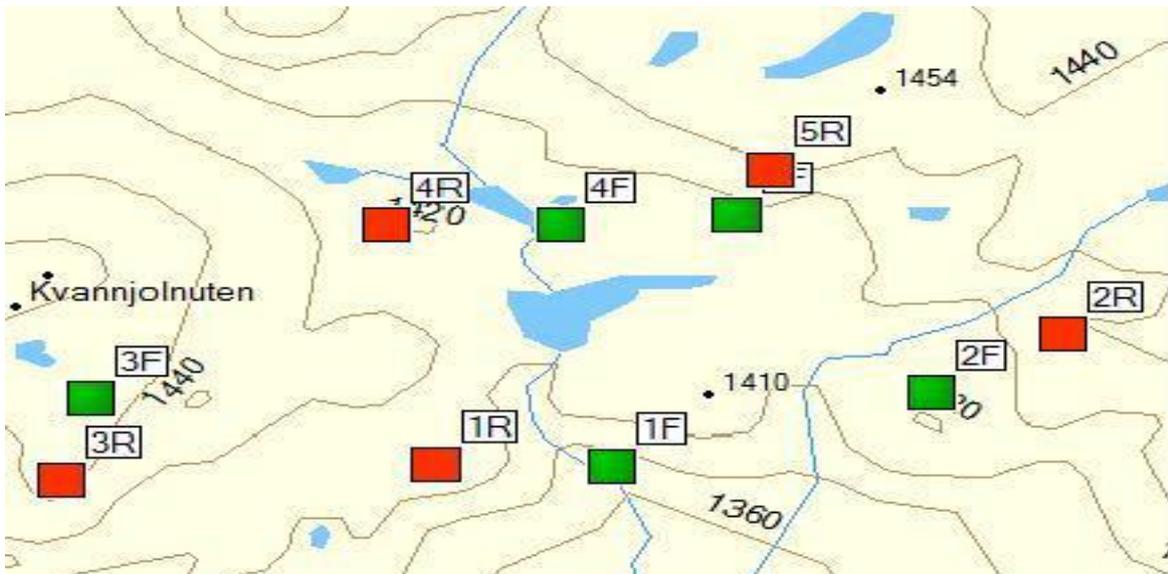


Figure 1: Map of the study site at Finse. Flat landscapes are marked with green squares and rough landscapes with red squares. (F = flat landscape, R = rough landscape).

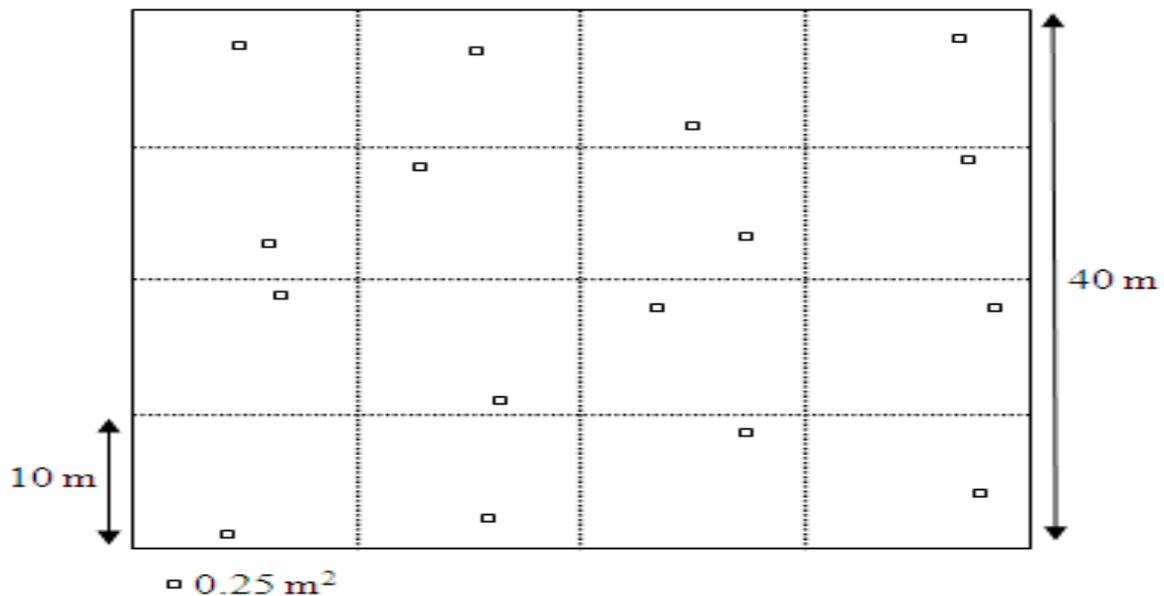


Figure 2: Sampling design for one landscape plot. Within each 10 x 10m quadrant, sample plots were placed using stratified random distribution. Bulbils were collected from plants closest to the centre of the plot.

On the 2nd August, 2012, the site was revisited by which time most of the study plants had produced enough bulbils for collection. A 0.5m x 0.5m quadrant was placed in each 10m x 10m landscape plot and plants closest to the centre of the plots were collected and taken to the laboratory. At most 15 bulbils were collected from each mother plant per plot and were packaged in paper bags, and stored in a cool, dry place before use. Soil moisture percentages were measured in all sample plots on the 10th of August after some light drizzle in the morning. This was to provide a fair idea of moisture variations on the various plots. Recordings were carried out with a soil moisture sensor (TRIME-PICO, IMKO GmbH, Ettlingen, Germany).

Green house experiment

In the green house, 480 pots each measuring 7cm in diameter were filled with potting soil. 160 pots were placed on each of three tables representing three different treatments of water, i.e., T1 = Wet moisture regime, T2 = medium moisture regime and T3 = dry moisture regime. The bulbils were sown such that each mother plant had its bulbils entering into all the three treatments. All the 480 pots were saturated with water. Excess water was allowed to remain around the pots for a day to maintain the moisture content of the soil. The room was then set to a temperature of 20°C to allow all the bulbils to sprout before the three treatment conditions could be applied. In effect, the full experiment resulted in a design of 80 replicates x 3 watering treatments x 2 landscapes = 480 plants in total.

Out of the 480 pots sown, only 106 pots (22%) had sprouting bulbils after 35 days. This resulted in an unbalanced treatment combination with some landscapes having far more individuals than others. Therefore, the study site was revisited in late September for new bulbils. *B. vivipara* plants were collected randomly from the 10m x 10m plots. The collected bulbils were sown in petri dishes with filter paper saturated with distilled water and kept at 20°C in the greenhouse. The bulbils had already shown signs of sprouting 3 days after sowing; in contrast to the 21 days it took for the first bulbils collected in early August to start sprouting.

Fourteen days after sowing, almost all bulbils had germinated. They were transplanted into 7cm diameter pots filled with potting soil and the set up described earlier was repeated. However, three plots had no bulbils at the time of collection reducing the total number of pots to 471 (i.e. of 80 replicates x 3 watering treatments x 2 landscapes = 480, minus 9 missing plants).

The pots were watered every second day until all seedlings had emerged successfully. Weeding was carried out after 14 days to ensure that each mother plant had only three seedlings, each entering into one of the three moisture regimes. Seedlings remained in the same conditions of light (MASTER SON-T PIA Plus 400W E E40, High Pressure Sodium lamp with clear tubular outer bulb), temperature (22°C and 16°C day and night respectively) and moisture for another 14 days before treatment conditions were applied. When the moisture treatment started most seedlings had 2 - 3 true leaves.

An automated watering system supplied the pots with water and the watering treatments were applied when soil moisture in T1, T2 and T3 had fallen below 70%, 50%, and 30%, threshold respectively. In practice, this meant that seedlings were given water on every 3rd day, 6th day and 9th day, respectively. These percentage values were chosen in accordance with the % moisture recordings taken from the various landscape plots during the collection of bulbils in August. The moisture levels were regulated using the soil moisture sensor (TRIME-PICO, IMKO GmbH, Ettlingen, Germany). After the fifth week treatment, there was infestation of the plants by insect larvae from the family (*Sciaridae*) and the watering treatment regimes were adjusted to 50%, 30% and 12% representing 6 days, 9days and 11 days of watering respectively. The treatment lasted for 8weeks with 25 plants dying of which 10 were from the flat landscapes and 15 from the rough landscapes.

Traits measurement

By the end of the experiment, the number of leaves per individual plant was counted. Digital calipers were used to measure the length and width of the largest leaf on each plant and the leaf shape was calculated as the leaf width / leaf length. The approximate mean leaf area was calculated as the products of the two measured parameters [i.e. approximate mean leaf area (^aMLA) = leaf length (mm) x leaf width (mm)]. We call this approximate because it actually overestimates the surface of the leaves and is hence not suitable for comparison with other studies calculating leaf area accurately. The approximate total leaf area was then calculated as the products of the leaf area and the total number of leaves [i.e. approximate total leaf area (^aTLA) = leaf area (mm²) x total number of leaves]. The above-and-below ground parts of the plants were separated and oven-dried at 60°C for 72 hours. They were weighed and the total plant biomass (dry weight, mg) and root : shoot ratio were determined. Other ratios such as approximate leaf area ratio (^aLAR) [approximate total leaf area per unit of plant biomass, mm² mg⁻¹] and approximate specific leaf area (^aSLA) [approximate leaf area per unit of leaf

biomass, $\text{mm}^2 \text{mg}^{-1}$] were calculated (Sultan & Bazzaz, 1993). Below is a table for the eight measured traits:

Trait	Unit
Approximate specific leaf area, ^a SLA	$\text{mm}^2 \text{mg}^{-1}$
Approximate leaf area ratio, ^a LAR	$\text{mm}^2 \text{mg}^{-1}$
Root : shoot ratio	
Total plant biomass	mg
Number of leaves	
Approximate mean leaf area, ^a MLA	mm^2
Approximate total leaf area, ^a TLA	mm^2
Leaf shape	

Statistical analyses

We tested variational properties in morphological traits in plants from both landscape types by using mixed effects models where site and treatment were entered as random factors. This was done to partition the variance between these different levels (Pélabon et al., 2013). The different components of variance were expressed as mean-squared scaled variances to enable comparison of the variance across morphological traits (Pélabon et al., 2013).

For plastic responses, the slope of the reaction norm of a given trait indicates its plasticity (Gianoli, 2001; Gianoli & González-Tuber, 2005). The degree of plasticity for each landscape type was investigated by calculating the absolute plasticity value for each morphological trait as the sum of the absolute difference between trait values in wet and medium treatments, and in dry and medium treatments: [Absolute plasticity (trait) = |trait value (wet) - trait value (medium)| + |trait value (dry) - trait value (medium)|]. The calculations were done for both landscape types and the analysis of variance model was run, where the absolute plasticity value was entered as the response variable with landscape type and "pair" as the predictor variables. "Pair" was not significant ($P > 0.1$) for each of the eight morphological traits evaluated. The simplified model resulted in the absolute plasticity value entered as the response variable with landscape type as the only predictor variable. Principal component analysis (PCA) was performed to identify possible correlations between traits.

Morphological traits with expected high or low responsiveness to soil moisture were investigated by comparing the mean plasticity values (steepness of the slope) and the mean-squared scaled variances of the traits. R version 2.15.2 (Foundation for Statistical Computing) was used for the statistical analyses.

RESULTS

Principal Components Analyses revealed that five out of the eight morphological traits evaluated: - ^aMLA, ^aTLA, number of leaves, root : shoot ratio and leaf shape, covaried closely. Total plant biomass, ^aSLA and ^aLAR turned out to be less correlated with the other traits (Figure 3). ^aMLA, ^aTLA and number of leaves loaded together positively on the first axis whereas root: shoot ratio and number of leaves loaded together negatively on the same axis. Also, the former traits responded positively to decreasing moisture whereas the latter ones responded negatively (Figure 4). Therefore, when analysing the phenotypic variation, we will have special focus on root : shoot ratio which is required for our predictions and acknowledge that the four other traits are correlated with this trait.

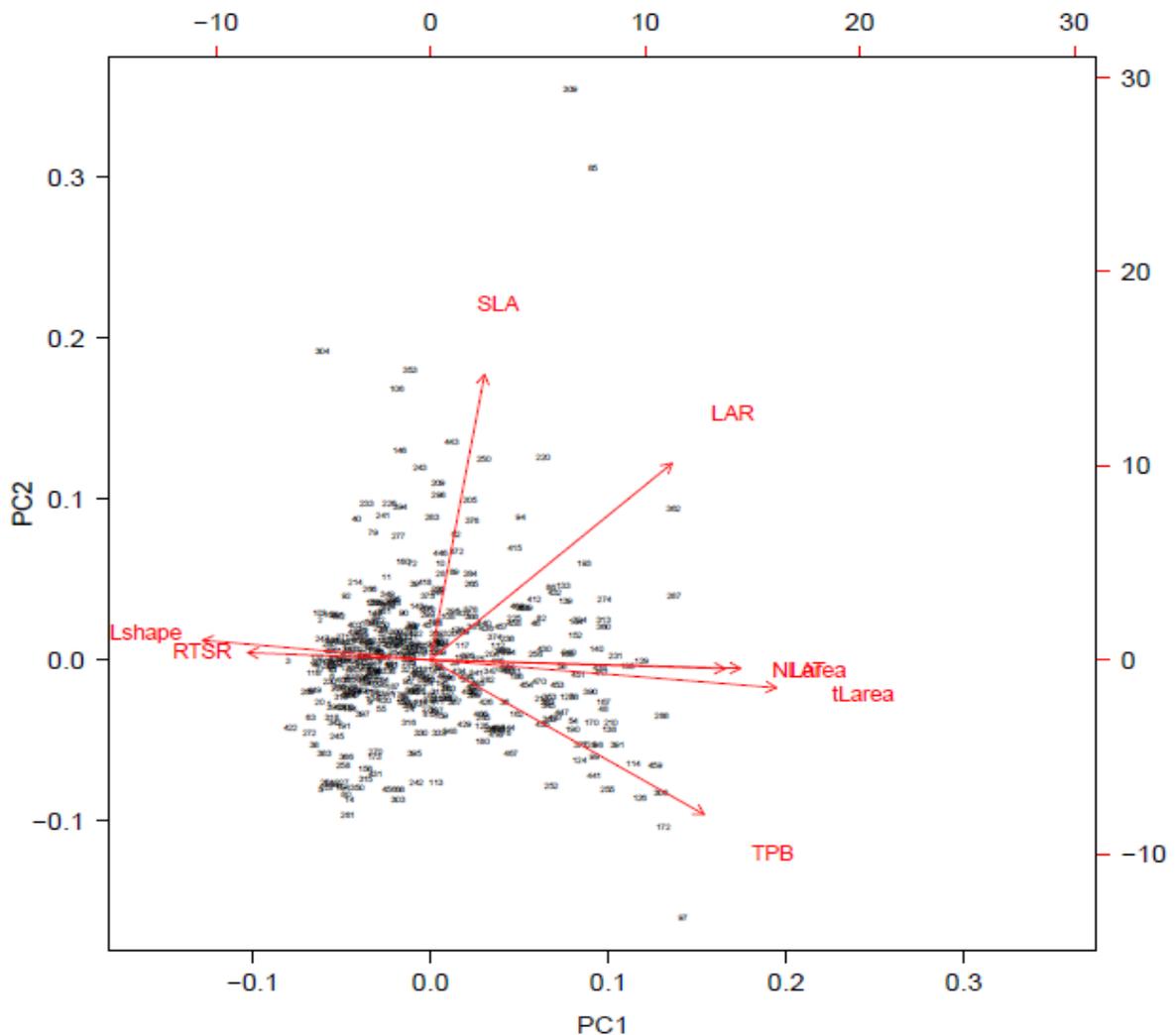


Figure 3. Biplot of principal components analysis (PCA) of morphological traits. (TPB = Total plant biomass, NLAT = Number of leaves, Larea = Approximate mean leaf area, tLarea = Approximate total leaf area, LAR = Approximate leaf area ratio, SLA = Approximate specific leaf area, Lshape = Leaf shape, RTSR = Root : shoot ratio). The first principal component (PC1) accounted for 49.9% while the second principal component (PC2) accounted for 16.9 % of the total variance in morphological traits.

Comparison of phenotypic variation between landscape types

Out of the eight morphological traits evaluated, plants from the flat landscapes showed the highest total phenotypic variation in the greenhouse. Of the eight morphological traits, a total mean squared scaled variance of 3.8 was generated by plants from the rough landscapes as compared to 4.3 generated by those from the flat landscapes (Table 1). Plants from the rough landscapes had a treatment variance of 0.2 (5.2%), a site variance of 0.15 (3.9%) and a residual variance of 3.4 (90.8%) for the eight traits. Plants from the flat landscapes exhibited a treatment variance of 0.06 (1.3%), a site variance of 0.27 (6.3%) and a residual of 3.9 (92.4%) for the eight traits.

Landscape type with greater phenotypic plasticity to soil moisture

Phenotypic plasticity in response to soil moisture was greater in plants from the rough landscapes than in plants from the flat landscapes (Table 1&2). Among the five traits that showed close correlation (^aMLA, ^aTLA, number of leaves, root-shoot ratio and leaf shape), and the three independent traits (total plant biomass, ^aSLA and ^aLAR) evaluated, all except leaf shape weakly showed greater phenotypic plasticity to moisture in plants from the rough landscapes compared to those from the flat landscapes (Table 2). However, in none of the morphological traits evaluated was this plastic response to soil moisture significantly different between the two landscapes (Table 2).

Table 1. *Components of the phenotypic variance in morphological traits*

Trait	Landscape type	Mean \pm s.e.	Treatment	Site	Residual	CV ²
^a SLA	Flat	25.62 \pm 1.65	0.011 8%	0 0%	0.13 92%	0.14
	Rough	25.41 \pm 1.80	0.013 7%	0 0%	0.172 93%	0.19
^a LAR	Flat	4.33 \pm 0.44	0.021 4%	0.019 3%	0.55 93%	0.59
	Rough	4.87 \pm 0.32	0.0025 0.4%	0.016 2.8%	0.56 97%	0.58
Root: shoot ratio	Flat	5.84 \pm 0.41	0 0%	0.046 10%	0.43 90%	0.48
	Rough	5.37 \pm 0.66	0.032 5%	0.023 3%	0.64 92%	0.70
Total plant biomass	Flat	64.22 \pm 6.37	0.011 1.49%	0.047 6.35%	0.682 92.16%	0.74
	Rough	68.21 \pm 10.93	0.064 14%	0.039 8%	0.365 78%	0.47
Number of leaves	Flat	2.66 \pm 0.22	0 0%	0.016 4%	0.342 96%	0.36
	Rough	2.87 \pm 0.22	0.021 7%	0.01 4%	0.259 89%	0.29
^a MLA	Flat	94.03 \pm 6.14	0.0004 0.1%	0.03 6%	0.474 93.9%	0.50
	Rough	106.16 \pm 8.92	0.012 3%	0.016 4%	0.42 93%	0.45
^a TLA	Flat	306.57 \pm 39.28	0.013 1%	0.1 7%	1.23 92%	1.30
	Rough	348.59 \pm 51.56	0.045 5%	0.042 4%	0.9 91%	0.99
Leaf Shape	Flat	0.52 \pm 0.02	0 0%	0.0081 6%	0.129 94%	0.14
	Rough	0.50 \pm 0.03	0.0064 5%	0 0%	0.112 95%	0.12

For both landscapes, the different variance components are given as mean-square scaled variances on the top line, and percentage of the total variance on the bottom line for each morphological trait. The given means are the estimates of the fixed effect in the mixed-effects models. The mean-squared scaled variances were obtained by scaling the variances by the square of the means.

Total CV² = CV² (Treatment) + CV² (Site) + CV² (Residual)

Flat landscape [overall sum (CV²) = 4.3] Rough landscape [overall sum (CV²) = 3.8]

Table 2. Analysis of variance for the effect of soil moisture on the phenotypic plasticity in *Bistorta vivipara* from topographically rough and flat landscapes

Response	Mean plasticity \pm s.e.		Effect	Df	Mean Square	F value	P
	Flat	Rough					
^a SLA	20.47 \pm 2.13	< 21.93 \pm 2.21	Landscape	1	70.2	0.224	0.637
			Residual	131	313.6		
^a LAR	5.54 \pm 0.68	< 6.76 \pm 0.71	Landscape	1	49.6	1.548	0.216
			Residual	131	32.0		
Root : shoot ratio	7.11 \pm 1.13	< 7.49 \pm 1.17	Landscape	1	4.8	0.054	0.816
			Residual	131	87.9		
Plant biomass	73.81 \pm 7.28	< 83.01 \pm 7.56	Landscape	1	2851.9	0.779	0.380
			Residual	131	3660.1		
Number of leaves	2.71 \pm 0.23	< 3.08 \pm 0.24	Landscape	1	4.5	1.267	0.262
			Residual	131	3.5		
^a MLA	94.54 \pm 10.46	< 107.02 \pm 10.86	Landscape	1	5178.7	0.687	0.409
			Residual	131	7543.1		
^a TLA	473.71 \pm 56.15	< 566.86 \pm 58.30	Landscape	1	288103	1.324	0.252
			Residual	131	217540		
Leaf shape	0.30 \pm 0.02	= 0.30 \pm 0.02	Landscape	1	0.000004	0.0001	0.992
			Residual	131	0.04		

Estimates (+ s.e.) obtained from the analysis of variance models (ANOVA) with mean plasticity entered as the response variable and the landscape type as the predictor variable. Mean plasticity values were obtained from the absolute plasticity values along the three moisture regimes [i.e., Absolute plasticity (trait) = |trait value (wet) - trait value (medium)| + |trait value (dry) - trait value (medium)|]

Trait responsiveness to soil moisture and topography

Plants from the rough landscapes showed relatively greater variance in the expression of ^aSLA than those from the flat landscapes (Table 1). Plasticity to moisture was also greater in plants originating from the rough than in those from flat landscapes. However the total variation in the ^aSLA was unexpectedly low. Looking at the total leaf area per plant biomass; ^aLAR , plants from both landscapes produced high variation and was approximately the same for both landscapes (Table 1). Plasticity to soil moisture was much higher in plants from the flat landscapes than those from the rough landscapes according to the variance coefficient analyses (Table 1). However, it was higher in plants from the rough landscapes than those from the flat landscapes according to the absolute plasticity analyses (table 2). Both ^aSLA and ^aLAR responded negatively to soil dryness (Figures 4).

Plants produced high variation in root : shoot ratio (Table 1). Phenotypic variation in the expression of root : shoot ratio was higher in plants from rough landscapes than those from flat landscapes (Table 1), and a higher share was explained by plasticity in the rough landscapes than in the flat (Table 2). The total variation in plant biomass was higher in plants from the flat landscapes than in those from the rough landscapes. However, plasticity to moisture was very high in plants from the rough landscapes, whereas plants from the flat landscapes showed much reduced or approximately no plasticity in plant biomass (Table 1&2). Plant biomass responded positively to decreasing soil moisture in plants from both landscapes (Figure 4). Of all traits tested, ^aTLA and total plant biomass in plants from the flat landscapes exhibited the greatest variation whereas ^aSLA exhibited the lowest variation in the same landscape. Plasticity to moisture was greatest in plant biomass but only in the plants from the rough landscapes. It was lowest in root : shoot ratio in plants from the flat landscapes.

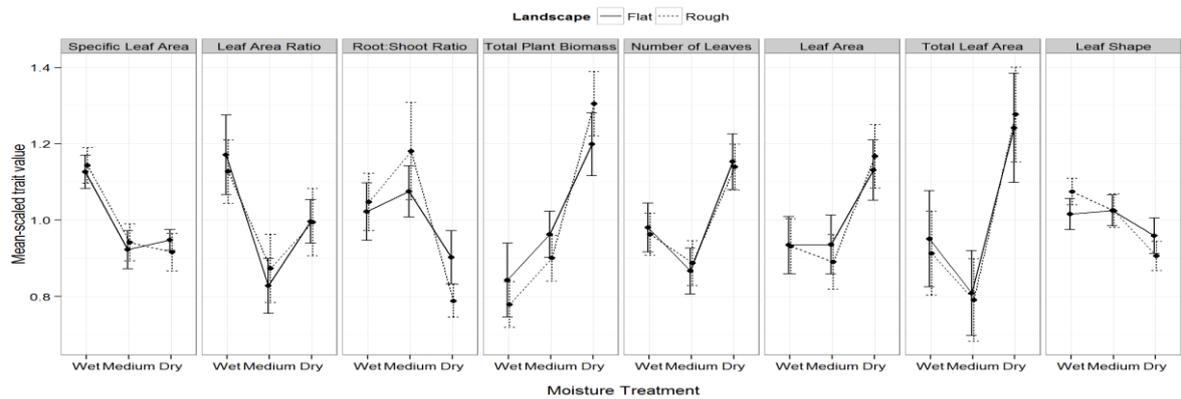
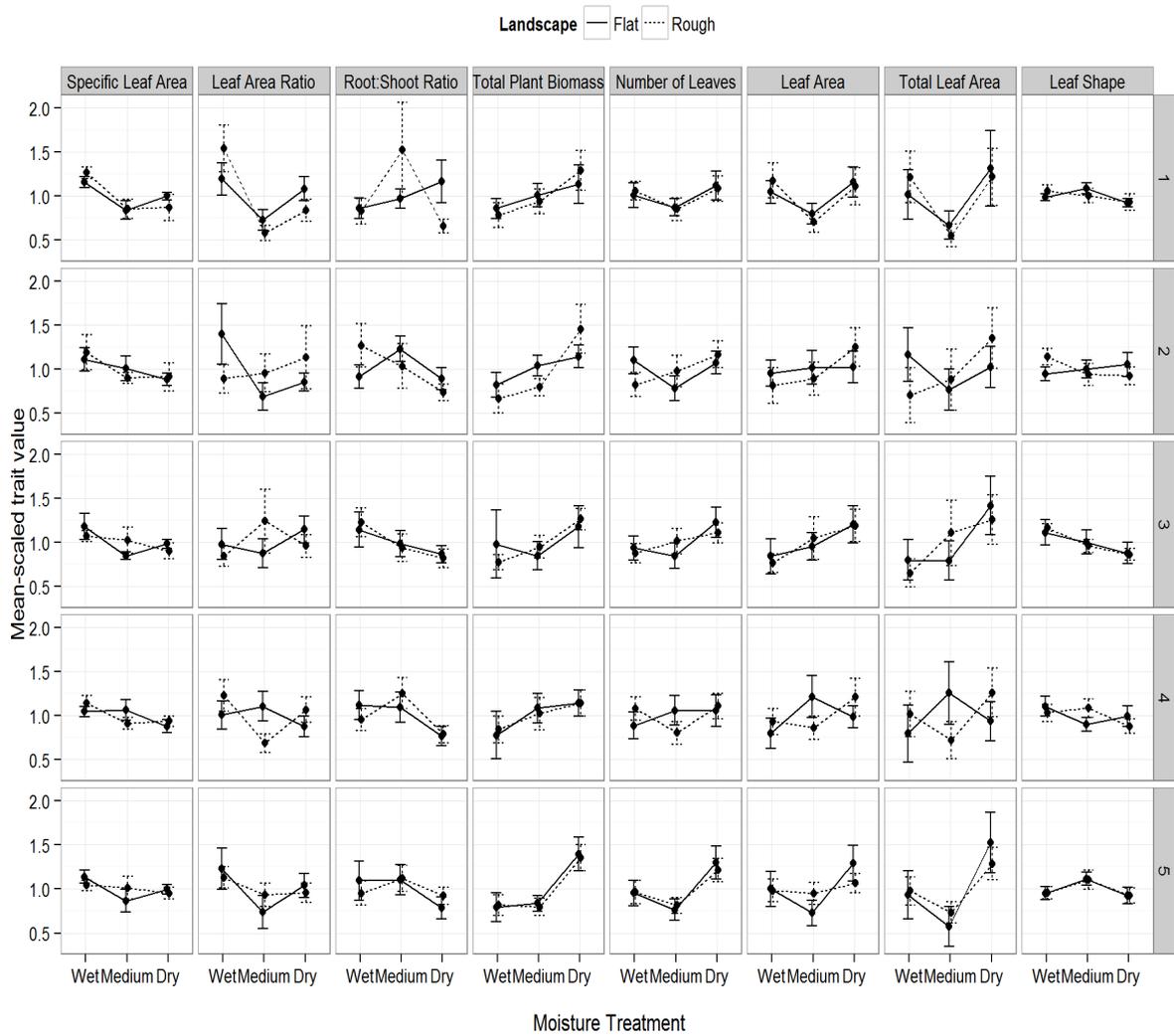


Figure 4. Comparison of phenotypic plasticity in *Bistorta vivipara* from rough and flat landscapes. Reaction norms of mean-scaled trait value to soil moisture (wet and dry moisture regimes) of plants from different landscapes. In the top figure, each panel contains reaction norms for plants from two site pairs (flat 1-rough 1-flat 5-rough 5) from top to bottom. Lines were made with mean-scaled trait values for 48 individuals, and represent the effect of moisture treatment on the various morphological traits. The bottom figure is the summary of the top figure combining the 5 landscape pairs (flat 1-rough 1-flat 5-rough 5) into 2 landscape types- Flat and Rough.

DISCUSSION

Comparison of phenotypic variation between landscape types

Plants from the flat landscapes demonstrated a greater phenotypic variation than those from the rough landscapes. However, variation due to plasticity was greater in plants from the rough landscapes. The reduced phenotypic variation in plants from the rough landscapes may be caused by frequent windy conditions spreading bulbils across the landscape such that similar genets inhabited the same site. Thus, there may have been reduced genetic diversity in plants from the rough landscapes possibly due to this dispersal patterns and/or a stronger selection for plasticity. This would explain why a greater percentage (6.3%) of the variation in plants originating from the flat landscapes was caused by differences between sites compared to 3.9% in plants from the rough landscapes. Although site variation may be caused by environmental or maternal effects, we think that maternal effect may be the cause and bulk of it may be genetic diversity. This is based on the earlier finding made by Diggle et al. (1998) who documented high levels of genetic diversity in *Bistorta vivipara*. Thus, mothers in different and especially in the flat landscapes showed higher variation in the expression of their morphological traits. The higher treatment variance in plants from the rough landscapes confirms their greater selection for plasticity.

Phenotypic integration may be another cause for the low phenotypic variance in plants from the rough landscapes. Particularly, in the four integrated traits (^aMLA, ^aTLA, number of leaves and leaf shape), phenotypic variation tended to be higher in plants from the flat landscapes. In most similar studies (e.g., Sultan & Bazzaz, 1993; Gianoli & González-Tuber, 2005), these traits have been reported to decrease in mean values with soil dryness. In contrast to this observation, all except leaf shape increased with soil dryness in the current study. Phenotypic integration has been reported to increase with environmental stress for several plant species (Schlichting, 1989; Waitt & Levin, 1993; Pigliucci, 2004; Gianoli, 2004). The moisture treatment levels used in the experiment were chosen in accordance with moisture percentages recorded in the field during early morning showers in August, 2012. It may be possible that plants especially those in the wet moisture treatment, received water above their field capacity resulting in water stress. The resulting decrease in squared coefficient of variation in each of the integrated traits especially in plants from the rough landscapes may be a mechanism driving adaptive evolution (Lande, 1980; 1984; Wagner

1988). The increase in squared coefficient of variation in each of the integrated traits in plants from the flat landscapes suggests their ability to cope with higher moisture levels.

Greater unexplained variance may be another possible reason for the lower phenotypic variance especially in plants from the rough landscapes. Unexplained variance was 92.4% in plants from the flat landscapes and 90.8% in the rough landscapes. Thus, a greater percentage of the phenotypic variance in the plants came from some other factors and was relatively higher in plants from flat landscapes.

Landscape type with greater phenotypic plasticity

We hypothesized that plants from rough landscapes would show greater phenotypic plasticity than those from flat landscapes. Although results obtained from ANOVA indicated that differences in plasticity to soil moisture was not statistically significant between plants from the two landscapes, the general trend weakly supports the hypothesis. Plants from the topographically rough landscapes demonstrated weakly higher plasticity for the ^aSLA, plant biomass and the closely correlated traits (e.g., root: shoot ratio) than plants from the flat landscapes. These three morphological traits have dominated in plastic response studies in plants where moisture was considered (e.g., Sultan and Bazzaz, 1993; Gianoli & González-Tuber, 2005; Mallitt, Bonser & Hunt, 2010). They are also the traits that climate change is predicted to affect, especially plant biomass and ^aSLA (Matesanz et al., 2010). This suggests that populations that occur in environments with heterogeneous moisture conditions may be able to cope because they have seemingly greater capacity for plasticity for these traits.

Trait responsiveness to soil moisture and topography

There was a greater variation, and plasticity to moisture for ^aSLA and root : shoot ratio in plants from the rough landscapes than in those from the flat landscapes. These traits are associated with water conservation and collection in plants respectively (Reich et al., 1997), and may be more important to species which inhabit environments with heterogeneous moisture conditions (e.g., rough landscapes). As expected, ^aSLA decreased with soil dryness. Studies which have similarly reported decrease in specific leaf area (here called ^aSLA) with soil dryness also reported decreases in plant biomass, leaf area (here called ^aMLA), total leaf area (here called ^aTLA) and number of leaves, and an increase in root : shoot ratio with soil dryness (Sultan & Bazzaz, 1993; Gianoli & González-Tuber, 2005; Mallitt et al., 2010).

The above results are in contrast to that of the current study, as the four former traits rather increased with soil dryness possibly due to phenotypic integration. Thus, plants especially those from the rough landscapes needed to balance photosynthesis (increased biomass production) and evapotranspiration (more leaves with large area) simultaneously in the dry soil. Hence, there was the need for thicker leaves (lower ^aSLA). The decreased allocation to root biomass in the dry moisture regime suggests that plants in the current study perhaps had high instantaneous water use efficiency which compensated for the low water acquisition capacity (Heschel et al., 2004). It is also possible that the drought threshold was not achieved such that plants in the dry moisture regime still had enough water whereas those in the wet regime received water above their field capacity. Therefore, there was no need for plants to increase resource allocation to root biomass. This explains why root : shoot ratio showed a negative relationship especially with the traits that demonstrated phenotypic integration. Thus, root : shoot ratio was traded-off to allow resource allocation particularly to the integrated trait which increased with soil dryness. The high plasticity of both traits in plants from the rough landscapes indicates their relatively higher capacity to cope with moisture variations than those from the flat landscapes.

A greater plasticity to moisture in plant biomass and its resulting increase with soil dryness suggests that plants from the rough landscapes have a higher fitness and tolerance to soil moisture variation compared to those from the flat landscapes. The low phenotypic variance for plant biomass suggests that plants in the rough landscapes responded similarly to moisture variation than those in the flat landscapes. This is possibly due to the decrease in genetic diversity of plants from the rough landscapes. Although there was approximately equal variance for ^aLAR in plants from both landscapes, there was no clear pattern as to which landscape plants exhibited a higher plasticity for the trait as the two analyses produced contrasting results (Table 1&2). Overall, results suggest that similar levels of stress tolerance may be achieved with varying underlying conditions of responses (Griffith, Kim & Donohue, 2004). Plants from rough landscapes are better adapted to heterogeneous moisture conditions than those from the flat landscapes.

The non-significant difference in plasticity between the two landscapes could be attributed to the fact that migration may have spread similar genets over the two landscapes. This is possibly due to efficient dispersing ability of *B. vivipara* (Bauert, 1996; Graae et al., 2004;

Gillis et al., 2005; Bruun et al., 2008) which may be enough to overcome the distances between site pairs. Thus, individuals may have become adapted differently to soil moisture variations but not so strongly as to create detectable significant difference in plasticity between the landscapes with a small experiment. This seems to suggest why *B. vivipara* was well represented in the two landscapes during bulbil collection. Plastic responses in plants in relation to landscape roughness may also be controlled by other environmental factors (e.g. soil nutrients, pH, temperature, light, bed rock composition etc.) which act synergistically with soil moisture. This suggests why the highest percentage of the phenotypic variation came from unexplained variance other than the two investigated variables: - moisture and topography. This supports Mallitt et al. (2010) who recommended the use of multi-environmental factors in plasticity experiments. They found that genotypes of pepper grass (*Lepidium bonariense*) demonstrated high degree of plasticity to combined effects of light and water availability than each of the single factors. Hence, the degree of plasticity may be underestimated in single-factor experiments.

Moreover, *B. vivipara* is a perennial species whose leaves require 4 years of initiation before reaching their functional and structural maturity. This development feature has profound consequences for dynamics of resource allocation and the timing of plant responses to environmental variation (Diggle, 1997). Significant differences in degree of plasticity to moisture in plants from the two landscapes may have shown up in later stages of development than the 3 months in the greenhouse. Additionally, the moisture treatment levels used in the experiment were chosen in accordance with moisture percentages recorded in the field during early morning showers in August, 2012. It is possible that plants in the greenhouse received wetter conditions than where they thrive best. Therefore, the true variation in magnitude of plasticity of plants from the two landscapes could not be revealed. The larval attack on the roots of the plants may have further decreased the growth potential of the plants. It is also noteworthy that what is actually considered as topographically rough or flat landscape may be subjected to questioning as it is difficult to find in nature, a 'true' flat and rough landscapes occurring in the same site (i.e., close to each other).

CONCLUSION AND FURTHER RESEARCH

The aim of the study was to investigate if *Bistorta vivipara* from topographically rough landscapes were more plastic to soil moisture than those from flat landscapes, and whether topographic effect on environmental conditions (soil moisture) will offer plants some buffering against future environmental changes. Results obtained weakly confirm our hypothesis and support the theoretical expectation that plasticity would be of a greater magnitude in more heterogeneous environments. Results weakly add to the existing evidence from studies that have documented greater fitness in plants occurring in heterogeneous environments compared to more homogeneous ones. Results further seemed to suggest that phenotypic variation may be lower in species from heterogeneous landscapes because of reduced genetic diversity and/or greater selection for plasticity, due to continuous spread of similar genets over the same site. Overall, results suggest that plants in heterogeneous landscapes are more plastic and therefore plants from such landscapes will cope better with climate change.

We recommend further research to use the F_2 generation to verify the presence of genetic variation. Including other environmental factors such as temperature, light and nutrients may reveal the true contrast in plasticity between plants from the two landscapes. We also recommend adjustment in moisture to 4 days, 8 days and 12 days. Finally, testing results on sexually reproducing species such as *Luzula spicata* may broaden our knowledge on how topographic effects on environmental conditions will affect plastic responses in different reproducing species. The intra-pair and inter-pair distances should be consistent and reasonably longer.

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Appendix 1

R script for the statistical analyses

ANOVA - Absolute plasticity analysis

```
model1 <- lm(Absolute plasticity of trait~Landscape type * pair, na.action=na.exclude)
```

```
model2 <- lm(Absolute plasticity of trait~Landscape type + pair, na.action=na.exclude)
```

```
model3 <- lm(Absolute plasticity of trait~Landscape type, na.action=na.exclude)
```

```
anova(model3)
```

```
summary(model3)
```

Variance components analysis

```
summary(lmer(Trait~1+(1|Treatment/Site),na.action=na.exclude,data=data [Landscape  
type=="Flat",]))
```

```
summary(lmer(Trait~1+(1|Treatment/Site),na.action=na.exclude,data=data [Landscape  
type=="Rough",]))
```

Mixed effects models - Absolute plasticity analysis

```
model1 <- lmer(Absolute plasticity of trait~Landscape type-1+(1|Pair/Site),  
na.action=na.exclude)
```

```
summary(model1)
```

Appendix 2

Effect of soil moisture on the phenotypic plasticity in Bistorta vivipara from topographically rough and flat landscapes

Response	Mean plasticity \pm s.e.		
	Flat		Rough
^a SLA	20.43 \pm 2.35	<	22.00 \pm 2.43
^a LAR	5.54 \pm 0.80	<	6.78 \pm 0.82
Root : shoot ratio	7.03 \pm 1.35	<	7.47 \pm 1.38
Plant biomass	74.37 \pm 8.83	<	83.01 \pm 9.05
Number of leaves	2.72 \pm 0.25	<	3.08 \pm 0.26
^a MLA	94.51 \pm 10.87	<	107.09 \pm 11.25
^a TLA	474.64 \pm 61.80	<	566.31 \pm 63.72
Leaf shape	0.30 \pm 0.02	=	0.30 \pm 0.03

Estimates (+ s.e.) obtained from the mixed effects models fitted with mean plasticity entered as the response variable, landscape type as the fixed factor and "pair" and "site" as random factors, with "site" nested within "pair". Mean plasticity values were obtained from the absolute plasticity values along the three moisture regimes [i.e., Absolute plasticity (trait) = | trait value (wet) - trait value (medium) | + | trait value (dry) - trait value (medium) |].

Appendix 3

For the five landscape pairs, mean, (standard deviation), coefficient of variation for each morphological trait in the three moisture treatments are given.

Table continues in the next page.

Trait	Treatment	1		2		3	
		F	R	F	R	F	R
^a SLA	wet	28.88	30.17	29.11	31.40	31.48	27.94
		(6.32)	(6.04)	(14.03)	(19.31)	(14.18)	(6.50)
	medium	0.22	0.2	0.48	0.61	0.45	0.23
		20.91	20.33	26.38	23.68	22.64	26.69
		(10.64)	(9.83)	(13.15)	(5.76)	(4.40)	(13.81)
		0.51	0.48	0.5	0.24	0.19	0.52
dry	24.89	20.70	23.12	24.06	26.28	23.54	
	(4.17)	(13.13)	(7.44)	(16.86)	(4.68)	(9.15)	
		0.17	0.63	0.32	0.7	0.18	0.39
^a LAR	wet	3.92	7.11	7.03	4.71	4.38	3.96
		(2.42)	(4.69)	(6.93)	(3.18)	(2.85)	(2.14)
	medium	0.62	0.66	0.99	0.68	0.54	0.54
		2.38	2.49	3.46	5.06	3.93	5.83
		(1.53)	(1.51)	(2.83)	(4.10)	(2.73)	(6.16)
		0.64	0.56	0.82	0.81	0.69	1.06
dry	3.55	3.88	4.29	6.00	5.14	4.51	
	(1.78)	(2.12)	(2.02)	(7.58)	(2.47)	(2.43)	
		0.5	0.55	0.47	1.23	0.48	0.54
Root : shoot ratio	wet	7.14	5.11	4.34	6.67	5.66	6.31
		(3.85)	(3.45)	(2.49)	(4.85)	(3.54)	(3.34)
	medium	0.54	0.68	1.57	0.73	0.62	0.53
		8.05	9.42	5.82	5.45	4.87	4.84
		(3.63)	(12.39)	(2.48)	(4.87)	(2.76)	(2.92)
		0.45	1.31	0.43	0.89	0.54	0.6
dry	9.66	4.05	4.22	3.87	4.28	4.23	
	(8.09)	(1.76)	(2.32)	(1.20)	(1.72)	(2.17)	
		0.83	0.43	0.55	0.52	0.4	0.51
Total plant biomass	wet	44.78	53.28	44.33	38.75	95.48	43.59
		(23.43)	(36.29)	(30.65)	(35.79)	(135.16)	(20.00)
	medium	0.52	0.68	0.69	0.92	0.92	0.46
		52.66	64.01	56.05	46.13	82.48	53.37
		(27.83)	(36.01)	(22.79)	(20.32)	(57.27)	(26.34)
		0.53	0.56	0.41	0.44	0.69	0.49
dry	59.08	87.81	61.69	84.48	114.58	71.10	
	(45.48)	(56.91)	(27.79)	(63.86)	(83.89)	(26.84)	
		0.77	0.65	0.45	0.76	0.73	0.38
Number of leaves	wet	2.31	2.93	2.81	2.07	3.00	2.13
		(1.30)	(1.16)	(1.56)	(1.27)	(1.58)	(1.09)
	medium	0.56	0.4	0.55	0.61	0.53	0.51
		2.00	2.36	2.00	2.46	2.71	2.46
		(0.89)	(1.34)	(1.29)	(1.61)	(1.73)	(1.27)
		0.45	0.57	0.65	0.66	0.64	0.51
dry	2.56	3.00	2.75	2.94	3.92	2.69	
	(1.50)	(1.52)	(1.29)	(1.53)	(1.98)	(1.08)	
		0.59	0.51	0.47	0.52	0.5	0.4

Trait	Treatment	4		5	
		F	R	F	R
^a SLA	wet	26.22	28.7	29.08	26.68
		(5.60)	(8.88)	(7.72)	(5.53)
		0.21	0.31	0.27	0.21
	medium	26.71	22.91	22.13	26.04
		(11.50)	(6.64)	(12.9)	(13.49)
		0.43	0.29	0.58	0.52
	dry	22.00	23.55	25.43	24.42
		(6.93)	(5.47)	(5.50)	(6.76)
		0.31	0.23	0.22	0.28
^a LAR	wet	4.20	5.53	5.89	5.97
		(2.56)	(3.27)	(4.50)	(2.34)
		0.61	0.59	0.76	0.39
	medium	4.61	3.09	3.53	4.94
		(2.75)	(1.85)	(3.58)	(2.79)
		0.6	0.6	1.01	0.56
	dry	3.65	4.81	4.99	5.06
		(1.82)	(2.55)	(2.53)	(2.27)
		0.5	0.53	0.51	0.45
Root : shoot ratio	wet	6.48	5.94	5.88	3.79
		(3.73)	(3.07)	(4.80)	(1.97)
		0.58	0.52	0.82	0.52
	medium	6.37	7.79	5.94	4.47
		(3.83)	(4.26)	(3.73)	(2.39)
		0.6	0.55	0.63	0.54
	dry	4.44	4.90	4.23	3.68
		(2.37)	(2.41)	(2.6)	(4.56)
		0.53	0.5	0.61	0.42
Total plant biomass	wet	51.45	55.54	42.59	77.36
		(69.13)	(40.41)	(35.74)	(40.01)
		1.34	0.73	0.84	0.52
	medium	71.73	67.33	44.99	75.36
		(43.39)	(47.27)	(19.84)	(37.14)
		0.6	0.7	0.44	0.49
	dry	75.52	75.33	75.05	128.47
		(36.31)	(38.60)	(39.41)	(57.52)
		0.48	0.51	0.53	0.45
Number of leaves	wet	2.69	3.31	2.50	3.46
		(1.08)	(1.62)	(1.51)	(1.29)
		0.66	0.49	0.6	0.49
	medium	2.87	2.47	2.00	2.94
		(1.77)	(1.55)	(1.26)	(1.29)
		0.62	0.63	0.63	0.44
	dry	2.86	3.40	3.4	4.38
		(1.83)	(1.68)	(1.92)	(1.89)
		0.64	0.49	0.56	0.43

^a MLA	wet	70.57	110.28	92.19	83.50	101.65	80.03
		(34.7)	(72.89)	(57.01)	(78.15)	(85.47)	(44.23)
	medium	0.49	0.66	0.62	0.94	0.84	0.55
		53.78	61.87	98.22	91.76	114.81	109.72
		(31.72)	(39.75)	(66.01)	(68.88)	(70.09)	(91.40)
		0.59	0.6	0.67	0.75	0.61	0.83
dry	77.21	104.04	98.67	128.66	145.11	124.35	
	(44.94)	(74.01)	(67.99)	(89.37)	(92.43)	(76.89)	
		0.59	0.71	0.69	0.69	0.64	0.62
^a TLA	wet	188.43	364.47	310.24	221.44	381.61	189.34
		(205.56)	(338.74)	(327.0)	(368.68)	(397.65)	(187.91)
	medium	1.09	0.93	1.05	1.66	1.04	0.99
		123.71	165.56	204.21	276.47	376.94	320.69
		(118.04)	(141.86)	(226.61)	(394.17)	(395.99)	(385.94)
		0.95	0.86	1.11	1.43	1.05	1.2
dry	243.43	345.41	272.01	423.47	674.38	363.77	
	(315.62)	(356.45)	(248.18)	(431.42)	(568.71)	(324.58)	
		1.3	1	0.91	1.02	0.84	0.89
Leaf shape	wet	0.6	0.53	0.49	0.57	0.55	0.62
		(0.09)	(0.13)	(0.16)	(0.17)	(0.26)	(0.098)
	medium	0.15	0.25	0.33	0.3	0.47	0.16
		0.66	0.5	0.52	0.47	0.5	0.51
		(0.15)	(0.16)	(0.19)	(0.22)	(0.25)	(0.14)
		0.23	0.31	0.37	0.47	0.5	0.27
dry	0.56	0.46	0.55	0.46	0.44	0.46	
	(0.11)	(0.17)	(0.28)	(0.2)	(0.21)	(0.14)	
		0.19	0.37	0.51	0.44	0.49	0.31

		78.73	85.89	86.63	135.91
	wet	(65.48)	(55.00)	72.19)	(64.07)
		0.83	0.64	0.81	0.47
^a MLA	medium	120.09	79.67	65.13	131.33
		(90.39)	(48.13)	(51.14)	(70.60)
		0.75	0.6	0.79	0.54
	dry	97.31	111.76	115.71	147.94
		(45.50)	(76.27)	(69.92)	(60.98)
		0.47	0.68	0.6	0.41
		258.43	337.94	274.94	502.0
	wet	(406.58)	(343.80)	(320.64)	(295.67)
		1.57	1.02	1.17	0.65
^a TLA	medium	407.59	239.33	169.21	376.23
		(445.91)	(269.55)	(267.72)	(244.29)
		1.09	1.13	1.58	0.65
	dry	303.61	419.79	450.34	659.93
		(268.18)	(355.41)	(388.89)	(376.61)
		0.88	0.85	0.86	0.57
		0.5(0.2)	0.51	0.51	0.44
	wet		(0.19)	(0.16)	(0.11)
		0.39	0.38	0.32	0.26
		0.41	0.53	0.59	0.51
Leaf shape	medium	(0.14)	(0.2)	(0.15)	(0.19)
		0.34	0.37	0.26	0.38
		0.45	0.43	0.49	0.43
	dry	(0.2)	(0.16)	(0.19)	(0.16)
		0.45	0.37	0.39	0.37