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Trait differentiation among *Stipa krylovii* populations in the InnerMongolia Steppe region

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ABSTRACT

Terrestrial plants in natural populations can adjust their morphologies and structures in response to different environmental conditions by genetic differentiation and environmental modification (phenotypic plasticity). The adaptive mechanisms for quantitative variation of 7 *Stipa krylovii* populations were evaluated using a combination of experiments performed in a field and a common garden. (1) In the common garden experiment, the quantitative traits of *S. krylovii* differed significantly ($P < 0.05$), and high overall differentiation (D) was found between populations ranging from 0.53 to 0.97; the first 3 principal components (PCs) could explain 78.4% of the variation among the populations, but only 3/39 pairwise variables showed significant correlations between PC scores and environmental variables. These results indicated that neutral processes might be mainly responsible for the contemporary patterns of phenotypic differentiation of *S. krylovii*. (2) In the field, populations of *S. krylovii* differed significantly for all traits ($P < 0.001$); the first 3 principal components could explain 80.8% of the variation among the populations, and 5/39 pairwise variables between PC scores and environmental variables showed significant correlations. Only two traits (the lengths of the first and the second glumes) showed similar phenotypic pattern (based on the results of Spearman's correlation) and similar values (based on the results of paired samples *t*-tests) between both conditions. These results indicated environmental modification (phenotypic plasticity) played a certain role for the phenotypic differentiation of *S. krylovii*. (3) Intra-population variability (CV_{intra}) of *S. krylovii* was systematically higher than inter-population variability (CV_{inter}) for all traits, except for the 1000-seed weights in the common garden experiment, suggesting a high potential capacity of *S. krylovii* to adapt to environmental changes. The results indicate the importance of combining data from field and common garden experiments in order to understand the ecological adaptive mechanism and evolutionary potential of such an important species in view of its phenotypic divergence.

1. Introduction

Over the last few decades, increasing attention has been paid to evolutionary responses to rapid global changes, including environmental changes and anthropogenic activities (Parmesan, 2006; Waples et al., 2008). Environmental changes (for example, climatic changes) have shifted plant distributions, resulting in plant species expansion in newly favourable areas and decline in increasingly unfavourable locations (Kelly and Goulden, 2008). Due to anthropogenic influences, many natural terrestrial communities throughout the world are becoming increasingly degraded or lost, especially in the arid and semi-arid grasslands (Gustafson et al., 2004; Li, 1997). The most important cause of species' geographic distribution limits is related to their capacity for adaptation across spatially variable environments (Eckhart et al., 2011). Environmental gradients provide an ideal experimental setting for investigating plants' adaptations to environmental changes (Lowry et al., 2014; Ravenscroft et al., 2014). Habitat fragmentation may limit the range shift (Davis and Shaw, 2001), although range shifts have been reported for many species in regions such as grassland ecosystems (Liu, 2003).

Terrestrial plants in natural populations can show morphological and structural changes in response to differing environmental conditions by genetic differentiation and environmental modification (phenotypic plasticity) (Martin and Asner, 2009; Wright et al., 2004). These 2 mechanisms enable plants to cope with rapid

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environmental changes like climate changes (Bresson et al., 2011). Genetic differentiation and environmental modification (phenotypic plasticity) can be distinguished by raising individual plants from different environments under the same conditions (i.e. in a common garden) (Alberto et al., 2011, 2013; Ballentine and Greenberg, 2010; Baruch et al., 2004; Vitasse et al., 2009). When phenotypic divergence disappears in common garden populations, this indicates that the phenotypic differences observed in the original habitats might be due to phenotypic plasticity resulting from environmental modification. When phenotypic divergence is maintained in common garden populations, this indicates that phenotypic differences might be due to underlying genetic differentiation (Alberto et al., 2011, 2013). Moreover, if environmental and morphometric differences are significantly correlated in a common garden, then phenotypic genetic differentiation might be under the local adaptation scenario; if not, then phenotypic genetic differentiation might be under neutral processes scenario, such as founder effect, genetic drift and migration (Baruch et al., 2004). In addition, if a significant correlation occurs between environmental and morphometric differences in natural conditions, such a correlation might contribute to the combined effects of environmental modification (phenotypic plasticity) and local adaptation.

As a main grassland of China and part of the Eurasian Steppe, the Inner Mongolia Steppe has becoming seriously fragmented and degraded because of climate changes and human activities (Jiang et al., 2006). Despite the large environmental variations, *Stipa krylovii* is indigenous to various types of terrains, including meadow steppe, typical steppe and desert steppe from east to west in the Inner Mongolia Steppe. Recent studies have demonstrated that the distribution of *S. krylovii* has rapidly increased, showing a pattern of eastward migration in the past 60 years (Liu, 2003). The dominance and widespread distribution of *S. krylovii*, coupled with a significant level of habitat heterogeneity, suggest substantial inter-population variation of this grass in the Inner Mongolia Steppe. The nature of the factors responsible for such variation can be inferred from the spatial variation patterns observed in morphological, structural, physiological, genetic, and environmental traits, as well as potential association between quantitative and environmental difference in their natural conditions (Jia et al., 2008; Wang et al., 2006; Zhao et al., 2003). Previous data also showed that traits related to growth (e.g. height of vegetative shoots, height of reproductive shoots, leaf length) decreased significantly across drought gradients, and that traits related to reproductive (e.g., length of calluses, length of seeds, length of awns, length of glumes) had no significant correlations with climatic factors (Jia et al., 2008; Wang et al., 2006; Zhao et al., 2003). A basic question regarding the large variations exhibited by *S. krylovii* between populations is to what extent these characteristics are determined by genetic differences or by phenotypic plasticity. Extensive studies showed inconsistent divergences between phenotypic traits and molecular markers of some species (Ballentine and Greenberg, 2010; Kurt et al., 2012; Latta and McKay, 2002a,b; Merilä and Crnokrak, 2001; Taylor et al., 2005). Molecular markers are often neutral, e.g. not responsive to natural selection, while quantitative traits do usually respond to selection, indicating that gene markers would be rarely good indicators of adaptive responses (Hall et al., 2007; Knapp and Rice, 1998). Therefore, in order to understand the mechanisms that some species use to adapt to diverse environmental conditions, common garden experiments for different populations should be performed. Moreover, as a native species to the Inner Mongolia Steppe, *S. krylovii* is considered a crucial species for ecological restoration. Therefore, it is urgent to understand how *S. krylovii* populations adapt to different conditions. Accordingly, by obtaining data from a common garden and natural habitats, we might reasonably speculate the adaptive mechanism(s) used by *S. krylovii* to thrive in variously habitats (Bresson et al., 2011). Such experiments might shed insight into the extent and nature of adaptive variation used by *S. krylovii*.

In this study, natural *S. krylovii* populations across aridity gradients were investigated, and 7 corresponding *S. krylovii* populations were grown in a common garden. Fifteen quantitative traits (including growth, seed related, and leaf function traits) from both sets of populations were measured in this study to analyze whether the quantitative trait differentiation of *S. krylovii* might be due to local adaptation to environmental conditions or not. In particular, we asked the following questions: Is there evidence of local adaptation to environmental variations among *S. krylovii* populations in the quantitative traits we observed, and are these traits associated with the environmental conditions of the population's 'home' site? To what extent is phenotypic variation of these traits driven by genetic differentiation based on measurements taken in the common garden and in the field?

2. Materials and methods

2.1. Species and population sampling

Stipa krylovii Roshev. is a perennial tussock-forming C_3 grass that grows 30–80 cm in height. It is wind-pollinated, flowers in mid to late July, and ripens in late August or early September.

Seven representative populations of *S. krylovii* were selected, which cover the main distribution area (113.82°–118.37E, 43.84°–49.51N) of *S. krylovii* in the Inner Mongolia Steppe (Table 1, Fig. 1). In this region, populations of *S. krylovii* are distributed in meadow steppe, typical steppe and desert steppe that are characterized by climatic variations, which provides an ideal system for studying genetic differentiation resulting from climatic 'local adaptation scenario' (Wang et al., 2006). Historical climatic records were used to evaluate the relationships between climatic and quantitative phenotypic differences. The climatic conditions at each site were characterized using digital maps and data generated from the WorldClim database (Hijmans et al., 2005), providing gridded estimates (with 1 km resolution) of precipitation and temperature during the period of 1950–2000. Variables that were highly correlated were excluded from our selection, and 8 bioclimatic variables (annual mean temperature, mean diurnal range (mean monthly maximum – minimum temperature), mean temperatures of the wettest and the driest quarters, annual precipitation, precipitation seasonality [coefficient of variation], and precipitation of the wettest and the driest quarters) (Table 1) were used to summarize the aspects of temperature and precipitation that were derived from the WorldClim database (Hijmans et al., 2005). Details for further description of these bioclimatic variables and how they are calculated could be found from the website (http://www.rforge.net/doc/packages/climates/html/bioclim.html).

In August of 2007, in each of the 7 sites, 50 individual plants were randomly collected at intervals of at least 10 m in order to avoid collecting ramets from the same genet. Quantitative traits for these individual plants were measured, and spikes were harvested, sorted by maternal plants and stored in −4 °C condition. At each site, soil samples were collected in triplicate at a depth of 10–15 cm in the rooting zone. The total soil C, N and P contents of the rooting zone were determined, and C:N and N:P ratios were calculated. The soil total C and N were measured using Elemetar analyzer (Vario EL III, Germany), and total P was extracted using $HClO_4$–H_2SO_4 and determined by colorimetric method.

2.2. Common garden experiment

Seeds (actually caryopses) collected in situ were sown in plastic pots (20 cm × 21 cm) filled with vermiculite in December 4, 2008,