

Geographic variation and local adaptation in *Oryza rufipogon* across its climatic range in China

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Summary

1. Variation in plant functional traits and demographic behaviour in populations of widely distributed species on geographic gradients may be key to understanding their geographic range limits. *Oryza rufipogon*, a wild progenitor of rice, occurs in discrete wetland populations over a wide latitudinal range in China.

2. We examined correlations between plant functional traits, latitude, longitude and climatic variables in 34 populations of *O. rufipogon* across its northern range limit. We also investigated the responses of individuals from all populations after transplantation to two experimental gardens: one north of its range and the other in the extreme south.

3. Seed mass in the field was positively correlated with latitude and longitude, whereas flag-leaf area was negatively correlated with latitude; both effects could be explained largely as a response to temperature. Transplantation revealed that both plasticity and population differentiation contributed to geographic variation. All populations reproduced and overwintered in the southern garden, but in the northern garden, only populations from the northern range were able to reproduce (65%) and survive the winter (58%).

4. Synthesis: Environmentally determined variation in plant functional traits is complex, with both plastic trade-offs between traits and genetic differentiation between populations contributing to the location of the northern limit of *Oryza rufipogon*.

Key-words: common-garden transplant, functional traits, geographic range, latitudinal limit, phenotypic plasticity, plant–climate interactions, population differentiation, temperature limitation

Introduction

Elucidating the mechanisms that shape demographic variation and local adaptation of species across geographic ranges is key to understanding what creates geographic range limits. The ability of plant species to adapt to local environmental conditions is an important determinant of their geographic range (Vergeer & Kunin 2013). Many studies have investigated how population dynamics, occupancy or abundance differ across the geographic ranges (Samis & Eckert 2007; Gerst, Angert & Venable 2011; Wagner *et al.* 2011), or between a few pairs of central and peripheral populations (Samis & Eckert 2009; Wagner *et al.* 2011). However, variation in the fitness expressed as local demographic behaviour must be underpinned by variation in morphologic and physiologic traits. This phenotypic variation across a geographic range may arise in fundamentally different ways: through phenotypic plasticity or local genetic differentiation.

Phenotypic plasticity can be sufficient to compensate functionally for the effects of resource limitation to maintain population growth rates of peripheral populations (Eckhart, Geber & McGuire 2004; Milla *et al.* 2009). For example, Doak & Morris (2010) reported that southernmost populations of two tundra species showed lower survival and recruitment, but the population growth ratio was compensated by greater growth of individual plants. Thus, the variation in local population abundance may be disconnected from variation in specific plant performance. On the other hand, phenotypic variation associated with genetic differentiation may enhance fitness across the range (Eckhart, Geber & McGuire 2004). A challenge for future work is to disentangle the roles of genetic differentiation vs. plasticity in producing phenotypic variation across the geographic range.

Identifying traits and characterizing their variation across geographic or environmental gradients is important in assessing how constraints on their evolution may ultimately determine geographic limits (Eckhart, Geber & McGuire 2004; Samis & Eckert 2007; Sexton *et al.* 2009). Transplant

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experiments are a powerful tool to discriminate between the contributions of genetic differentiation and phenotypic plasticity (Bennington & McGraw 1995; Donohue *et al.* 2001; Eckhart, Geber & McGuire 2004) and to evaluate whether traits can potentially adapt beyond current range boundaries (Samis & Eckert 2009; Doak & Morris 2010).

This study uses both approaches to investigate the nature of geographic variation in individual performance and climatic limitation in common wild rice, *Oryza rufipogon*, throughout its northern range in China. Studies of populations in situ were combined with transplant experiments to two common gardens: one beyond its northern range limit and the other in the southernmost part of China. We chose readily quantifiable functional traits, including seed mass and morphologic characteristics of the flag leaf, as the main response variables (Nicotra *et al.* 2010). Seed size variation within species can be adaptive in heterogeneous landscapes and has been found to be associated with latitude (Winn & Gross 1993; Koenig *et al.* 2009) and elevation (Baker 1972; Holm 1994; Guo, Mazer & Du 2010). The flag leaf of rice contributes most to grain yield and is one of the most important targets for crop breeders (Misra 1986).

At the scale of this study, climatic factors, including temperature, solar radiation and rainfall, will probably be the main constraints on the performance and distribution of *O. rufipogon*. We address the following primary questions: (i) Is there an ecologic gradient in seed mass and other functional traits across the northern geographic range? (ii) What are the relative contributions of plasticity and genetic differentiation to observed adaptive patterns of response? (iii) How do such patterns of variation contribute to determining the northern range limit?

Materials and methods

STUDY SPECIES

Oryza rufipogon is a spreading, perennial grass with clonal growth. Most populations are discrete and grow in marshes, open ditches, swampy grassland, ponds, river banks and margins of rice fields. *Oryza rufipogon* is considered to be the ancestor of Asian cultivated rice (*Oryza sativa*), and it is the most important germplasm for rice improvement (Oka 1988). Flowering is in response to shortening of days. The initial tasselling stages are seen from late August (northern populations) to early October (southern populations), changing with day length (Gao, Ge & Hong 2000). It has open panicles with slender spikelets and produces dark-purple caryopses, with long awns that dehisce readily. *Oryza rufipogon* is widely distributed in the tropics and subtropics of Asia (Vaughan 1994) from southern China (28 °N) to northern Australia (22 °S). It is reported to occur in eight provinces in south China, including Guangdong, Guangxi, Hainan, Yunnan, Hunan, Jiangxi, Fujian and Taiwan (populations in Fujian and Taiwan disappeared in 1978 and 2000, respectively). The range of *O. rufipogon* in China stretches from 18°09' N to 28°14' N and from 100°40' E to 121°15' E. The Dongxiang populations (Jiangxi Province) are the most northerly populations in its entire range. Because of habitat deterioration and fragmentation in recent decades, many populations are now small, isolated and in danger of extinction (Gao 2004).

FIELD SURVEY AND SAMPLING

Nearly all known locations for *O. rufipogon* in China were explored between September and November 2010. Seed and rhizome samples were obtained from 34 natural populations, including all surviving populations from its peripheral distribution (Yunnan, Hunan and Jiangxi provinces) and representing the geographic distribution of *O. rufipogon* in China. The altitudes of collection sites range from 3 to 165 m above sea level, with the exception of a population in Yunnan Province (787 m). Clonal fragments, each consisting of a tiller with stem base with attached roots, were taken from five randomly selected clonal individuals in each population. Seed samples were obtained from 21 of the populations, as seeds of the other 13 populations had already been shed.

We collected further seed samples from each population in August to October 2011, when seeds were mature and vegetative extension had finished. A mixed sample of seeds was collected from all available individuals (a minimum of 20) in each population. Seed dry mass was determined using eight replicates of 100 caryopses, after drying for a minimum of 48 h at 60 °C. We made the following measurements on a single tasselling tiller from each of 5 to 15 randomly selected average-size individuals per population: length and width of flag leaf, panicle length, and number of fertile and infertile spikelets per panicle. Flag-leaf area was determined using an Li-3000 Portable Area Meter (Li-3000; Li-Cor, Inc., Lincoln, NE, USA). Then, each flag leaf was oven-dried for a minimum of 48 h at 60 °C and weighed.

TRANSPLANT EXPERIMENT

We carried out transplant experiments at two geographically extreme common gardens (rice paddies): the northern transplant site, situated at the experimental farm of Huazhong Agricultural University, Wuhan, China (30 °29' N, 114 °19' W), was north of the current range limit of *Oryza rufipogon*; the southern transplant site, located in an abandoned rice field (Lingshui County, Hainan Province, Hainan Island; 18 °34' N, 110 °02' W) was at the extreme south of its Chinese distribution (Fig. 1a).

The rhizome fragments collected from the field were planted individually in pots in September–November 2010 and grown in the glasshouse at Wuhan Botanical Garden (CAS). In early May 2011, the two strongest tillers were taken from each of the resulting clonal individuals to give two genetically matched batches of ramets, each batch comprising 170 individuals (34 populations × 5 replicate clones).

One batch was transplanted to the northern experimental garden and the other to the southern one, in a completely randomized block design with five blocks (for replicate clones) per garden. Each block was subdivided into 34 2 × 2 m cells (for populations), and a single ramet was planted randomly into each. The gardens were 1000 m² in area and surrounded with barbed-wire fences to exclude grazing cattle. They were watered regularly to maintain a water level of c. 20 cm above the soil surface. The survival and tasselling of each individual were recorded monthly from June to November. Seeds and flag leaves were collected at maturity and measured by the same methods as in the natural population survey. The overwinter survival was assessed by the presence of one or more green leaves after the beginning of growth during the spring of the second season in 2012.

CLIMATIC DATA

Monthly temperature and rainfall data were obtained from the WORLDCLIM version 1.4 data set, which is based on weather conditions

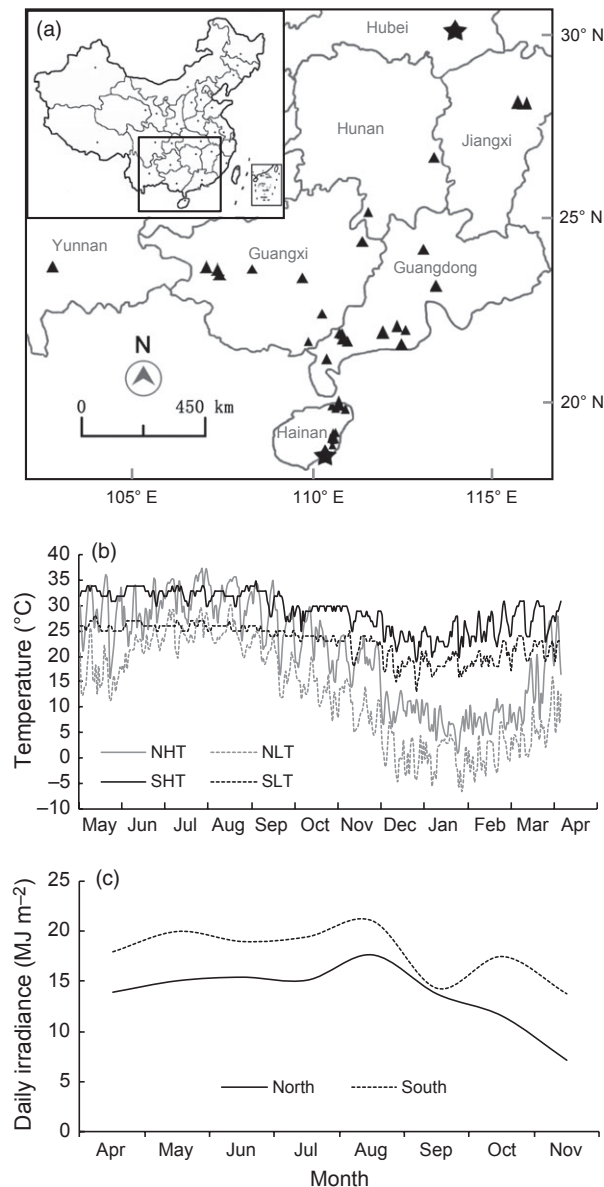


Fig. 1. (a) Location of the natural populations of *Oryza rufipogon* (triangles) and the two experimental gardens (pentangles) in China. (b) Daily averages of the maximum air temperature (unbroken line) and the minimum air temperature (broken line) at northern (NHT, NLT) and southern (SHT, SLT) experimental gardens for the period between 1 May 2011 and 4 April 2012. (c) Daily irradiance at the two experimental gardens from April to November 2011 (northern garden, unbroken line; southern garden, broken line).

recorded 1950–2000 and subsequently interpolated to a grid-cell resolution of 30 arc seconds (Hijmans *et al.* 2005; <http://www.worldclim.org>). The monthly solar radiation data for the period 1971–2000 were compiled from Chinese Ecosystem Research Network data set (<http://www.cern.ac.cn>) at 1×1 km resolution in ArcGIS 9.1. A total of 18 variables were obtained for six features of temperature ($^{\circ}\text{C}$), radiation ($\text{MJ m}^{-2} \text{ day}^{-1}$) and rainfall (mm). For all three climatic factors, these comprised the annual mean (mean of all monthly mean values), the annual seasonality (coefficient of variation in all monthly mean values) and the mean values for the wettest, driest, warmest and coldest quarters. The latter include extremes representing seasons favourable or stressful for plant growth (Murray *et al.* 2004).

STATISTICAL ANALYSES

Given the high degree of cross-correlation between climatic variables (Murray *et al.* 2004), principal components analysis (PCA) was used to reduce the dimensionality of the climatic data set. We used Pearson correlations to test the associations between the seed mass and the geographic and climatic variables. Where seed mass was significantly correlated with a geographic variable, a partial correlation analysis was carried out to clarify the relationship between response variable and geographic variable by controlling for the three climatic principal components (PC1 to PC3). This analysis allowed us to determine whether the response variable–geographic variable relationship could be construed as direct or indirect (i.e. mediated by climatic variables). Because of the collinearity of climatic variables, we also directly estimated their independent contributions of the most significant climatic variables to variation in the plant traits using hierarchical partitioning analysis (R 2.11.1, the R Foundation for Statistical Computing) with a general linear model and bootstrapping (Chevan & Sutherland 1991; MacNally 2002; MacNally & Walsh 2004).

We used linear regression to analyse the relationships between plant traits (seed mass, flag-leaf area, flag-leaf length, flag-leaf width, flag-leaf shape and spikelet number of per panicle) and latitude and longitude of origin in all natural and transplanted populations. In addition, we tested the variation in these plant traits among the natural and transplanted populations using one-way ANOVAs with Tukey–Kramer HSD contrasts. Population \times Environment interactions were tested by two-way ANOVA (linear model). The effects of latitude of origin on reproductive success and overwinter survival at the northern transplant garden were examined using logistic regression. Except where otherwise stated, all analyses were performed using SPSS version 16.0.

Results

CLIMATIC VARIATION

All 18 climatic variables were significantly related to either or both of latitude and longitude (Table 1). From the PCA analysis with varimax rotation of the intercorrelated traits, three principal components, which accounted for 82% of the total variance were extracted (Table 1). The first principal component, PC1, had its loadings for temperature (annual mean, seasonality, and mean of driest and coldest quarters) and radiation (annual mean) and explained 52% of the variance. Mean temperature of wettest quarter primarily determined PC2, which contributed to 18% of the variance. PC3 had high loading on precipitation (annual mean and mean of wettest quarters). All three principal components were significantly correlated with longitude (PC1: $r = 0.49$, $P < 0.01$; PC2: $r = -0.39$, $P < 0.05$; PC3: $r = -0.46$, $P < 0.01$), but only PC1 and PC3 were significantly correlated with latitude (PC1: $r = 0.93$, $P < 0.001$; PC3: $r = 0.36$, $P < 0.05$).

GEOGRAPHIC AND CLIMATIC VARIATION IN PLANT PERFORMANCE

Many of the plant traits measured on natural populations in 2011 were significantly correlated with their latitude of origin (Table 2): seed mass, and flag-leaf width, area and shape (length/width ratio). In addition, seed mass and leaf shape

Table 1. Principal components analysis (PC1, PC2 and PC3) for 18 climatic variables estimated for collection provenances ($n = 34$ sites) of *Oryza rufipogon*. Correlation coefficients (r) are presented for relationships between bioclimatic variables and latitude and longitude

	PC1	PC2	PC3	Latitude	Longitude
Eigenvalue	9.4	3.3	2.1		
Percentage variation explained	52.4	18.1	11.6		
Eigenvectors					
Annual mean temperature	0.90	0.41	0.10	-0.96***	-0.55***
Temperature seasonality	-0.91	-0.34	-0.10	0.97***	0.53***
Mean temperature of wettest quarter	0.39	0.90	0.05	-0.63***	-0.64***
Mean temperature of driest quarter	0.93	0.13	0.07	-0.85***	-0.37*
Mean temperature of warmest quarter	0.15	0.19	0.06	-0.22	-0.10
Mean temperature of coldest quarter	0.90	0.36	0.11	-0.97***	-0.53***
Annual mean radiation	0.92	-0.03	0.25	-0.86***	-0.11
Radiation seasonality	-0.87	-0.26	0.05	0.77***	0.42**
Radiation of wettest quarter	0.35	-0.02	0.48	-0.41*	-0.34*
Radiation of the driest quarter	0.81	0.43	-0.03	-0.80***	-0.46**
Radiation of warmest quarter	0.82	0.23	0.30	-0.88***	0.15
Radiation of coldest quarter	0.67	-0.20	-0.13	-0.42*	-0.12
Annual mean precipitation	0.18	-0.12	0.95	-0.34*	0.35*
Precipitation seasonality	0.19	0.81	0.33	-0.40*	-0.47**
Precipitation of wettest quarter	0.10	0.10	0.97	-0.31	0.18
Precipitation of the driest quarter	-0.54	-0.77	0.30	0.59***	0.68***
Precipitation of warmest quarter	-0.20	0.57	0.72	-0.12	-0.01
Precipitation of coldest quarter	-0.63	-0.70	0.18	0.71***	0.70***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

were positively correlated with longitude of origin. Significant regressions on latitude and longitude accounted for 13–69% of phenotypic variation, depending on the trait (Fig. 2).

However, all of these variables and the number of spikelets per panicle were also highly correlated with the first principal component of climatic variation (PC1), but not with PC2 or PC3 (Table 2). The geographically significant correlations largely disappeared (except for opposing trends in flag-leaf width and shape), however, when the effects of PC1 were partialled out, but they generally persisted when the effects of PC2 and PC3 were controlled for (Table 2). Thus, the climatic variables represented by PC1 (mainly temperature and radiation variables, see Table 1) were more influential on the latitudinal and longitudinal gradients in plant traits than those represented by PC2 (mainly mean temperature of wettest quarter) or PC3 (mainly rainfall variables).

Hierarchical partitioning of the effects of the nine variables most highly correlated with plant traits when considered individually (Table 3) revealed that mean temperature of the warmest quarter had the only significant effect on flag-leaf area and length, and number of spikelets per panicle. It also provided the strongest independent effect on seed mass and almost the strongest effect on flag-leaf width. Flag-leaf width and shape appeared to be influenced independently by several temperature variables. Seed mass was the only trait to be influenced significantly by precipitation independent of temperature variables (Table 3).

PLANT RESPONSES TO TRANSPLANTATION

Unlike natural populations, those transplanted to the southern experimental garden showed no significant correlation of seed mass with latitude or longitude of origin (Fig. 2a). Those transplanted to the northern experimental garden produced insufficient seed for this analysis. Similarly, neither were there any significant relationships between flag-leaf area (Fig. 2b) or length (Fig. 2c) and latitude or longitude in either of the two transplant treatments. However, flag-leaf width retained a negative relationship (Fig. 2d) and flag-leaf shape (ratio of length to width) retained a positive relationship (Fig. 2e) with latitude of origin in the southern garden, but not in the northern one. Transplantation to both southern and northern gardens revealed negative relationships between the number of spikelets per panicle that had not been apparent in the in situ populations. No relationships with longitude of origin were apparent in either transplant garden.

Overall, across populations, there were substantial phenotypic variations between plants grown in their natural habitat and those in the northern and southern transplant gardens. Flag-leaf area, flag-leaf length, flag-leaf width and spikelet number per panicle were all significantly larger when transplanted northwards and smaller when transplanted southwards; mean seed mass was greater when populations were transplanted northward, in the populations that could produce seed in the northern garden, but was not affected by transplantation southward (Fig. 3). No significant variations were detected in mean ratio of flag-leaf length to width between the three types of environment. Examination of norms of reaction for these characteristics in response to transplantation (Fig. 3) revealed more complex relationships, suggesting

Table 2. Correlation coefficients (r) between seed mass and flag-leaf mass of natural populations and latitude, longitude and bioclimatic principal components (PC1, PC2 and PC3). Partial correlation coefficients represent the contributions of latitude and longitude to seed mass variation after controlling for the effects of PC1, PC2 and PC3

	Seed mass	Flag-leaf area	Flag-leaf length	Flag-leaf width	Flag-leaf length/width	Number of spikelets
Latitude	0.54**	-0.38*	0.11	-0.65***	0.82***	-0.26
Latitude (control PC1)	-0.13	0.23	0.43*	0.01	0.59***	0.32
Latitude (control PC2)	0.60***	-0.34	0.18	-0.67***	0.84***	-0.22
Latitude (control PC3)	0.64***	-0.30	0.20	-0.62***	0.85***	-0.21
Longitude	0.49**	-0.10	0.14	-0.22	0.43*	-0.19
Longitude (control PC1)	0.13	0.01	0.23	-0.11	0.57**	-0.09
Longitude (control PC2)	0.44*	-0.20	0.24	-0.44*	0.72***	-0.24
Longitude (control PC3)	0.42*	-0.27	0.22	-0.54**	0.80***	-0.25
PC1	0.66***	-0.43*	-0.03	-0.65***	0.70***	-0.35*
PC2	-0.18	0.22	0.20	0.17	0.03	0.24
PC3	-0.18	-0.16	-0.09	-0.20	0.04	0.01

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

considerable genotypic differences between populations for all of the traits, in concert with environmental effects; two-way ANOVA models revealed highly significant Population \times Environment interactions for all six traits (Table 4), providing strong evidence for local genetic adaptation.

REPRODUCTIVE SUCCESS AND OVERWINTER SURVIVAL IN THE NORTHERN TRANSPLANT GARDEN

There were striking differences between populations in their reproductive success and overwinter survival when transplanted to the northern common garden (Fig. 4). No population originating from south of 21 °N successfully reproduced or survived the subsequent winter, whereas all populations originating north of 24 °N reproduced and survived. One population (from 21.33 °N) failed to reproduce but survived the winter, and three populations (21.80, 21.95 and 23.67 °N) successfully seeded but did not survive the winter.

The absolute minimum temperature recorded historically at the sites of origin declined from 5.1 °C in the south to -12.4 °C in the north (Fig. 4). Logistic regression indicated that a 50% probability of both reproductive success and survival corresponded with a latitude of origin where the absolute minimum recorded temperature was *c.* 0 °C. During the experiment, the daily minimum air temperature fell below zero for a total of 43 of days at the northern transplant garden, whereas at the southern transplant garden it remained above 16 °C for the entire experiment period, and all transplanted populations successfully reproduced and overwintered there.

Discussion

Species with wide geographic distributions and historically stable range limits, such as *Oryza rufipogon*, can provide

valuable insights into the role of climate in determining distribution. This involves understanding how traits related to fitness vary along climatic gradients, both within the range and beyond its limit (Sexton *et al.* 2009). A main focus of interest with *O. rufipogon* is its northern limit, as its distribution is bounded in the south by the South China Sea rather than climate. Although as a wetland plant, it occurs as discrete populations, its variation across a latitudinal range of 1250 km and a longitudinal range of 1400 km was essentially clinal, as might be expected for responses to climate.

The trend to increasing individual seed mass at higher latitude is not consistent with a previously reported pattern of heavier seeds at low latitudes, at both community (Lord *et al.* 1997; Murray *et al.* 2004) and global scales (Moles & Westoby 2003; Moles *et al.* 2005, 2007). In multispecies comparisons, many factors can affect seed mass, not least phylogenetic constraints and the evolution of dispersal mechanisms, but even within *O. rufipogon*, the regressions on latitude and longitude only accounted for *c.* 25% of its variation. Latitudinal and longitudinal gradients reflect complex and collaterally varying combinations of climatic variables, as was demonstrated by the PCA. Smaller seeds at higher latitudes might have been expected as a response to limitation in resource availability: lower solar radiation and temperature at higher latitudes would decrease photosynthetic rates and shorten the growing season, thus reducing both seed provisioning and development time (Baker 1972; Moles & Westoby 2003). However, the significant decline in the number of spikelets per panicle with latitude of origin in both transplant gardens might signal the well-known trade-off between seed size and seed number (Shipley & Dion 1992; Moles *et al.* 2004). The identification, by hierarchical partitioning, of temperature in the warmest quarter and precipitation in the driest and coldest quarters as key independent effects on seed mass

Fig. 2. Relationships between plant traits and latitude (left) and longitude (right) of origin, in natural and transplanted populations of *Oryza rufipogon* (a) individual seed mass, (b) flag-leaf area, (c) flag-leaf length, (d) flag-leaf width, (e) ratio of flag-leaf length/width and (f) number of spikelets per panicle. Natural populations (NP, closed circles, dark continuous line); southern-transplanted (SEP, open diamonds, dark broken line); and northern-transplanted (NEP, open triangles, grey continuous line) populations. Regression lines and coefficients of determination are given for significant relationships.

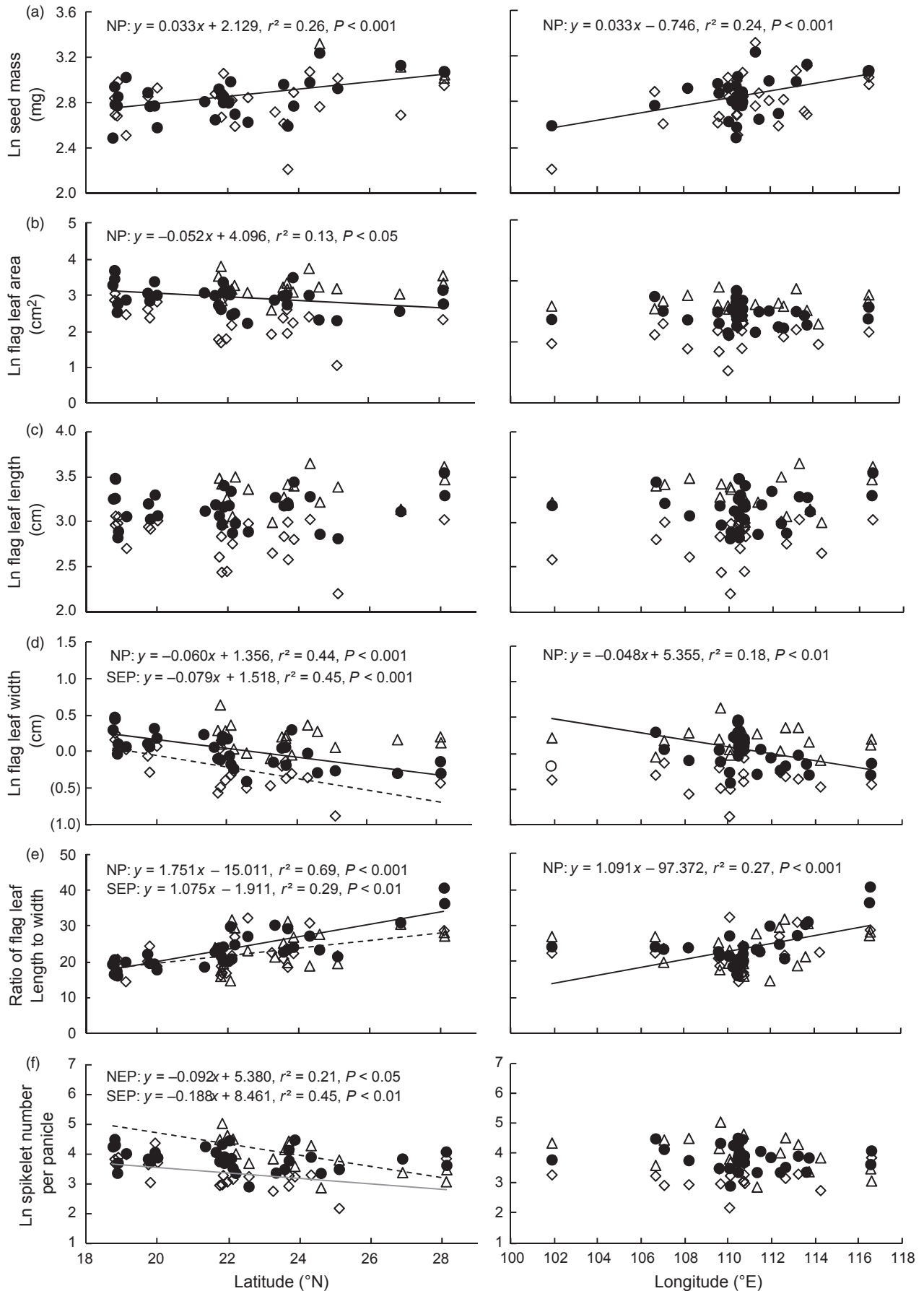


Table 3. Hierarchical partitioning of the independent effects of nine climatic variables on six plant traits in 34 natural populations of *Oryza rufipogon*

	Fraction of regression relationship (%) attributable independently to environmental variables for each trait					
	Seed mass	Flag-leaf area	Flag-leaf length	Flag-leaf width	Flag-leaf length/width	Number of spikelets
Annual mean temperature				20.9	–23.4	
Mean temperature of wettest quarter					–13.8	
Mean temperature of driest quarter				16.7	–14.8	
Mean temperature of warmest quarter	21.2	32.0	30.4	20.5		44.1
Mean temperature of coldest quarter				19.1	–23.0	
Precipitation in wettest quarter						
Precipitation in driest quarter	18.3					
Precipitation in warmest quarter						
Precipitation in coldest quarter	18.4					

Nonsignificant contributions ($P > 0.05$) are omitted. Probabilities are based on bootstrapping with 1000 repetitions. Negative relationships are indicated with a minus sign.

does imply resource limitation. Thus, the results may support the hypothesis that natural selection favours larger seeds towards the geographic limit, because better-provisioned seeds can increase the probability of establishment and confer greater resistance to hazards on seedlings (Pluess, Schutz & Stöcklin 2005; Metz *et al.* 2010). Seed mass has previously been found to increase along altitudinal gradients (Holm 1994; Pluess, Schutz & Stöcklin 2005; Alexander *et al.* 2009). Nevertheless, a population of *O. rufipogon* from the exceptional altitude of 800 m in Yunnan Province (23.68 °N) had the smallest seed mass and was clearly an outlier in the regressions on latitude and longitude (Fig. 2a,b).

An overall decline in resources for seed production at higher latitude also seems to be indicated by the reduced flag-leaf area. It is well-established that the flag leaf of rice provides the primary source of photosynthate for seed development, because of its proximity to the panicle and persistent photosynthetic activity during grain filling (Misra 1986). Mean temperature of the warmest quarter was identified as the only significant independent influence on flag-leaf area. Jordan & Hill (1994) also found that mean temperature of the warmest quarter was a major determinant of leaf length in *Nothofagus cunninghamii*. Reduction in leaf area in *O. rufipogon* was in fact largely the result of the leaves becoming narrower. The narrower leaves may be less susceptible to damage by low temperatures, as leaves became narrower with increasing altitude in *N. cunninghamii* (Hovenden & Vander Schoor 2003).

Species with wide geographic distributions typically display considerable phenotypic variation, some of which may be genetically based (e.g. Banta *et al.* 2012). Trait variation across the distribution of *O. rufipogon* can be attributed in

part to phenotypic plasticity in response to the climatic gradients. The fact that none of the significant associations with longitude of origin and only some associations with latitude of origin were sustained when plants were transferred to either experimental garden attests to the direct influence of environment on morphology. Also, there were consistent phenotypic effects of transplantation, averaged over all populations. Another wetland plant, *Potamogeton pectinatus*, similarly, showed great flexibility of traits when transplanted across the whole latitudinal range of Europe (Santamaria *et al.* 2003). Nevertheless, relationships with other traits in *O. rufipogon* were sustained in the southern experimental garden but not in the northern one, suggesting the existence genetic differentiation. Furthermore, an association between numbers of spikelet per panicle and latitude was uncovered by transplantation to both common gardens, even though it was not evident in field populations; this could be interpreted as convergent plastic responses in the field masking underlying genotypic differences. The clearest evidence for locally adapted genotypic diversity in *O. rufipogon*, however, comes from the reaction norms of various traits in the transplantation experiment, which yielded significant Genotype \times Environment interactions. Local genetic differentiation of traits has been reported in various wide-ranging species, particularly where there is limited gene flow between populations. In *Carlina vulgaris*, the decline in fitness was linearly related to distance of transplantation across Europe (Becker *et al.* 2006). Early- and late-flowering genotypes of *Arabidopsis thaliana* had different modelled climatic envelopes that resulted in significantly different potential ranges size and niche breadth (Banta *et al.* 2012). Examination of the perennial *Arabidopsis lyrata* across its north-western European range has also found

Fig. 3. Comparison of mean plant traits (left-hand panels) and their reaction norms (right-hand panels), for 34 populations of *Oryza rufipogon* in their natural habitats (NP) and transplanted to northern (NEP) and southern gardens (SEP): (a) seed mass, (b) flag-leaf area, (c) flag-leaf length, (d) flag-leaf width, (e) ratio of flag-leaf length/width and (f) number of spikelets per panicle. Box plots show median values, 25th and 75th percentiles, and 5th and 95th percentiles. Results from one-way ANOVA with Tukey–Kramer multiple-contrast tests are shown. Treatments indicated by different letters are significantly different ($P < 0.05$).

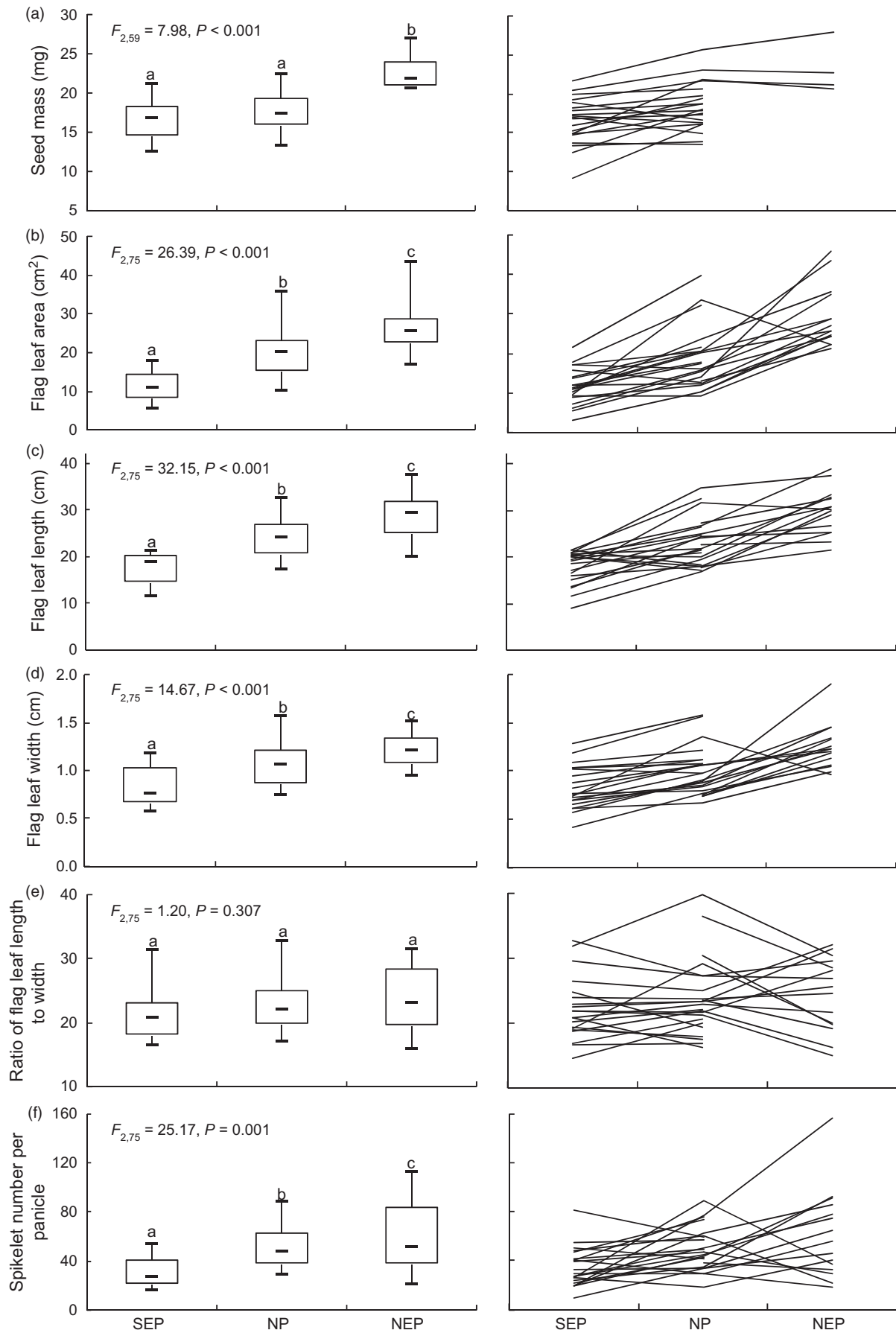


Table 4. Summaries of two-way anovas of the effects of environment and population of origin on plant traits in 34 populations of *Oryza rufipogon* grown in three environments (their natural environments, and northern and southern transplant gardens)

	Environment (E)			Population (P)			E × P		
	d.f.	F	P	d.f.	F	P	d.f.	F	P
Seed mass	2	46.14	<0.001	36	27.98	<0.001	24	8.31	<0.001
Flag-leaf area	2	144.95	<0.001	36	10.05	<0.001	33	4.67	<0.001
Flag-leaf length	2	81.95	<0.001	36	6.02	<0.001	33	3.84	<0.001
Flag-leaf width	2	169.93	<0.001	36	19.78	<0.001	33	4.90	<0.001
Ratio of flag-leaf length/width	2	4.53	0.011	36	12.67	<0.001	33	2.68	<0.001
Number of spikelets per panicle	2	130.32	<0.001	36	7.93	<0.001	33	4.02	<0.001

All linear models and their intercepts were significant ($P < 0.001$). Replication (clones per treatment): Transplant gardens, $n = 5$; natural sites, $n = 5$ –15. Only 20 populations tasselled and therefore produced flag leaves and seeds at the northern garden.

considerable local adaptation of populations, as well as declining performance towards both the northern and southern limits (Vergeer & Kunin 2013).

Rigorous evidence for the physiologic and demographic mechanisms controlling range limits can be provided by experimental transplantation to or beyond the current range limits (e.g. Prince & Carter 1985; Griffith & Watson 2005; Stanton-Geddes, Tiffin & Shaw, 2012). Although the northern transplant site for *O. rufipogon* was *c.* 2 °N of the northernmost natural population, only populations of southern provenance were unable either to reproduce or to survive the winter, whereas the populations of more northern provenance could generally do both. This difference in fitness is compelling further evidence for local genetic adaptation in *O. rufipogon*. As a 50% probability of flowering or survival corresponded with a low temperature extreme of about −4 °C at the latitude of origin, frost tolerance appears to be a significant factor and at least two distinct genotypes for this exist in Chinese populations. The lethal effects of low temperature are only one possible mechanism for limitation, as northern range limits have also been associated with reduced seed production or reproductive failure arising from low-temperature limitation of pollen-tube growth (Woodward 1990).

Such variations in fitness do not in themselves, however, explain the northern distribution limit in *O. rufipogon*, because 20 of the populations could reproduce beyond its current range and 18 of them were able to survive the winter there. Several mechanisms may contribute to limit range expansion, including genetic constraints, demographic instability and historic constraints. Range-edge populations may fail to adapt to adverse environmental conditions because of reduced genetic diversity and/or swamping by immigration of less well-adapted genotypes (Gaston 2009). A previous study of 47 populations of Chinese *O. rufipogon* with similar geographic origins to our material revealed that peripheral populations have similar genetic diversity to core populations (Gao 2004), providing little support for this mechanism. Demographic instability might include increased mortality, reduced reproduction or inadequate immigration to sink populations (Gaston 2009). As *O. rufipogon* exists as metapopulations in more or less discrete wetlands within the landscape, dispersal

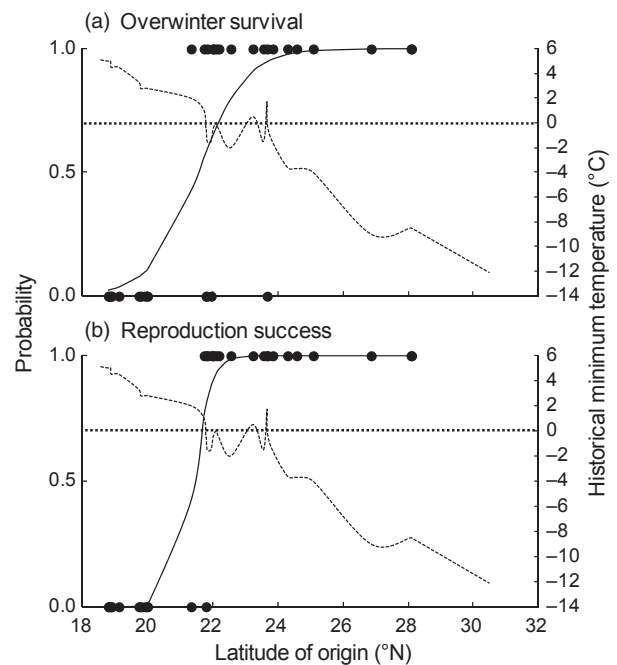


Fig. 4. Probability of (a) overwinter survival and (b) reproductive success of 34 populations of *Oryza rufipogon* transplanted to the northern experimental garden, in relation to their latitude of origin. The historical minimum temperature (broken line) at the sites of origin is also shown. Overwinter survival was assessed by the presence of one or more green leaves for each individual at the beginning of second growth season. Reproductive success was assessed by the presence of one or more tassel for each population.

may play a role in maintaining viable populations. Longer-term transplantation experiments beyond distribution limits have also shown that the performance of the species was similar to that in its natural populations, and suggest that abiotic conditions are generally suitable for the species beyond the periphery (G. Liu, W. Zhou & W. Liu, unpubl. data). A range limit due to historical factors, reflecting that a species has not yet spread beyond its present boundary (Gaston 2009), could also be considered. The detailed history of *O. rufipogon* in southern China is not known, but archaeological evidence sug-

gests that its domesticated derivative (*O. sativa*) has occurred in this area for approximately 10 000 years (Jiang & Liu 2006). Thus, temporal constraints are unlikely to be the cause for the northern range limits.

Given the dominating influence of temperature variables on the trait variation observed across the geographic range of *O. rufipogon*, factors affecting the length and conduciveness to productivity of the growing season are likely to affect both reproductive success and winter survival, at least in the exceptionally adverse years that could limit its spread. Latitudinal variations in other plants have been associated with decreases in the length and average temperature of the growing season (e.g. Qian *et al.* 2003; Root *et al.* 2003). Latitudinally opposing variation and trade-offs between different functional traits may contribute to northern limits. As a photoperiod-sensitive species, *O. rufipogon* flowers in response to day lengths of about 15 h or less. Thus, its initial tasselling stage occurs in late August in northern-edge populations, but in early October in southern populations (Gao, Ge & Hong 2000), which allows less time for preceding vegetative growth in the north. The relatively harsh conditions at high latitudes appear to favour larger seeds and hence a greater requirement for photosynthate for grain filling and sufficient time for seed development. On the other hand, they also favour smaller flag-leaf area, restricting the source of photosynthate over the shorter growing season. Such trade-offs between adaptive plasticity (providing a fitness benefit) and nonadaptive plasticity (responses to physical or resource limitations) may play a role in determining species' distribution limits. The potential of predicted climatic warming for driving changes in the ranges of different plant species is complex and uncertain (Thomas 2010; Donnelly *et al.* 2012), but *O. rufipogon* is clearly highly sensitive to temperature across its geographic range.

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