Regional gradients in intraspecific seed mass variation are associated with species biotic attributes and niche breadth

Xiaomei Kang1,*, Jieyang Zhou1,*, Abuman1, Yanjun Liu1, Shiting Zhang1, Wei Liu1, Haiyan Bu1 and Wei Qi2,†

1State Key Laboratory of Grassland Agro-ecosystems, College of Ecology, Lanzhou University, Lanzhou 730000, China
2Gansu Provincial Extension Station of Grassland Techniques, Lanzhou 730000, China
*Corresponding author’s e-mail address: qiw@lzu.edu.cn
†X.K. and J.Z. contributed equally to this paper.

Abstract
Quantifying intraspecific trait variation (ITV) is crucial for understanding species local adaptation and regional distribution. Intraspecific seed mass variation (ITVsm) is expected to vary with environmental gradients or co-vary with related biotic attributes, but these relationships are not well known in a multispecies space. We performed interspecific and phylogenetic analyses to evaluate the relative power of three species biotic attributes and four niche breadth traits in explaining ITVsm variation for 434 eastern Qinghai–Tibetan species. We showed a positive relationship between species’ ITVsm and their niche breadth in the light, moisture and disturbance dimensions, supporting the idea that high ITV allows species to match their traits to different habitat conditions and thus to distribute in a wide range of environments. However, we did not find significant direct effect of species’ thermal niche on individual seed mass variation. Meanwhile, we showed significant effects of seed dispersal mode, but not of life form and pollination type, on ITVsm. This suggests that the covariation or co-evolution between seed and disperser was related to the pattern and magnitude of ITVsm, but not to plant lifespan, the quality and allocation pattern of available resources and the availability of pollination vector. Lastly, all multivariate models showed a significant combined contribution of species’ biotic attributes and niche breadth to their ITVsm, implying that intrinsic biotic limitations and extrinsic abiotic pressures may operate simultaneously in controlling regional-scale intraspecific seed development.

Keywords: Intraspecific trait variation; life form; light niche; niche breadth; Qinghai–Tibet Plateau; seed dispersal mode; seed mass; thermal niche.

Introduction
Reproductive success of most plants depends on their seeds (Baskin and Baskin 2014; Igea et al. 2017). Seed mass generally indicates the substantial nutrient reserves in seed, and thus is considered to be pivotal to early plant survival (Moles 2018; Vandelook et al. 2018; Kang et al. 2021). Because of the limitation of available resources for seed development in a growing season, plants can produce either many small seeds to increase an opportunity of distributing widespread or being dispersed to more habitats or sites (Leishman and Westoby 1994; Igea et al. 2017), or few large seeds with more resources to increase a chance of good early seedling establishment (Moles et al. 2005; Moles and Westoby 2006; Baskin and Baskin 2014; Moles 2018). Consequently, different populations of a species under contrasting environmental conditions may exhibit different seed mass due to diverging selective pressures (Veloso et al. 2017; Fricke et al. 2019). The previous studies have reported that the magnitude of intraspecific trait variation (ITV) in response to environmental gradients can vary strongly among species with different biotic attributes or evolutionary history (Hera et al. 2017; Zhang et al. 2020; Westerband et al. 2021), suggesting that species intrinsic biotic attributes may contribute at least partly to the pattern of ITV. Thus, intraspecific seed mass variation (ITVsm) is expected to be affected by both the environmental and biotic factors (Sack 2004; Fricke et al. 2019).

Due to a tight trait–environment relationship, it seems intuitive to think that the species’ niche breadth is positively correlated with their ITV (Violle and Jiang 2009; Violle et al. 2012; Sides et al. 2014; Fajardo and Siefert 2018, 2019); that is, a large intraspecific variation translates into a strong capacity of species to prevail in a wide range of environments. Hence, plant species can occur over relatively broad spatial scales because high ITV allows them to match their traits to different environmental conditions (Violle et al. 2012; Sides et al. 2014; Fajardo and Siefert 2019). The extent of ITVsm is likely to be positively related to the species’ niche breadth. Among niche dimensions, the species’ light and moisture variation (ITV) in response to environmental gradients can...
niches, representing their adaption to the light and moisture conditions in a habitat, can be considered first in examining ITVs because these habitat conditions directly affect the early-stage plant life-history traits related to seed mass, e.g. seed germination and seedling performance, both in within-species studies (Baskin and Baskin 2014; Veloso et al. 2017) and between-species researches (Leishman and Westoby 1994; Vandeloek et al. 2018). Normally, especially in most large-scale interspecific studies, large seeds are common in low-light and low-moisture conditions to strengthen seedling growth and survival under shade and drought habitats (Sack et al. 2004; Moles and Westoby 2006; Igea et al. 2017; Vandeloek et al. 2018). However, the low-light or low-moisture conditions in some regions, representing suboptimal resource availability for reproductive output and seed development, may select for small-seed species or populations (Sánchez-Gómez et al. 2006; Moles 2018; Kang et al. 2021). These opposite forces may result in an unclear species niche–seed mass relationship. Species thermal niche, generally defined by local thermal conditions or temperature levels, has a dominant effect on species distributions and individual plant growth (Fernández-Pascual et al. 2019; Löfler and Pape 2020). Hence, it is easy to deduce that species with the wide thermal niche breadth can have large among-population variation in individual size as the response to the variation in local thermal energy, indirectly causing large ITVs. Moreover, disturbance is often regarded as an environmental factor that is proven to restrict the survival of large-seed individuals of a species in high-disturbance, and of small-seed ones in low-disturbance surroundings (Wu et al. 2015; Phartyal et al. 2020). Thus, the species disturbance niche breadth is expected to correlate positively with ITVs.

Among the intrinsic factors possibly associated with ITVs, seed dispersal mode and life form can be considered first because their evolutionary divergences have been thought to be the drivers of the geographic distribution and evolution in seed mass. For example, some interspecific studies have found that the divergence of life form, especially it of woody and herbaceous plants, is the primary factor affecting global seed mass divergence (Moles et al. 2005; Moles and Westoby 2006), whereas others have confirmed the evolution/divergence of seed dispersal mode, especially in case of vertebrate dispersal, as the main force of global distribution and evolution in seed mass (Tiffney 2004; Zhang et al. 2020). Compared with most species whose seed mass is controlled mainly by genetic variation and available resources (Venable 1992; Paul-Victor and Turnbull 2009; Li et al. 2019), the mass of anemochorous and zoochorous seeds is also affected by seed dispersers (e.g. wind and animals; Butler et al. 2007; Zhang et al. 2020). Thus, we can expect that these seeds show a smaller intraspecific variation than autochorous seeds because the mass variation of the former needs to match structurally the size of dispersal appendages such as wings, hairs, pappus, hooks, spines, juicy aril or flesh (Lord et al. 1997; Eriksson 2016; Kang et al. 2021). We also expect woody species to have smaller ITVs than herbaceous species because the former have larger individual biomass and the ability to flower early (Bolmgren and Cowan 2008; Du et al. 2020), which may reduce the dependence of their seed development on the growing season, available resources and (or) habitat conditions (Butler et al. 2007; Qi et al. 2015). In addition, because of the significant difference among pollination types in their pollination efficiency in different environmental conditions, and the significant effect of pollination efficiency on species reproductive success and individual seed number (Totland and Eide 1999; Knight et al. 2005), we can expect significant influences of pollination system on ITVs through a seed size/number trade-off (Zhang et al. 2020).

Relative to most of the other regions in the world, the Qinghai–Tibet Plateau (QTP) varies significantly in climate parameters (Kang et al. 2010). The dramatic climate changes accelerate the alterations in regional or local vegetation features and environmental conditions, which, ultimately, force a rapid variation in plant traits in response to these changes. Thus, QTP is the ideal place to evaluate the current or forecast the future effects of environmental variations on the species distribution and variation in plant traits (Favre et al. 2015). Here, using a database of 434 generalist angiosperm species in the eastern part of QTP, we focused on the main factors, including three species attributes and four niche breadth traits, potentially contributing to ITVs in the regional flora. This is, to our knowledge, the first study to explicitly disentangle the various abiotic and biotic drivers of the intraspecific variation in a trait across species of a regional flora by using a large database with a wide environmental span. The study will help reconcile two sides of argument caused mainly by previous few-species or few-factor researches whether co-varying with biotic attributes or plastic response to environmental gradients is the determinant of regional patterns of intraspecific trait variation. Specifically, we addressed the following questions:

1) Do species with large niche breadth have large ITVs? What are the major niche factors that correlate with ITVs: temperature, light, moisture and/or disturbance? 2) Does the extent of ITVs vary significantly among species with different life forms, seed dispersal modes or pollination types?

To answer these questions, we determined the relative importance of intrinsic biotic attributes and niche breadth traits in ITVs by using both interspecific and phylogenetic analyses. Based on these analyses, we expected (i) a positive ITVs–niche breadth correlation both with and without controlling for phylogeny and (ii) a smaller ITVs for woody and zoochorous species than for others.

Materials and Methods

Study site

The study area is located on the north-east verge of Tibet Plateau (100°50′~103°40′E, 33°50′~35°70′N; 1700~4100 m a.s.l.), and belong to a transitional area of Qinghai–Tibetan Plateau, Qinling mountain and Loess Plateau. This region spans a large climate range, with a mean annual temperature from −1.7 to 13.2 °C, with mean annual precipitation from 343 to 709 mm.

Species studied

Through 16 years’ (2001–16) seed collecting and field investigation, we are able to gather a large and unprecedented comprehensive data set of a flora (~1580 species and 9800 populations; part of data are unpublished, while other data are showed in Qi et al. 2014, 2015 and Kang et al. 2021). For 417 populations (belonging to 352 species, being included
in the analysis) for which seeds were sampled for no fewer than 3 years, we did not find significant seed mass difference among years (results not shown), suggesting that the temporal effect (i.e. the effect caused by annual changes in climatic conditions, such as temperature and precipitation) on iTVsm is weak. Therefore, we did not need to consider annual variation of seed mass in this study. Finally, we selected a large data subset of 434 species for which seed mass had been measured in no fewer than seven populations each (altogether 4769 populations) [see Supporting Information—Supplementary Material and Appendix S3]. We used this species selection criterion because (i) the selected species had a large distribution range (i.e. regional widespread species) and (ii) the other species sample size was considered too low for testing intra-specific trait variation reliably. These species cover a wide taxonomic range, being derived from 199 genera and 58 families which based on Angiosperm Phylogeny Group IV system.

Data source
Seed mass was defined as the weight of the embryo and endospem, plus the seed coat. Other structures contributing to dispersal were not included as part of the seed. Seeds (n = 100 whenever possible) from each population were air-dried weighed three times, and the mean weights were used in further calculation (Qi et al. 2014, 2015; Kang et al. 2021). For every species, we evaluated three intrinsic biotic attributes, including life form (being classified as annual, herbaceous perennial and woody perennial), seed dispersal mode (autochory, anemochory and zoochory (ectozoochory and endozoochory)) and pollination type (anemophily and entomophily). For every population, we recorded its temperature regime, disturbance degree of the habitat as well as light and moisture levels to determine its niche positions in the light, moisture, disturbance and thermal dimensions (five levels for each niche dimension) [see Supporting Information—Appendix S1]. Specifically, we classified population’s thermal niche position based on the thermal level (i.e. thermal climatic zone; including alpine, cold-subalpine, warm-subalpine, cold-temperature and warm-temperature zone) it survives. Light/moisture/disturbance niche of a population was defined according to the light/moisture/disturbance level of habitat where more than its 70 % individuals (or more than its 70 % individuals that were collected seeds) occupied. In order to meet the requirement of calculating species niche breadth (each population having only one niche level in each dimension), we excluded the population whose <70 % individuals lived in one niche level. The details of the category and the measurement of biotic attributes of every species and the niche positions of every population were described in Supporting Information—Appendix S1.

Quantifying iTVsm and species niche breadth
For each species, we quantified its iTVsm by using the coefficient of variation (CV) among populations [see Supporting Information—Supplementary Material]. The CV was chosen as an index of relative rather than absolute variation, thus allowing direct comparison among species with different mean seed mass. The CV values of among-population seed mass variation were denoted CVsm. For each species, we calculated niche breadth in each dimension (light, moisture, thermal and disturbance) by using Levins’ B index based on the niche positions of every population (Papacostas and Freestone 2016).

Levins’ B of every species is calculated as: \( 1/\sum p_i^2 \), where \( p_i \) is the proportion of niche position (i.e. light, moisture, thermal or disturbance level) \( i \). The values of this index range from 1 to \( N \) (number of niche positions), and large values indicate a wider niche breadth.

Statistical analyses
All analyses below were carried out using R version 4.0.2 or SPSS 24.0 unless stated otherwise.

Interspecific analysis. Firstly, we evaluated the single effect of species biotic attributes (seed dispersal mode, life form and pollination types; as categorical variables) and niche breadth traits (light, moisture, thermal and disturbance niche breadth; as continuous variables) on CVsm by using one-way ANOVA and simple linear regression, respectively. Then, we performed generalized linear models (GLM) to test for the integrative effect of species biotic traits (as fixed factors) and niche breadth traits (as covariate) on CVsm. We also performed phylogenetic analysis (PA), but current implementations of the standard PA cannot deal with unordered categorical independent variables. Thus, for a direct comparison of PA and GLM, life form was treated as two binary variables in these analyses: xylophyta (woody/herbaceous: 1/0) and lifespan (perennial/annual: 1/0), and seed dispersal mode as: anemochory (yes/no: 1/0) and zoochory (yes/no: 1/0). In addition, entomophily/anemophily was coded as 1/0 in PA and GLM.

To better assess the factors affecting CVsm, we carried out regression tree models with species all biotic attributes and niche breadth traits as predictor variables. The trees can deal with non-linear and hierarchical relationships and provide reliable parameter estimates because the method guards against the elimination of variables that are good predictors of the response but are correlated with other predictors (Pyšek et al. 2012). The trees were constructed in CART by binary recursive partitioning, using the default ‘Gini’ impurity measure with a minimum child node of 10 species. Tenfold cross-validation was used to improve the accuracy of the tree.

Phylogenetic analysis. Phylogenetic analysis is an effective method to assess whether interspecific relationship is independent of species’ phylogeny. The comparison between interspecies and phylogenetic analyses can also help assess whether present-day trait relationship is a consistent pattern during trait evolutionary divergence. The analyses need a working evolutionary tree, which was obtained from an online website (http://phylodiversity.net) based on a comprehensive angiosperm phylogeny from Zanne et al. (2014). Prior to analyses, we examined the phylogenetic signal strength by estimating Pagel’s \( \lambda \) for CVsm and all related biotic and niche traits in the R package ‘phytools’ (Revell 2012). We used a maximum likelihood framework to estimate the parameter \( \lambda \), which can vary from 0 (no influence of phylogeny) to 1 (maximum phylogenetic influence). The package also provides \( P \)-values by performing a likelihood ratio test against the null hypothesis that \( \lambda = 0 \) [see Supporting Information—Table S1]. In phylogenetic analyses, the effect of each of species biotic attributes (xylophyta, lifespan, anemochory, zoochory and pollination types; as binary variables) and niche breadth traits (light, moisture, thermal and disturbance niche breadth; as continuous variables) on CVsm was determined by using phylogenetically independent contrasts (PICs) and
phylogenetic regression (PR; a regression analysis on the PICs of each trait against its standard deviation (SD)), respectively. Phylogenetically independent contrasts were calculated using the ‘Analysis of Traits’ model in Phylocom (Webb et al. 2008); whereas PR (i.e. regression analysis of standardized contrasts (forced through the origin)) was performed using SMA (standardized major axis) analysis in R.

Results
Across all species, the mean (± SD) of CVsm and light, moisture, thermal and disturbance niche breadth was 0.207 (0.070), 1.812 (0.479), 1.834 (0.449), 2.628 (0.681) and 1.958 (0.436), respectively. Meanwhile, phylogenetic signal of pollination type ($\lambda = 0.999$), xylophyta ($\lambda = 0.999$), anemochory ($\lambda = 0.881$) and zoochory ($\lambda = 0.817$) was significant and strong [see Supporting Information—Table S1], of lifespan ($\lambda = 0.494$), light niche breadth ($\lambda = 0.444$) and moisture niche breadth ($\lambda = 0.279$) was significant and weak, of CVsm ($\lambda = 0.140$) was marginally significant (0.05 < $P < 0.1$), but of disturbance and thermal niche breadth was non-significant (both $\lambda < 0.001$ and $P = 1$) [see Supporting Information—Table S1].

The association of CVsm with niche breadth in light (Fig. 1A), moisture (Fig. 1B) and disturbance (Fig. 1D) dimensions was significantly positive, but was non-significant with thermal niche breadth (Fig. 1C). The CVsm was non-significantly different among species with different life forms (Fig. 2A) and pollination types (Fig. 2C), but varied significantly among species with different seed dispersal modes (Fig. 2B), with zoochorous (ectozoochorous and endozoochorous) species having smaller CVsm than others. The PR confirmed the significantly positive association of CVsm with light niche breadth (Fig. 3A) and moisture niche breadth (Fig. 3B) and the non-significant association of CVsm with thermal niche breadth (Fig. 3C), but did not confirm the positive CVsm–disturbance niche breadth relationship (Fig. 3D). Phylogenetically independent contrasts showed significantly lower divergence in CVsm for zoochory than other seed dispersal modes (paired $t$-test, the same below; $N = 26$, mean = $-0.032$, $P = 0.010$). In contrast, the divergence in CVsm was not affected significantly by xylophyta ($N = 16$, mean = $0.019$, $P = 0.343$), lifespan ($N = 36$, mean = $0.001$, $P = 0.925$), anemochory ($N = 18$, mean = $0.029$, $P = 0.063$) or pollination types ($N = 8$, mean = $0.017$, $P = 0.476$).

Multivariate analysis (GLM) identified moisture niche breadth as the strongest factor influencing CVsm (Table 1), following by disturbance niche breadth, zoochory, anemochory and light niche breadth. In contrast, the effect of xylophyta, lifespan, pollination types and thermal niche breadth on CVsm
In the regression tree model, disturbance niche breadth was the first dividing criterion for exploring CVsm, in which species with high disturbance niche breadth had high CVsm (Fig. 4). In the second level of the regression tree, seed dispersal model was the dividing criterion in the group of species with high disturbance niche breadth.
to be influenced by abiotic and biotic factors (Violle et al. 2012; Sides et al. 2014; Vandelook et al. 2018; Fajardo and Siefert 2019; Fricke et al. 2019; Westerband et al. 2021). This is supported by our findings that the variation in ITVsm is associated with species biotic attributes and niche breadth. However, the effects of different biotic attributes or different dimensions of niche breadth of species on ITVsm were significantly different, suggesting that multiple mechanisms may operate simultaneously in governing seed development. Below, we discuss our findings as well as offer potential explanations for some of the unexpected results.

In congruent with the most findings and our first expectation, we showed that species with wide light and moisture niche breadths had high ITVsm, supporting the common hypothesis that high phenotypic plasticity or genotypic variability enhances species niche breadth by allowing species to express advantageous phenotypes or genotypes in a broad range of habitats (Violle et al. 2012; Sides et al. 2014; Fajardo and Siefert 2019). However, the interpretive force of these two niche traits to ITVsm was different, in which moisture niche breadth is the stronger predictor of ITVsm. The reason may be that habitat moisture conditions can affect intensely early-stage plant life-history processes such as seed germination and seedling survival or establishment. These processes are affected by seed mass to some extent (other seed traits, such as seed nutrient content and seed metabolic rate, can also be influential; Sack et al. 2004; Moles and Westoby 2006; Baskin and Baskin 2014; Vandelook et al. 2018). Thus, to get the local optima in these processes, species may develop different seed mass in response to the variation in the habitat moisture conditions. In contrast, the habitat light conditions (representing the amount of light resources available for plant growth) may affect mainly plant growth rate rather than seedling establishment, and thus, weakening the dependence of ITVsm on habitat light variation.

In contrast to our first expectation, the relationship between niche breadth thermal and ITVsm was not significant. In the present study, the thermal niche of species or population was defined according to the local thermal or temperature level, whereas the term is often used to represent the amount of thermal energy available for individual plant development. Thus, the non-significant relationship may imply that plant species respond to thermal gradients mainly by changing

Table 1. Results from GLM of various predictors on the coefficient of variation of intraspecific seed mass (CVsm). For binary (xylophyta, lifespan, anemochory, zoochory and pollination type) and continuous (light niche breadth, moisture niche breadth, thermal niche breadth and disturbance niche breadth) predictors, column ‘B’ represented the mean CVsm difference between groups (group ‘1’ minus group ‘0’) and regression slope, respectively. %SS, percentage of total sum of squares explained. *P < 0.05, **P < 0.01 and ***P < 0.001, respectively.

| Predictor | B       | F     | %SS |
|-----------|---------|-------|-----|
| Models    | Adjusted $R^2 = 0.184$ |       |     |
| Xylophyta | 0.017   | 1.91  | 0.4 |
| Lifespan  | -0.002  | 0.03  | 0.0 |
| Anemochory| 0.027   | 12.65** | 2.9 |
| Zoochory  | -0.039  | 16.35** | 3.6 |
| Pollination type | 0.000 |       | 0.0 |
| Light niche breadth | 0.022 | 10.80** | 2.5 |
| Moisture niche breadth | 0.034 | 22.92** | 5.0 |
| Thermal niche breadth | -0.001 | 0.01  | 0.0 |
| Disturbance niche breadth | 0.032 | 18.14** | 4.0 |

Discussion
The reasons for intraspecific variation in a certain plant trait are multiple; such variability arises from a combination of genetic variation, developmental instability and phenotypic plasticity due to environmental change, and thus, is expected

Figure 4. Results of the regression tree analysis of the relationship between CVsm (the coefficient of variation of among-population seed mass) and seven biotic or niche breadth predictors. In the decision of tree size, 10-fold cross-validations and a minimum child node of 10 sampling size are applied, and the Gini index is used as impurity.

breedth, in which the zoochorous species subgroup had low CVsm. For the subgroup of non-zoochorous species with high disturbance niche breadth, species with low moisture niche breadth had low CVsm (Fig. 4).

In addition, the validity of the above results was confirmed by similar trends in the variation of ITVsm when Gini coefficient value of among-population seed mass variation (GCsm) was used instead of CVsm in all of our analyses [see Supporting Information—Appendix S2].
their individual size, total reproductive biomass and/or seed number rather than the size of individual seed (Venable 1992; Fernández-Pascual et al. 2019; Kang et al. 2021). However, due to the lack of data on plant growth and reproduction characteristics, we could not determine which factor contributed more to our findings.

We are the first to analyse the relationship between disturbance niche breadth and ITV. Plant species often vary significantly in reproductive strategies to respond to different disturbance levels. For example, many species tend to reproduce early, shorten fruit development time or increase seed number as adaptation to high disturbance frequency and severity (Kühner and Kleyer 2008; Mabry and Fraterrigo 2009; Johnson and Miyashita 2010; Veloso et al. 2017). The variation in reproductive strategies is expected to affect seed mass by varying the reproductive output, pattern of reproductive allocation and the seed mass/number trade-off of individual plant (Wu et al. 2015; Herben et al. 2018). Thus, the positive interspecific relationship between disturbance niche breadth and ITVs may imply that species with variable reproductive strategies have the capacity to distribute widely along disturbance gradients. The positive relationship, however, was not supported by phylogenetic analysis, suggesting the cross-species correlation between species disturbance niche breadth and their intraspecific variation in reproductive strategies or seed mass and should be driven mainly by one or more large divergences deep in the phylogeny rather than by a consistent trait association throughout the evolutionary history of the clade.

Both interspecific and phylogenetic analyses showed a significantly lower ITVs in zoochorous species than others, supporting our expectation that the size variation of zoochorous seeds is restricted because they need to match structurally the size of animal-dispersal appendages and/or seed dispersers’ organ. However, the ITVs of anemochorous species was not different from that of autochorous species, implying no obvious structural limitation on intraspecific anemochorous seed size variation. Moreover, relative to zoochorous seeds, the dispersal distance of anemochorous seeds was generally short, and determined mainly by wind speed, the shape and type of dispersal appendages and seed mass (Greene and Johnson 1993; Savage et al. 2014; Zhou et al. 2019). Therefore, a strong seed mass variation helps maternal plants of anemochorous species spread seeds to different locations to avoid sibling competition (Savage et al. 2014; Traveset et al. 2014; Zhang et al. 2020). Surprisingly, we did not find significant difference in ITVs among life forms. Because life forms differ in plant lifespan, the quality of available resources and the way of partitioning and storing resources (Campanella and Bertiller 2008; Qi et al. 2014; Zhang et al. 2020), this finding suggests a lack of direct effects of plant lifespan, and resource availability and allocation patterns on the development of individual seed.

The non-significant effect of pollination type on ITVs may imply little, if any, pollen limitation on reproductive success and seed output and development (Walsh et al. 2014), which makes among-population seed mass variation less dependent on pollination vector. In addition, some specific pollination strategies, such as facultative or delayed autogamy in alpine zones (Sun et al. 2005; Dainese and Bragazza 2012; Xiong et al. 2013), can provide plants with substantial reproductive insurance in cases of low pollinator activity, which further weakens the effect of pollination vector on the difference in seed output among populations.

Conclusion

These results partly support our expectations. First, the ITVs—species niche breadth is significantly positive in the habitat-scale light, moisture and disturbance dimensions, but not in the local-scale thermal dimension, suggesting a stronger effect of habitat conditions than locally available resources on the among-population difference in seed mass. Then, seed dispersal mode, but not of life form and pollination type, is the only biotic attributes affecting significantly ITVs, implying that the covariation or co-evolution between seed and disperser, rather than plant vegetative growth characteristics and reproductive strategies, is strongly related to the pattern and magnitude of ITVs. Moreover, the multivariate models show significant combined effects of the species biotic attributes and the niche breadth on ITVs, supporting the idea of a multi-factor control on intraspecific seed development (Sides et al. 2014; Fricke et al. 2019). Notably, because our study was observational, we cannot conclude to what extent the measured ITVs represents genetic variation or phenotypic plasticity, but given the higher contribution of the species niche breadth traits (vs. biotic attributes) in ITVs and a non-significant or weak phylogenetic signal in these traits (light, moisture, disturbance and thermal niche breadth, GCsm and CVsm), it is likely that plasticity plays an important role.

Supporting Information

The following additional information is available in the online version of this article—

Table S1. Phylogenetic signal for ITVs (intraspecific seed mass variation) and each of its related biotic attributes and niche breadth traits.

Appendix S1. The methods of seed mass-related biotic attributes and plant niche traits measurement.

Appendix S2. The results of interspecific and phylogenetic analysis that use Gini coefficient value of among-population seed mass variation (GCsm) as the index of intraspecific seed mass variation (ITVs).

Appendix S3. List of intraspecific seed mass variation, three biotic attributes and four niche breadth traits of 434 angiosperm species. CVsm and GCsm are separately the CV (coefficient of variation) and GC (Gini coefficient) value of among-population seed mass variation. LF, life form; SDM, seed dispersal mode; PT, pollination type; An, annual; Hp, herbaceous perennial; Wp, woody perennial.

Supplementary Material. List of seed mass and seven related biotic attributes and niche traits of 434 angiosperm species (4769 populations). SM, seed mass (mg); LF, life form; SDM, seed dispersal mode; PT, pollination type; LN, light niche; MN, moisture niche; TN, thermal niche; DN, disturbance niche; An, annual; Hp, herbaceous perennial; Wp, woody perennial; Aut, autochory; Anem, anemochory; Ect, ectozoochory; End, endozoochory; Anem, anemophily; Entom, entomophily.

Sources of Funding

The study is supported by the Project of the National Natural Science Foundation of China (31770448, 32171518), the Science and Technology Support Project of Ecological Grassland Restoration and Management in Gansu Province in 2020 granted to W.Q. (letter of Forestry and grassland Bureau of Gansu Province: [2020]72) and the Natural Science
Foundation of Gansu Province granted to W.L. [21JR7RA482]. The study is also supported by Gannan Grassland Ecosystem National Observation and Research Station.

Conflict of Interest
None declared.

Contributions by the Authors
X.K. and W.Q. conceived the ideas; all authors but J.Z. and Y.L. collected the data; X.K. and J.Z. analysed the data. X.K., J.Z. and W.Q. contributed to the writing of the manuscript.

Acknowledgements
The authors would like to express their gratitude to EditSprings (https://www.editsprings.com/) for the expert linguistic services provided.

Data Availability
The data that support the findings of this study are openly available at: https://fitchshare.com/s/94672a4f1a8c1397791e5.

Literature Cited
Baskin CC, Baskin JM. 2014. Seeds: ecology, biogeography, and evolution of dormancy and germination, 2nd edn. London: Academic Press.
Bolmgren K, Cowan PD. 2008. Time-size tradeoffs: a phylogenetic comparative study of flowering time, plant height and seed mass in a north-temperature flora. Oikos 117:424–429.
Butler DW, Green RJ, Lamb D, Mcdonald WJF, Forster PI. 2007. Biological Ecology 106:1–18.
Campanella MV, Bertrill MB. 2008. Plant phenology, leaf traits and leaf litterfall of contrasting life forms in the arid Patagonian Monte, Argentina. Journal of Vegetation Science 19:75–85.
Dainese M, Bragazza L. 2012. Plant traits across different habitats of the Italian Alps: a comparative analysis between native and alien species. Alpine Botany 122:11–21.
Du YJ, Mao LF, Queenborough SA, Primack R, Comita LS, Hampe A, Ma KP. 2020. Macro-scale variation and environmental predictors of flowering and fruiting phenology in the Chinese angiosperm flora. Journal of Biogeography 47:2303–2314.
Erlksson O. 2016. Evolution of angiosperm seed disperser mutualisms: the timing of origins and their consequences for coevolutionary interactions between angiosperms and frugivores. Biological Reviews 91:168–186.
Fajardo A, Sievert A. 2018. Intraspecific trait variation and the leaf economics spectrum across resource gradients and levels of organization. Ecology 99:1024–1030.
Fajardo A, Sievert A. 2019. The interplay among intraspecific leaf trait variation, niche breadth and species abundance along light and soil nutrient gradients. Oikos 128:881–891.
Favre A, Packert M, Pauls SU, Jahngic SC, Uhl D, Michalak I, Muellner-Riehl AN. 2015. The role of the uplift of the Qinghai–Tibetan plateau for the evolution of Tibetan biotas. Biological Reviews 90:236–253.
Fernández-Pascual E, Mattana E, Pritchard HW. 2019. Seeds of future past: climate change and the thermal memory of plant reproductive traits. Biological Reviews 94:439–456.
Fricke EC, Tewksbury JJ, Rogers HS. 2019. Linking intra-specific trait variation and plant function: seed size mediates performance tradeoffs within species. Oikos 128:1716–1725.
Greene DF, Johnson EA. 1993. Seed mass and dispersal capacity in wind-dispersed diaspors. Oikos 67:69–74.
Herben T, Klimešová J, Chytrý M. 2018. Effects of disturbance frequency and severity on plant traits: an assessment across a temperate flora. Functional Ecology 32:799–808.
Herz K, Dietz S, Haider S, Jandt U, Scheel D, Brueheide H. 2017. Drivers of intraspecific trait variation of grass and forb species in German meadows and pastures. Journal of Vegetation Science 28:705–716.
Igea J, Miller EP, Papadopouios AST, Tanentzap AJ. 2017. Seed size and its rate of evolution correlate with species diversification across angiosperms. PLoS Biology 15:e2002792.
Johnson EA, Miyaniishi K, eds. 2010. Plant disturbance ecology: the process and the response. Amsterdam, Holland: Elsevier/AP.
Kang SC, Xu YW, You QL, Flugel WA, Pepin N, Yao TD. 2010. Review of climate and cryospheric change in the Tibetan Plateau. Environmental Research Letters 5:015101.
Kang XM, Zhou JY, Abuman, Du GZ, Qi W. 2021. Multi-factor control of seed mass of species on the eastern part of the Qinghai–Tibetan Plateau: integration of environmental filters, local adaptation and correlated evolution. Environmental and Experimental Botany 187:104471.
Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman TL. 2005. Pollen limitation of plant reproduction: pattern and process. Annual Review of Ecology Evolution and Systematics 36:467–497.
Kühner A, Kleyer M. 2008. A parsimonious combination of functional traits predicting plant response to disturbance and soil fertility. Journal of Vegetation Science 19:681–692.
Leishman MR, Westoby M. 1994. Hypotheses on seed size: tests using the semiarid flora of western New South Wales. Australia. American Naturalist 143:890–906.
Li N, Xu R, Li YH. 2019. Molecular networks of seed size control in plants. Annual Review of Plant Biology 70:435–463.
Loiffer J, Pape R. 2020. Thermal niche predictors of alpine plant species. Ecology 101:e02891.
Lord J, Egan J, Clifford T, Jurado E, Leishman M, Williams D, Westoby M. 1997. Larger seeds in tropical floras: consistent patterns independent of growth form and dispersal mode. Journal of Biogeography 24:205–211.
Mabry CM, Fraterrigo JM. 2009. Species traits as generalized predictors of forest community response to human disturbance. Forest Ecology and Management 257:723–730.
Moles AT. 2018. Being John Harper: using evolutionary ideas to improve understanding of global patterns in plant traits. Journal of Ecology 106:1–18.
Moles AT, Ackerly DD, Webb CO, Tweddle JC, Dickie JB, Pitman AJ, Westoby M. 2005. Factors that shape seed mass evolution. Proceedings of the National Academy of Sciences of the United States of America 102:10540–10544.
Moles AT, Westoby M. 2006. Seed size and plant strategy across the whole life cycle. Oikos 113:91–105.
Papacostas KJ, Freestones AL. 2016. Latitudinal gradient in crab nache breadth. Global Ecology and Biogeography 25:207–217.
Paul-Victor C, Turnbull LA. 2009. The effect of growth conditions on the seed size/number trade-off. PLoS One 4:e6917.
Phartyal SS, Rosbakh S, Ritz C, Poschlo P. 2020. Ready for change: seed traits contribute to the high adaptability of mudflat species to their unpredictable habitat. Journal of Vegetation Science 31:331–342.
Pyšek P, Chytrý M, Pergl J, Sadlo J, Wild J. 2012. Plant invasions in the Czech Republic: current state, introduction dynamics, invasive species and invaded habitats. Preslia 94:575–629.
Qi W, Bu HY, Cornelissen JHC, Zhang CH, Guo SQ, Wang JH, Zhou XH, Li WJ, Du GZ. 2015. Untangling interacting mechanisms of seed mass variation with elevation: insights from the comparison of inter-specific and intra-specific studies on eastern Tibetan angiosperm species. Plant Ecology 216:283–292.
Qi W, Guo SQ, Chen XL, Cornelissen JHC, Bu HY, Du GZ, Cui XL, Li WJ, Liu K. 2014. Disentangling ecological, allometric and evolutionary determinants of the relationship between seed mass and elevation: insights from multiple analyses of 1355 angiosperm species on the eastern Tibetan Plateau. *Oikos* 123:23–32.

Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3:217–223.

Sack L. 2004. Responses of temperate woody seedlings to shade and drought: do trade-offs limit potential niche differentiation? *Oikos* 107:110–127.

Sánchez-Gómez D, Valladares F, Zavala MA. 2006. Performance of seedlings of Mediterranean woody species under experimental gradients of irradiance and water availability: trade-offs and evidence for niche differentiation. *New Phytologist* 170:795–806.

Savage D, Borger CP, Renton M. 2014. Orientation and speed of wind gusts causing abscission of wind-dispersed seeds influences dispersal distance. *Functional Ecology* 28:973–981.

Sides CB, Enquist BJ, Ebersole JJ, Smith MN, Henderson AN, Sloat LL. 2014. Revisiting Darwin’s hypothesis: does greater intraspecific variability increase species ecological breadth? *American Journal of Botany* 101:56–62.

Sun SG, Guo YH, Gituru RW, Huang SQ. 2005. Corolla wilting facilitates delayed autonomous self-pollination in *Pedicularis dunniana* (Orobanchaceae). *Plant Systematics and Evolution* 231:229–237.

Tiffney BH. 2004. Vertebrate dispersal of seed plants through time. *Annual Review of Ecology, Evolution, and Systematics* 35:1–29.

Totland O, Eide W. 1999. Environmentally-dependent pollen limitation on seed production in alpine *Ranunculus acris*. *Ecoscience* 6:173–179.

Traveset A, Heleno R, Nogales M. 2014. The ecology of seed dispersal. In: Gallagher RS, ed. *Seeds—the ecology of regeneration in plant communities*. Wallingford, UK: CABI, 62–93.

Vandelook F, Janssens SB, Matthies D. 2018. Ecological niche and phylogenetics explain distribution of seed mass in the central European flora. *Oikos* 127:1410–1421.

Veloso A, Silva PS, Siqueira WK, Duarte KLR, Gomes ILV, Santos HT, Fagundes M. 2017. Intraspecific variation in seed size and light intensity affect seed germination and initial seedling growth of a tropical shrub. *Acta Botanica Brasilica* 31:736–741.

Venable DL. 1992. Size-number trade-offs and the variation of seed size with plant resource status. *The American Naturalist* 140:287–304.

Violle C, Enquist BJ, Mcgill BJ, Jiang L, Albert CH, Hulshof C, Jung V, Messier J. 2012. The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution* 27:244–252.

Violle C, Jiang L. 2009. Towards a trait-based quantification of species niche. *Journal of Plant Ecology* 2:87–93.

Walsh RP, Arnold PM, Michaels HJ. 2014. Effects of pollination limitation and seed predation on female reproductive success of a deceptive orchid. *AoB Plants* 6:plu031; doi:10.1093/aobpla/plu031.

Webb CO, Ackerly DD, Kembel SW. 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* 24:2098–2100.

Westerband AC, Knight TM, Barton KE. 2021. Intraspecific trait variation and reversals of trait strategies across key climate gradients in native Hawaiian plants and non-native invaders. *Annals of Botany* 127:553–564.

Wu GL, Shang ZH, Zhu YJ, Ding LM, Wang D. 2015. Species-abundance–seed-size patterns within a plant community affected by grazing disturbance. *Ecological Applications* 25:848–855.

Xiong YZ, Fang Q, Huang SQ. 2013. Pollinator scarcity drives the shift to delayed selfing in Himalayan mayapple *Podophyllum hexandrum* (Berberidaceae). *AoB Plants* 5:plt037; doi:10.1093/aobpla/plt037.

Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, Fitzjohn RG, Mcglinn DJ, O’Meara BC, Moles AT, Reich PB, Royer DL, Soltis DE, Stevens PF, Westoby M, Wright IJ, Aarssen L, Bertin RI, Calaminus A, Govaerts R, Hemmings F, Leishman MR, Oleksyn J, Soltis PS, Swenson NG, Warman L, Beaulieu JM. 2014. Three keys to the radiation of angiosperms into freezing environments. *Nature* 506:89–92.

Zhang C, Li J, Willis CG, Ma Z. 2020. Among-population variation in seed mass for 190 Tibetan plant species: phylogenetic pattern and ecological correlates. *Global Ecology and Conservation* 23:e01163.

Zhou QL, Liu ZM, Xin ZM, Daryanto S, Wang LX, Qian JQ, Wang YC, Liang W, Qin XP, Zhao YM, Li XL, Cui X, Liu MH. 2019. Relationship between seed morphological traits and wind dispersal trajectory. *Functional Plant Biology* 46:1063–1071.