

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

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Abstract With increasing elevation, seed mass is expected to be either larger for its advantage during seedling establishment in stressful high-elevation environments (“stress-tolerance” mechanism) or smaller due to energy constraints. Based on the combination of inter- and intra-specific analyses on 4,023 populations of 320 Tibetan plant species, we found an overall positive within species but negative among species seed mass–elevation relationship, suggesting that regional seed mass distribution with elevation is affected mainly by the energy-constraint mechanism at among-species level, but by the stress-tolerance mechanism at within-

species level. Moreover, both intra- and inter-specific analyses revealed a mass-dependent seed mass variation along elevation gradients: small seeds tended to increase but large seeds tended to decrease with increasing elevation, indicating that the stress-tolerance (respectively energy-constraint) mechanism may exert a stronger effect on elevational distribution of small-seeded (respectively large-seeded) populations or species. The mass-dependent seed mass variation along elevation gradients, however, was absent within woody and zoochorous species, implying substantial available resources and long time for seed development, and the covariation or coevolution between the mass of zoochorous seeds and their dispersers may allow for a (partial) decoupling of species’ seed mass and their intra-specific variation with elevation. Together our results call for more comparative analyses at different taxonomic levels in detecting geographic variation in a trait.

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Introduction

Seed mass generally indicates the amount of reserves that a seed contains, which can influence seed dispersal, germination, seedling growth and, ultimately, fitness of a plant (Coomes and Grubb 2003; Moles and Westoby 2003). For a given amount of resource, small-seeded species are able to produce more seeds than large-seeded species, whereas large-seeded species can support seedlings to better tolerate environmental stresses encountered during seedling establishment (Smith and Fretwell 1974; Moles and Westoby 2003, 2006). Thus, to allocate limited resource into fewer but larger seeds or into more but smaller ones follows a fundamental trade-off (Westoby et al. 1996; Muller-Landau 2010). Some studies or hypotheses predicted that the optimum and range of species’ seed mass would be evolutionarily stable and dominated by the trade-off (for details see the evolutionarily stable seed mass model of Geritz 1995 and Smith–Fretwell model 1974).

In mountain areas, elevation is one of the most striking factors for trait distribution because it presents environmental gradients in temperature, water, and soil nutrients (Wang et al. 2007; Qi et al. 2014a). Despite a long-held interest, our understanding of elevation gradients in seed mass is still incomplete, with conflicting findings emerging (Baker 1972; Blionis and Vokou 2002). For example, some studies found an increase in seed mass with elevation (Boulli et al. 2001; Pluess et al. 2005), supporting the “stress-tolerance” hypothesis that large seeds have a great advantage during seedling establishment in the stressful high elevations. In contrast, other scholars found a negative relationship between seed mass and elevation, supporting the “energy-constraints” hypothesis that low temperatures and short growing seasons at high elevations may reduce photosynthetic rates, the energy for seed development, and seed provisioning (Baker 1972; Bu et al. 2007; Guo et al. 2010). Plastic developmental responses or allometric constraints on seed mass (i.e. small plants cannot support and nourish large fruits with large seeds), would be consistent with

this hypothesis (Thompson and Rabinowitz 1989; Cornelissen 1999).

In a recent inter-specific study from the northeastern Tibetan Plateau (Qi et al. 2014b), we found an overall balance between the two opposing mechanisms when controlling for species’ phylogeny and a shift in the balance toward increased energy constraint (respectively stress tolerance) with the increase (respectively decrease) in seed mass. We also found no consistent seed mass–elevation correlation among species with different life forms or different dispersal modes. Together the above findings indicate that seed mass itself can affect the inter-specific responses of seed mass to elevation, while the life history cycle and availability of dispersal agents may play a role too.

Geographic variation in seed mass, however, may show different pattern within and among plant species. For example, Pluess et al. (2005) found that seed mass in the Swiss Alps increases with elevation between related species but not among populations of individual species. Other scholars found a negative relationship between seed mass and latitude both within and among species, but the intra-specific slope was 3.6-fold smaller than the inter-specific slope and showed some notable exceptions (Moles and Westoby 2003; Murray et al. 2004). These findings suggest that intra-specific seed mass variation may be associated with a suite of intrinsic factors, such as dispersal, seed dormancy, fruit type, fruit size, flowering time, and ovule number per carpel (Hodgson and Mackey 1986; Rees 1997; Pluess et al. 2005; Bolmgren and Cowan 2008; Du and Qi 2010). These intrinsic factors hardly vary at the within-species level, thus they may counteract selection pressures among populations at different elevations.

Among the intrinsic factors possibly associated with within-species elevational variation in seed mass, seed dispersal mode and life form should be considered first because the evolutionary divergences of them have been thought to be the drivers of the geographic distribution and evolution in seed mass (Tiffney 2004; Moles et al. 2005; Moles and Westoby 2006). Moreover, pollination type and (or) pollination efficiency have significant effects on reproductive success, seed development, and thus possibly seed mass (Totland and Eide 1999; Knight et al. 2005). In this case, including these three traits in a statistical model may help clarify the ecological mechanisms underpinning elevational variation in seed mass.

Due to the possibly different responses of seed mass to elevation between within- and among-species level, the explicit comparison of intra- and inter-specific analysis can provide a more complete view of the ecological and evolutionary seed mass–elevation relationship. Here, we synthesize both the intra- and inter-specific seed mass–elevation relationships of 320 species, with at least ten populations each, across a more than 2,500 m gradient of elevation in the northeastern Tibetan Plateau. We address the following primary questions. (1) Is there an overall similar intra- and inter-specific seed mass–elevation relationship? (2) Do the two opposing mechanisms, i.e. stress tolerance and energy constraints, operate simultaneously both at within- and among-species levels? And if so, in which cases does each of these mechanisms exert a stronger effect on seed mass? (3) Is there a consistent within-species pattern when species' life form, dispersal mode, and (or) pollination type are considered or controlled for statistically?

This is, to our knowledge, the first study to combine and compare within- and among-species elevation pattern in seed mass using a large database with a wide elevation span. This way, we try to examine the potential mechanisms underpinning the elevational variation in seed mass.

Methods

Study area

The study area is located on the northeastern verge of the Tibetan Plateau in China (101°05'E–104°40'E, 32°60'N–35°30'N, about 50,000 km²), where elevation is the strongest determinant of bioclimatic gradients. In this region, from 1,700 to 4,200 m a.s.l, one can move from the subtropical via the temperate vegetation zone to intermittent tundra (more details see Du and Qi 2010; Qi et al. 2014a).

Fieldwork and study species

Fieldwork was conducted over 11 years (2001–2011). In every summer and autumn, seeds were sampled and collected at the start of natural dispersal. In every winter, seeds were air-dried to a constant mass at room temperature (approximately 15°). We weighed 100 seeds from pooled collections three times for each

sample of each species whenever possible, and then took the mean as seed mass. Besides, we evaluated (seed) dispersal mode (autochory, anemochory, ectozoochory, and endozoochory), life form (annual, herbaceous perennial, and woody perennial), and pollination system (anemophily and entomophily) for every species. Details about methods of trait measurement and categorization were described in Qi et al. (2014b).

Eleven years' work allowed us to gather a large database (approximately 1,500 species, 8,700 populations, and 13,500 samples). For 337 populations (belonging to 292 species) 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 seed mass variation is weak, and can be not considered in this study.

For this study, we selected a large and diverse subset of 320 species for which seed mass had been measured in no fewer than ten populations each (altogether 4,023 populations, see Appendix 1). We used this selection criterion because (1) the selected species had a large elevation range (average 1,100 vs. 600 m for other species), and (2) the other species sample size was considered too low for testing intra-specific relations reliably.

Statistical analyses

Prior to analyses, seed mass (mg) was log-transformed to optimize normality of frequency distributions. For a species, the mean value of seed mass and elevation across populations was used in inter-specific analyses. Summary statistics and graphics were conducted in SPSS v16.0 (SPSS Inc., Chicago, IL, USA) or R v3.0.2 (<http://www.R-project.org/>).

Inter-specific analyses

Relative to our recent inter-specific study (Qi et al. 2014b, 1,355 species), only a subset (320) species could be used for the present study. To remove the possible different results due to a biased sample, we performed similar inter-specific analyses as Qi et al. (2014b) did on the 320 species. Specifically, we used quantile regression to estimate the relationship

between elevation (x) and seed mass (y). Quantile regression extends classical least squares regression by estimating slopes through the median (or mean) as well as through each quantile (or percentile) of a bivariate relationship, and thus can identify the limits, boundaries, and shifting relationship within our bivariate distribution. We estimated the quantile regression coefficients for the 2.5th through the 97.5th quantiles.

To control for phylogeny, the seed mass–elevation correlation was also assessed by using phylogenetically independent contrasts (PICs), with elevation as independent variable and seed mass as dependent variable. As the independent variable was continuous, PICs calculated standardized independent contrasts across all nodes. These contrasts were then integrated to produce an ordinary least square linear regression through the origin and return a Pearson correlation coefficient (PIC r). The phylogenetic tree used for the PICs was based on APG III data (R20120829.new; available at <http://www.phylodiversity.net>). Branch lengths according to fossil data (Bell et al. 2010; Smith et al. 2010) were assigned using the BLADJ algorithm.

Intra-specific analyses

For every species, we first performed a simple linear regression between seed mass (y , log-mg) and elevation (x , km). The slope of the regression line was taken as an index ($RS_{SM/E}$ for short) representing the direction and magnitude of intra-specific changes in seed mass with elevation. Therefore, based on a two-tailed one-sample t test at $\alpha = 0.05$, if $RS_{SM/E}$ of 320 species is significantly higher or lower than zero, there is evidence for an overall intra-specific positive or negative seed mass–elevation relationship, respectively.

Then, we examined the effects of related traits, including species' seed mass average (SSMA), dispersal mode, life form, and pollination system, on $RS_{SM/E}$. The single effects were analyzed by simple line regressions for continuous variables (SSMA) or one-way ANOVA for categorical variables, while their integrative effects were examined by general linear model (GLM) with SSMA as covariate and other related traits as factors. Because traits of related taxa may be similar due to common ancestry and hence are not statistically independent, we repeated model selection using a phylogenetic generalized least-

squares model (PGLM) framework (Freckleton et al. 2002). This method uses the phylogenetic variance/covariance matrix estimated from the phylogeny to adjust for correlated error structure. The parameter lambda (λ) measures the degree of phylogenetic autocorrelation (from 0 implying no autocorrelation to 1 implying maximum autocorrelation). The phylogenetic tree for the PGLM was based on APG III data (R20120829.new), and branch lengths were assigned using the BLADJ algorithm.

Finally, we performed simple linear regression between $RS_{SM/E}$ and SSMA for each species subgroup with a given life form, dispersal mode, or pollination type to examine whether the relationship between $RS_{SM/E}$ and SSMA for different plant types (i.e. plant species with different life forms, dispersal modes, or pollination types) was similar.

Results

Across all species, there was an overall significant negative inter-specific relationship between seed mass and elevation, with geometric mean seed mass decreasing 1.8-fold along each 1,000 m elevation gradient [$R^2 = 0.022$, regression slope (α) = -0.256 (\log_{10} mg km $^{-1}$), $p = 0.009$; Fig. 1a]. The linear coefficient in quantile regression analyses steadily declined as the quantiles increased (Fig. 1a). Only the coefficients corresponding to the 3rd through the 24th quantiles were not significantly different from zero (based on the quadratic coefficient, result not shown). Thus, there was a shift from a slightly but significantly positive slope in the lower quantiles to a significantly negative slope in the upper quantiles. In contrast, there was no significant inter-specific seed mass–elevation relationship after controlling for phylogeny (based on PICs analysis; Fig. 1b).

Intra-specific changes in seed mass along elevational gradient covered a large range, where $RS_{SM/E}$ (\log_{10} mg km $^{-1}$) varied from -0.304 (*Impatiens noli-tangere*) to 0.451 (*Trisetum sibiricum*), indicating that the seeds of the high-elevation populations were from $0.497\times$ to $2.824\times$ heavier than the seeds of their conspecific lowland ones for each 1,000 m elevation interval. Across all species, intra-specific seed mass variation showed a significant but slight increase (mean $RS_{SM/E} = 0.022$; 95 % CI 0.010, 0.033; Fig. 2) with increasing elevation, i.e. seeds of high-elevation

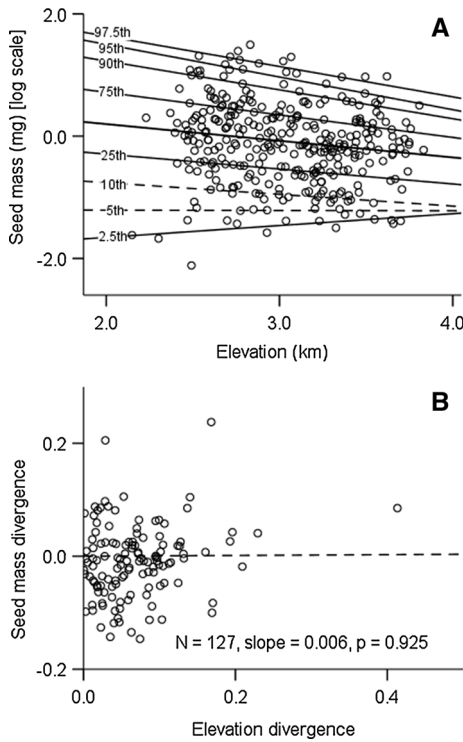


Fig. 1 The inter-specific relationships between seed mass and elevation estimated from quantile regression (a also showing the 2.5th, 5th, 10th, 25th, 75th, 90th, 95th, and 97.5th quantiles) and between seed mass divergence ($\log_{10} \text{mg Ma}^{-0.5}$) and elevation divergence ($\text{km Ma}^{-0.5}$) estimated from phylogenetically independent contrasts (PICs) analysis (b also showing the number of contrasts (N), the linear regression slope (slope), and the p values). Dashed line denotes a non-significant relationship (at $\alpha = 0.05$)

populations were on average 5.1 % (95 % CI 2.2, 7.9 %) larger than seeds of their conspecific lowland ones for each 1,000 m elevation interval.

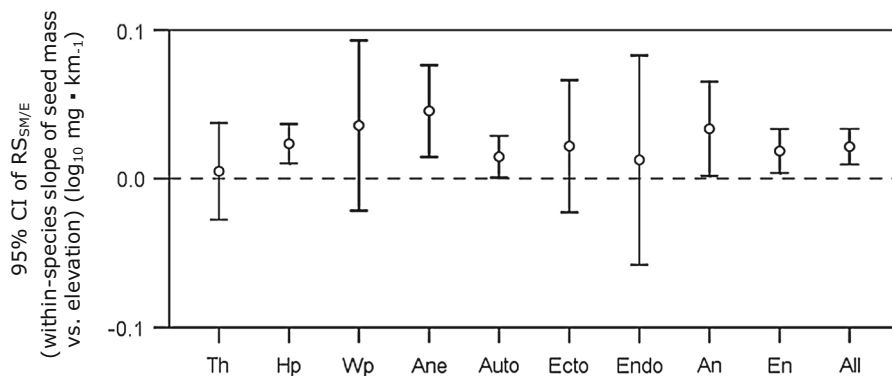


Fig. 2 Within-species regression slope of log-scale seed mass on elevation ($RS_{SM/E}$) by species' life form (*Th* annual, *Hp* herbaceous perennial, *Wp* woody perennial), dispersal mode

We did not find significant relationship between $RS_{SM/E}$ and life forms (Fig. 2; $p = 0.460$), dispersal modes (Fig. 2; $p = 0.252$), or pollination systems (Fig. 2; $p = 0.814$). The relationship between $RS_{SM/E}$ and SSMA, however, was significantly negative (Table 1; Fig. 3; $R^2 = 0.038$, $p = 0.001$), and its regression line intersected the line of $RS_{SM/E} = 0$ at $SSMA = 0.56$ (3.6 mg). When all related traits were considered, the GLM approach was congruent with simple line regressions or one-way ANOVA, where SSMA was the only significant variable in predicting intra-specific seed mass variation along elevation gradients (Table 2). In PGLM, lambda estimates were not different from zero (mean = 0.004; 95 % CI 0.000, 0.077) and SSMA was also the only significant predicted variable (Table 2).

The significance of the relationship between $RS_{SM/E}$ and SSMA differed among species' subgroups with different growth forms or dispersal modes (Table 1). For example, the annual, herbaceous perennial, and autochorous species subgroups presented similarly negative relationship as the full sample, while no such relationship was found in the woody, ectozoochorous, endozoochorous, and anemochorous species subgroups. In contrast, the relationship for anemophilous species and entomophilous species was both significantly negative (Table 1).

Discussion

Interpreting geographic variation in a trait may be difficult as a series of interact mechanisms (hypotheses) may operate synchronously. For example, if a

(*Ane* anemochory, *Auto* autochory, *Ecto* ectozoochory, *Endo* endozoochory), pollination system (*An* anemophily, *En* entomophily) and all species ("All" for short)

Table 1 Results of the line regression between within-species regression slope of log-scale seed mass on elevation ($RS_{SM/E}$) and species' seed mass average (SSMA) for all species and for

each species subgroup with single life form, dispersal mode, or pollination type. Regression slopes that are significantly different from zero (at $p < 0.05$) are in boldface

| Species (sub)groups | <i>n</i> | Intercept | Slope (α) | 95 %CI of α |
|---------------------------|----------|-----------|--------------------|--------------------|
| All species | 320 | 0.018 | -0.032 | -0.050; -0.014 |
| Life form | | | | |
| Annual (Th) | 48 | -0.015 | -0.056 | -0.100; -0.011 |
| Herbaceous perennial (Hp) | 251 | 0.020 | -0.038 | -0.060; -0.017 |
| Woody perennial (Wp) | 21 | 0.035 | 0.004 | -0.064; 0.072 |
| Dispersal mode | | | | |
| Anemochory (Ane) | 70 | 0.027 | -0.044 | -0.098; 0.011 |
| Autochory (Auto) | 214 | 0.009 | -0.037 | -0.059; -0.015 |
| Ectozoochory (Ecto) | 21 | 0.029 | -0.013 | -0.117; 0.091 |
| Endozoochory (Endo) | 15 | 0.044 | -0.031 | -0.180; 0.118 |
| Pollination system | | | | |
| Anemophily (An) | 64 | 0.011 | -0.058 | -0.103; -0.013 |
| Entomophily (En) | 256 | 0.018 | -0.027 | -0.047; -0.007 |

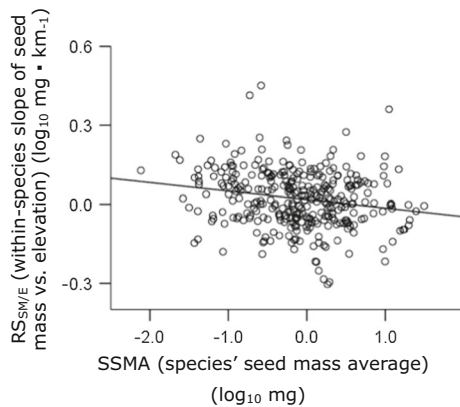


Fig. 3 The relationship between within-species regression slope of log-scale seed mass on elevation ($RS_{SM/E}$) and species' seed mass average (SSMA)

negative relationship between seed mass and elevation is observed, the 'energy constraints' hypothesis could be consistent with three different mechanisms by which shorter growing seasons or cooler temperatures at higher elevations may induce an decrease in seed mass, i.e. through (1) favoring smaller-seeded genotypes (natural selection), (2) excluding large-seeded taxa (species sorting), and (or) (3) decreasing maternal plant size (phenotypic plasticity or allometric constraints) may be expected (Baker 1972; Bu et al. 2007; Guo et al. 2010; Qi et al. 2014b). These mechanisms may not be distinguished from each other by examining only a single taxonomic level. In this case, the

combination of intra- and inter-specific analyses may allow a clearer understanding of elevational variation in seed mass.

Similar to our previous study with larger samples (Qi et al. 2014b), the inter-specific pattern in seed mass exhibited a slight but significant decrease with increasing elevation, suggesting that the 'energy constraints' hypotheses may play a major role in seed mass variation at among-species level. However, the negative seed mass–elevation relationship was no longer significant after controlling for phylogeny. The conflict between analyses with and without controlling for phylogeny implies that the effect of 'energy constraints' mechanism on inter-specific seed mass variation may only result from the species in our data having strong phylogenetic dependence (Freckleton 2009; Qi et al. 2014b) and uneven distribution of species numbers in different taxa (e.g. genera, families, or orders; i.e. taxa whose seed mass decreases with elevation generally have more species numbers than other taxa), or alternatively from a species sorting process, in which more lineages (e.g. species) of large-seeded taxa are allowed to be sorted into low-elevation habitats with higher energy levels (e.g. higher net primary productivity) and less environmental stress.

Opposite to the inter-specific pattern, the intra-specific pattern in seed mass showed a slight but significant increase with increasing elevation. As an overall reduction in plant size is the most conspicuous structural alteration for a species along elevation

Table 2 Results of the effects of species' seed mass average (SSMA), dispersal mode, pollination system, and life form on the within-species regression slope of log-scale seed mass onelevation ($RS_{SM/E}$) in general linear mode (GLM) and in phylogenetic general linear mode (PGLM)

| Sources | General linear mode | | Phylogenetic general linear mode | | |
|----------------------------|---------------------|-----------|----------------------------------|---------|-----------|
| | df | F | Estimate | SE | <i>t</i> |
| Life form | 2 | 1.257 | 0.02728 | 0.01497 | 1.822 |
| Dispersal mode | 3 | 1.044 | 0.00163 | 0.01009 | 0.162 |
| Pollination system | 1 | 0.173 | 0.00060 | 0.01516 | 0.040 |
| Species' seed mass average | 1 | 14.267*** | −0.03653 | 0.01065 | −3.429*** |

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

gradients (Körner 2003; Pluess et al. 2005), the positive intra-specific seed mass–elevation relationship should not be caused by phenotypic plasticity. Or, at least, the influence of phenotypic plasticity on intra-specific seed mass variation is weaker than that of the mechanisms causing an increase in seed mass with elevation. One of these mechanisms may be the 'stress-tolerance' hypothesis that larger-seeded genotypes evolve by natural selection at high-elevation zone because they exhibit superior survivorship during seedling establishment in stressful environments (Boullin et al. 2001; Pluess et al. 2005; Muller-Landau 2010; Qi et al. 2014b). The other mechanism may be that some proven intra-specific variations in life history strategies, such as increasing reproductive allocation or (and) decreasing seed number per plant individual with increasing elevation (Fabbro and Körner 2004; Guo et al. 2010), may exert a strong and positive effect on individual seed mass, resulting in an increase in it, even though maternal size may decrease with elevation. However, we cannot conclude which of the two reasons is responsible for intra-specific seed mass variation, or to what extent the two reasons contribute to the variation, because we have no data concerning plant size, reproductive allocation, and seed number per plant individual of all populations, and also no direct evidence that high-elevation populations have larger-seeded genotypes.

In accordance with our previous study with larger samples (Qi et al. 2014b), quantile regression analysis showed a triangular distribution for the inter-specific seed mass–elevation relationship, in which the slope of the relationship varied across quantiles, shifting from a slightly but significantly positive slope in the lower quantiles to a significantly negative slope in the upper quantiles. This suggests a mass-dependent inter-specific seed mass variation along elevation gradients:

large seeds (seed mass near the upper edges of the triangular distribution; e.g. 97.5th quantile) tended to decrease but small seeds (seed mass near the lower edges of the triangular distribution) tended to increase with increasing elevation. To our surprise, the mass-dependent seed mass variation was also found in intra-specific analysis, where within-species regression slope of seed mass on elevation ($RS_{SM/E}$) was significantly negative associated with SSMA, but independent of species' life form, dispersal mode, and pollination type. The similarity between intra- and inter-specific analyses demonstrates that the trade-off between energy-constraint and stress-tolerance mechanisms drives the elevational variation in seed mass at different taxonomic levels, and that the influence of energy-constraint (respectively stress-tolerance) mechanism on elevational distribution of seed mass increases with increasing (respectively decreasing) seed mass. To explicitly explain the pattern, we develop a hypothesis (details in Fig. 4) according to the evolutionarily stable (ESS) seed mass model (Geritz 1995). Our hypothesis is based on seed mass being restricted by resource or (and) availability of seed development time and environmental stress increasing significantly with elevation. Thus, the hypothesis might not be realistic in tropical and subtropical low mountains which presumably have neither significant resource- or (and) time-constraints on seed development, nor a significant elevational gradient in environmental stress.

In this study, caution should be taken to explain the difference in the significance of $RS_{SM/E}$ versus SSMA for species' subgroups with single life form or with single dispersal mode because the regression slopes of the relationship were statistically homogeneous (at $p = 0.05$) among them. This may be partly due to small sample sizes in some species subgroups (e.g.

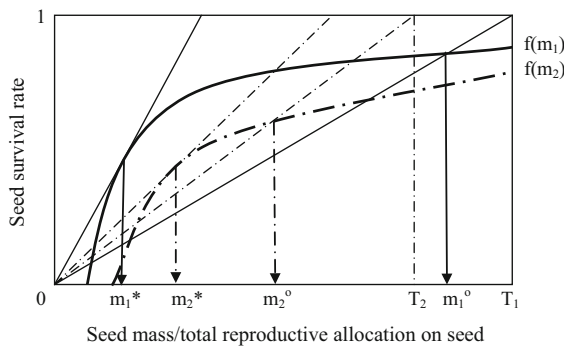


Fig. 4 Evolutionarily stable seed mass range model (ESS model, Geritz 1995) of conspecific populations (or congeneric species) in moderate low-elevation environment, $f(m_1)$ (real line), and in stressful high-elevation one, $f(m_2)$ (dashed line), while T_1 and T_2 represent their total reproductive allocation on seeds, respectively. The ESS model includes all individual seed masses between the Smith–Fretwell optimum (1974), m^* and maximum, m^0 , which is given the intersection of the graph of $f(m)$ with the main diagonal. Thus, $m_2^* > m_1^*$ is for environmental stress, but $m_2^0 < m_1^0$ is for energy constraint

anemochory and endozoochory), resulting in a large range (95 % CI) of regression slope for them. Besides, woody and zoochorous (ectozoochorous and endozoochorous) species generally develop some specific attributes or life history strategies, which may reduce the dependence of within-species seed mass variation with elevation on SSMA. Firstly, for woody species, because of their large biomass and the ability of early flowering (Sola and Ehrlén 2007; Bolmgren and Cowan 2008; Du and Qi 2010), there may be no or less increase in time or energy constraint on their seed development with increasing elevation even when their seeds are large. Then, the mass of zoochorous seeds (mainly large seeds) generally co-varies or co-evolves with the body size of seed dispersers (principally birds and mammals; Johnson et al. 1985; Leishman and Westoby 1994; Gros et al. 2006), but the within-species variation of the latter tends to increase from warmer areas (e.g. low elevation) to cooler areas (e.g. high elevation; Bergmann’s rule; Mayr 1963; Ashton 2001, 2002), which, thus, may exert another effect opposite to the influence of energy-constraints mechanism on within-species mass variation of zoochorous seeds with elevation.

As Geritz (1995) mentioned, evolutionarily stable seed mass of each species (or higher taxon) should be distributed between the Smith and Fretwell (1974) optimum, m^* , and the maximum, m^0 . m^* is always a

single value that maximizes fitness (survivable seed number) in the absence of competition, and thus coincides with the point of contact where a straight line through the origin touches the graph of $f(m)$ (Fig. 4), the expected survival rate per seed of mass m . m^0 is the intersection of the graph of $f(m)$ with the main diagonal [straight line through the origin and $(T, 1)$; T , total reproductive allocation to seed] (Fig. 4), representing over it individuals that would not produce enough survivable seeds (<1) to replace themselves. In the r/K -strategist model, r -strategists always have better colonization ability and maximum reproductive yield, while k -strategist always have better seedling survival and maximum competitive ability. Therefore, in the case of limited resources and asymmetric competition in seed mass, seeds of r -strategists are generally small and close to m^* , while those of k -strategists are generally larger and close to m^0 .

Also, as shown in Fig. 4, $f(m_1)$ and $f(m_2)$ represent the graphs of expected survival rate per seed mass of conspecific populations (or congeneric species) in moderate low-elevation environments and in stressful high-elevation ones (suggested by the Smith–Fretwell model), respectively. At one extreme, the Smith–Fretwell optimum (absolute r -strategist’s) seed mass in high-elevation environments, m_2^* , is larger than that in low-elevation environments, m_1^* , owing to higher seedling mortality, resulting in stress-tolerance adaptation. At the other extreme, the maximum (absolute k -strategist’s) seed mass in high-elevation environments, m_2^0 , is smaller than that in low-elevation environments, m_1^0 , because fewer resources are available for seed development ($T_2 < T_1$), conforming to the energy-constraints mechanism. Between the two extremes, individual seed mass responses to elevation gradients can be roughly predicted from the relative positioning of various species along an r - K continuum, which allows for various points of balance between the two opposite mechanisms.

However, for most species, even k -strategist’s species (e.g. high herbaceous and woody species), whose actual individual seed mass is far less than m^0 because, compared with total reproductive biomass, energy cost for individual seed mass is extremely small. Thus, the maximum individual seed mass for a population or taxon should be limited by the time available for resource allocation to seed/fruit

development (Moles and Westoby 2003; Murray et al. 2004), which is tightly associated with growing season length, and thus, elevation.

Conclusion

Based on the comparison of inter- and intra-specific analyses, this study provides theoretical and empirical underpinning of some likely mechanisms responsible for the seed mass–elevation relationship in a north-eastern Tibetan flora. The striking difference between the two analyses is that, overall, the relationship is significantly positive in intra-specific analysis but negative in inter-specific analysis, suggesting regional seed mass distribution with elevation is affected mainly by energy-constraint mechanisms at among-species level, but by stress-tolerance mechanisms or the variation in plant reproductive strategies at within-species level. The striking similarity, however, is that both intra- and inter-specific analyses support a mass-dependent seed mass variation with elevation, suggesting that elevational variation in seed mass is the outcome of the trade-off between energy-constraint and stress-tolerance mechanisms operating simultaneously at different taxonomic levels. Because of the complex results, our study, thus, calls for more comparative analyses at different taxonomic levels in detecting geographic variation in a trait to help prevent the often incomplete interpretation caused by the analyses at a single taxonomic level.

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