Mineral nutrient stoichiometric variability in *Hedera helix* (Araliaceae) seeds

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### INTRODUCTION

Allocation of resources to seeds is a key phase in a plant’s life history due to its influence on the likelihood of seedling emergence, establishment and survival, and its subsequent effects on maternal fitness. The dependence of offspring fitness on seed size has been extensively supported by many studies documenting the fact that larger seeds confer benefits to seedlings against nutrient limitation, drought, shading, herbivory and competition [e.g. Bond et al. (1999), Jakobsson and Eriksson (2000) and Leishman et al. (2000) for across species comparisons; Seiwa (2000), Seiwa et al. (2002) and Gómez (2004) for within species comparisons].

Probably as a result of this ecological importance, seed size variability is well-documented. Within an individual species, most variability occurs among the seeds produced by an individual plant (Michaels et al., 1988; Obeso and Herrera, 1994), suggesting that seed size is a character which shows certain phenotypic plasticity. In fact, an increase in the nutrient concentration of the growing environment often leads to the production of heavier seeds that contain higher levels of mineral nutrients (reviewed in Fenner, 1992). The main sources of within-plant variation in seed size are paternal genetic effects (Sundaresan, 2005), timing of flowering and fecundation, position effects (within plant and within fruit; Diggle, 2005), brood size (Obeso, 1993) and sibling rivalry (Obeso, 2004).

However, despite the considerable information available about seed-mass variation, and the fact that most studies consider seed mass to be an integrated measure of allocation, little is known about the allocation of different resources. Furthermore, the importance of seed mineral content on seedling performance has mainly been documented for cultivated species (Stoddard, 1999; Bramble et al., 2002; Calderini and Ortíz-Monasterio, 2003). Although positive effects of size on fitness are difficult to separate from those of mineral nutrient concentration due to the obvious correlation between both variables, some studies have been able to clarify certain effects. It is known, for example, that phosphorus (P) concentration affects seedling growth rate in *Rorippa nasturtium aquaticum* in P-deficient growth conditions, independent of seed size (Austin, 1966). Similarly, seeds of *Trifolium subterraneum* with higher P concentrations but of the same average size have been found to produce plants of greater biomass (Bolland and Paynter, 1990). In *Medicago polymorpha*, the P concentration of seed was significantly associated with final adult size and seed production, while seed size was only associated with greater biomass (Bolland and Paynter, 1990).

Despite that allometric growth may explain a great amount of the variability in allocation patterns at the plant level (Weiner, 2004), there is relatively little information on stoichiometry at seed level or the consequences of the allometric trajectory on the distribution of resources within seed. In this respect, Krannitz (1997) reported that larger seeds of the shrub *Purshia tridentata* not only had greater absolute quantities of nitrogen (N) and magnesium but also a higher concentration of N, suggesting a clear advantage for heavier seeds. On the contrary, in wheat grains, N concentration was constant...
across the entire spike despite position effects on grain mass (Stoddard, 1999).

Due to the well-known trade-off between size and number (Smith and Fretwell, 1974), the number of seeds per fruit (brood size) is an important source of variability in seed mass. Then brood size might potentially explain deviations in allocation from those predicted by the allometric relationships. Furthermore, differences in seed provisioning due to within-fruit position effects, embryo genotype and sibling rivalry can also contribute to within-fruit variability in seed mass (Uma Shaanker et al., 1988; Domínguez, 1995; Obeso, 2004). In spite of its importance, there is a lack of information about mineral nutrient allocation in relation to the number of seeds per fruit and sibling rivalry within fruit. Several theoretical models predict the distribution of resources among siblings in terms of seed mass (e.g. Parker et al., 1989; Mock and Parker, 1997, 1998) and these predictions were empirically examined for seed mass (Obeso, 2004), but to my knowledge there is no information about distribution of other important compounds such as P, which is essential to plants in regard to energy transfer, activation of proteins and regulation of metabolic processes (Agren, 2004). Furthermore, seed P concentration influences seedling growth rate (Austin, 1966; Parrish and Bazzaz, 1985; DeMarco, 1990); however, the information about factors controlling P concentration on individual seeds is amazingly scarce.

The objective of this study was to examine these relationships using field-collected seeds of Hedera helix. The first objective was to describe the patterns of carbon (C), N, sulfur (S) and P allocation in relation to seed mass and to estimate the percentage of variance in mineral nutrient content that is accounted for by the allometric relationship. When an important proportion of the variance is not explained by the allometric function, like it was in the case of P, the second objective was to determine to what extent the variance in P allocation, not explained by seed mass, can be explained by brood size. Specifically it can be predicted that as a result of resource limitation, siblings from larger broods would face more intense competition and thus contain relatively less P than those from smaller broods.

MATERIALS AND METHODS

Plant material and chemical analyses

Ivy, Hedera helix, is a woody climber native to Europe, western Asia and North Africa that is also very invasive as an exotic in North America (Thomas, 1998). It produces hermaphrodite flowers on globose umbels. The ovary is 5-lobed and contains five ovules (Tutin et al., 1968). The fruit is a black berry containing one to four seeds (five-seeded fruits are extremely rare; 5 in 700 fruits). The seeds are radially oriented in relation to the peduncle; therefore there are no position effects in relation to their access to maternal resources. This species is not pollen limited (Jacobs et al., 2009). The seeds are dispersed by frugivorous birds and martens that fed on fruits during winter. As the droppings contain several seeds, clusters of seedlings are frequent and seedling recruitment frequently forms a dense ground cover in woodland habitats (Metcalf, 2005).

Samples of >100 fruits were collected from each of 14 individual ivy plants in a natural population living on hedgerow trees located in the Puerto San Lorenzo valley, Teverga, Asturias (northern Spain). Fruits were picked from as many infrutescences and plant positions as possible to reduce possible bias derived from position effects. Fruit samples were oven-dried at 60 °C for a minimum of 7 d. The dry pulp was manually removed and each individual seed was weighed to the nearest 0-1 mg and the seeds within the same fruit were ranked by seed mass. Sib ranks were assigned within fruit from the heaviest seed (A-sib) to the lightest one (D-sib in the case of four-seeded fruits). B-sib is the second in the hierarchy of masses and C-sib the third.

A subsample of 56 seeds, which included four seeds from each of the 14 plants, was separated for chemical analyses. The sample included seeds of different sizes in order to examine the allometric relationships, and the seeds of all plants were combined. Each of these seeds was weighed individually and analysed for C, N and S content by combustion using a standard automated CNHS procedure (PE 2400 Series II, CNHS/O).

Taking into account that P cannot be analysed in individual seeds at the same time as CNHS analyses, a different subsample of 201 seeds, was used for P analyses. This subsample consisted of seeds from 81 fruits (six fruits per plant in 11 plants and five fruits per plant in three plants): 16 one-seeded, 24 two-seeded, 27 three-seeded and 14 four-seeded fruits. The sample included seeds from all different brood sizes (one-, two-, three- and four-seeded fruits) from each of the 14 plants and 12–16 seeds per plant. P content (total content of P per seed) was determined by spectrophotometer after acid digestion of ashed samples (Niklas and Cobb, 2005).

Statistical analyses

Type II models [reduced major axis (RMA), the slopes denoted as $\alpha_{\text{RMA}}$] were used to determine the scaling exponents of C, N, P and S versus seed mass and N versus C since functional (rather than predictive) relationships were sought between variables that are biologically interdependent and subject to measurement error (Niklas, 1994). As standard error for $\alpha_{\text{RMA}}$ the same standard error as the ordinary least squares estimate was used (Quinn and Keough, 2002). To determine whether the slopes differed significantly from 1-0 (isometry), t-tests were used. The coefficients of regression ($r^2$) were used as a measure of the proportion of the total variation in Y that is explained by its linear relationship with X. In the case of P, to consider the hierarchical sampling design, the identity of the mother plant was also included as a random factor using linear mixed-effect models. The random slope and random intercept model showed with the lowest AIC (Akaike Information Criterion) estimated the same slope (0.818 ± 0.062) as the linear model (0.819 ± 0.064; Table 1). One-way ANOVAs were performed to examine the effect of brood size and seed rank on seed mass, P content and P concentration. When F-tests were significant, Tukey tests were performed to separate different means. ANCOVA using the seed mass as a covariate was performed to test
differences among plants in allocation to P. The statistical analyses were performed with R (R Development Core Team, 2009).

RESULTS

C and N allocation varied isometrically with seed mass (Table 1 and Fig. 1) and this variable explained 98.8% and 83.9% of the total variation in C and N content of seeds, respectively. In addition, N allocation varied isometrically with C content and this relationship explained 81.0% of the total variance (Table 1).

However, P and S varied allometrically with seed mass, with the power of the relationship being slightly greater than 1.0 in the case of P and lower than 1.0 in the case of S content (Table 1 and Fig. 1) and seed mass only explained 37.5% and 37.3% of the total variance in P and S allocation, respectively.

Brood size had a significant effect on mean seed mass per fruit (F<sub>3,197</sub> = 4.404, P = 0.005), and mean P content per seed (F<sub>3,197</sub> = 10.547, P < 0.001). Seeds from four-seeded fruits showed a significantly lower mean seed mass and P content than seeds from smaller brood sizes (Fig. 2). P concentration (mg P mg<sup>-1</sup> dry matter) varied significantly with brood size (F<sub>3,197</sub> = 5.276, P = 0.002) and showed significantly lower values for seeds from four-seeded fruits (Fig. 2).

When seed mass was included as a covariate, neither individual plants (F<sub>13,186</sub> = 1.161, P = 0.077) nor differences between plant slopes (interaction plant x seed mass; F<sub>13,186</sub> = 0.946, P = 0.504; for four-seeded fruits F<sub>2,81</sub> = 0.507) accounted significantly for variability in P allocation. After removing the non-significant interaction, differences among mother plants became significant (F<sub>13,186</sub> = 12.718, P < 0.001) and explained 28% of total variance in P allocation.

When examining, for all fruits combined, different brood sizes separately, P concentration of seeds (mg P mg<sup>-1</sup> dry matter) did not vary with sib rank within fruit (for two-seeded F<sub>1,48</sub> = 0.454, P = 0.504; for three-seeded F<sub>2,81</sub> = 0.544, P = 0.582; for four-seeded fruits F<sub>3,48</sub> = 2.422, P = 0.077). However, A-sib P concentration varied with brood size (F<sub>3,77</sub> = 5.479, P = 0.0018), significantly decreasing within four-seeded fruits (Fig. 3). Following a similar pattern, the P concentration of B-sibs also decreased within four-seeded fruits (F<sub>2,63</sub> = 3.851, P = 0.026; Fig. 3) but, C-sib P concentration did not vary with brood size (three- versus four-seeded fruits, F<sub>1,39</sub> = 3.179, P = 0.082; Fig. 3).

| Table 1. Summary of reduced major axis (RMA) regression analyses of log<sub>10</sub>-transformed data |
|---------------------------------------------------|-------------------------------|------------------|------------------|-----------------|------------------|
| Log P vs log X | r | α<sub>RMA</sub> ± s.e. | t-Test | Significance |
| C vs seed mass | 0.994 | 0.993 ± 0.014 | 0.500 | 0.309 |
| N vs seed mass | 0.916 | 1.020 ± 0.056 | 0.357 | 0.361 |
| S vs seed mass | 0.613 | 0.819 ± 0.088 | 0.021 | 0.198 |
| P vs seed mass | 0.611 | 1.191 ± 0.061 | 3.131 | 0.001 |
| N vs C | 0.900 | 1.027 ± 0.061 | 0.442 | 0.330 |

<i>t</i>-Tests examine whether α<sub>RMA</sub> significantly differs from 1 (isometry). d.f. = 54, except for P versus dry mass where d.f. = 199.

DISCUSSION

This study documents that variability in allocation of different essential elements (N, C) to seeds may be almost exclusively attributed to allometric growth and that deviations from the allometric trajectory, like the case of P, can be explained by brood size.

Seed mass is extensively used as an estimate of offspring fitness and is usually used as an integrated measure of allocation under the assumption that it represents an accurate estimate of the allocation of different essential elements. However, present results showed that there are substantial differences in the patterns of scaling of different mineral nutrients in <i>Hedera helix</i> seeds. Whereas the allocation of C and N scales isometrically with increasing seed mass, the allocation
of P and S scales allometrically. Whereas most of the variation in allocation of C and N may be explained by variation in seed mass, an important portion of the variation in allocation of S and P still remains unexplained after removing this effect. N and C follow an allometric trajectory that allows us to predict allocation of these elements to seeds after considering any factor that affects seed mass. However, we need an alternative explanation for variability in P allocation. The P concentration of seeds from large broods was reduced and the sibs occupying the highest ranks in the hierarchy of masses (sibs A and B; Fig. 3) were found to obtain an additional advantage in small broods in terms of P concentration.

The balance of N and P in plant tissues plays a pivotal role in plant biology. N allocation reflects investment in proteins, which are particularly rich in N. It is P allocation, however, that represents the capacity to produce proteins. The amount of P should correlate with the amount of ribosomes (a metabolically important sink of P), which represent the machinery to produce proteins and is thought to be related to plant growth rate (Kerkhoff et al., 2006; Niklas, 2006). Specifically, growth rates should correlate positively with increasing ribosomal RNA (and P) investments relative to protein (and N) investments (Niklas, 2006). The positive effect of seed P concentration on seedling and plant growth and even on plant fecundity has in fact been documented in several species (e.g. Austin, 1966; Bolland and Paynter, 1990). Furthermore, P content was found to be more important than seed mass in determining final plant mass of *Trifolium subterraneum* (Bolland and Paynter, 1990) although, when comparing plants of the same species growing at different rates, Matzek and Vitousek (2009) found that faster-growing plants did not have consistently lower N : P ratios.

Given that N content varied isometrically whilst P content varied allometrically with seed mass it can be concluded that a significant proportion of the variance in P allocation is not explained by N allocation. It therefore follows that there may be variability in P content for a fixed amount of N

![Fig. 2. Mean seed mass, mean P content and mean P concentration for seeds from different brood sizes (one- to four-seeded fruits). Bars indicate ± s.e. and significantly different means (P < 0.05, Tukey test) are indicated by different letters.](image)

![Fig. 3. Mean seed P concentration (mg mg\(^{-1}\) dry matter) for sibs of the same rank in fruits of different brood sizes (one- to four-seeded fruits). A-sib (the heaviest seed within fruit) in one- to four-seeded fruits, B-sib (the second seed in the hierarchy of masses within fruit) in two- to four seeded fruits and C-sib (the third seed in the hierarchy of masses within fruit) in three- and four-seeded fruits. Bars indicate ± s.e. and significantly different means (P < 0.05, Tukey test) are indicated by different letters.](image)
which might be indicative of differences between seeds in the potential growth rate of their future seedlings. In the case of *H. helix*, the potential growth rate of seedlings seems to favour larger seeds from small broods, which exhibit higher P: N ratios and probably faster growth.

Following across-species comparisons, it has been established that total leaf P content scales as the 4/3 power of the total plant mass for non-woody plants. Similarly, plant growth rate should scale across all species as the three-quarters power of total leaf P per plant (Niklas, 2006). If we assume that across species allometrical relationship can be translated to within-species comparisons (for *Eranthis hyemalis*, see Niklas and Cobb, 2006)) and from the whole plant to different plant tissues (Vrede et al., 2004; Niklas, 2006), P content within seed tissues of *H. helix* should scale as the 4/3 power of seed mass. However, results presented here show that the exponent was significantly >1 and <4/3 in accordance with the different stoichiometric scaling reported for reproductive tissues (Kerkhoff et al., 2006) and the suggestions of Ågren (2004).

S content has been related to the synthesis of proteins containing sulfur amino acids. The allometric allocation of this element to *H. helix* seeds did not follow the same pattern as P allocation, and a slight proportional advantage seems to be conferred on smaller seeds in terms of S content. The complex interaction between S and N content makes it difficult to interpret the patterns of allocation to S (Zhao et al., 1997; Scherer, 2001).

After removing the effect of seed mass and mother plant identity, 34 % of variance in P allocation remains unexplained. Hence, some of the variability in P allocation that is independent of seed mass or mother plant must be explained at the fruit level. Patterns of seed mass, and consequently, N and C allocation can be interpreted as a physiological constraint leading to sibling competition. Seed P content increases as the brood size increases from one to four, hence larger fruits are stronger sinks of P, but this P has to be shared between more seeds. Furthermore, resources are not equally distributed within broods (for instance, in four-seeded fruits D-sib achieve on average 67 % of A-sib weight) which can be interpreted as the consequence of competition among developing sibs. As a result of the physiological constraint and subsequent competition, smaller broods have proportionally more nutrients per sib and the optimum for an individual seed is a fruit containing two seeds.

The fact that A-sibs increased their P concentration in small broods might open the ground for fierce sibling rivalry, which may result in brood reduction, which would result in an advantage for A- and B-sibs from small broods in terms of P concentration and probably in terms of seedling growth rate (Parish and Bazzaz, 1985; DeMarco, 1990). This phenomenon probably entails two ecological implications. First, seed development is a stage of the life cycle with great mortality for many angiosperms and only a small proportion of the embryos develop into seeds (57-5 % of the ovules developed into seeds in the mature fruits of Ivy). Thus natural selection operating very early in the sporophytic phase might be driven, at least partially, by competition for P. Secondly, brood reduction increases the P:N ratio of the surviving seeds which may additionally enhance the growth rate and competitiveness of the seedlings.

In summary, although in this species seed mass can be used as a surrogate of investment in C and N, it is not an adequate estimate of investment in P and S. P allocation to seeds is not only determined by the allometric trajectory but also the consequence of competition for this nutrient among developing seeds as confirmed by the fact that larger seeds increased their P concentration when found in smaller broods.

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**LITERATURE CITED**


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