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Short research contribution

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EFFECT OF GENETIC RELATEDNESS ON THE ALLOMETRIC RELATIONSHIP BETWEEN BIOMASS INVESTMENT AND SEXUAL REPRODUCTION IN CLONAL PLANT

ABSTRACT: In order to analyze changes in biomass allocation patterns across genetically structured populations where plants are competing for access to light, we performed glasshouse experiment with controlled genetic identity of competitors. Clonal replicates of 17 *Lamium maculatum* (L.) genotypes were grown in three treatments: control (low competition for light availability), intraclonal and interclonal competition. It was shown that competition between genetically unrelated individuals (interclonal treatment) was the most stressful environment for Spotted Dead Nettle. Results of allometry analyses, based on standardized major axis (SMA) mathematical procedure, have shown the smallest differences in relative investment to sexual reproduction between larger and smaller individuals when competing with unrelated plants. Our results indicate that allometric relationships between vegetative and reproductive traits could be strongly affected by genetic relatedness of competing plants.

KEY WORDS: allometry, allocation, genetic relatedness

Allometry is the quantitative relationship between growth and biomass allocation (Weiner 2004). According to Stearns (1992), “allocation decisions between two or more processes that compete directly with

one another for limited resources within a single individual” imply mutually exclusive allocation, or physiological trade-off. Although allometric relationships between measures of plant size have often been treated as genetically fixed characteristics of species, or the products of biomechanical or other physical constraints (Givnish 1986), it was well documented that genetic correlations between traits could be greatly altered across environments (Via and Lande 1985, Cheplick 1995, Tucić and Stojković 2001). In other words, allometry can be plastic and trade-offs may vary with resource levels (Cheplick 1995, Weiner 2004). For plants, it is generally assumed that competitive interactions can modify the biomass proportion invested to sexual reproduction relative to vegetative growth (Prati and Schmid 2000, Van Kleunen *et al.* 2002, Bonser and Aarssen 2003). Ecological dynamics of plant population in a competitive context could depend on these changes in reproductive function, and may be greatly influenced by local demographic structure because: 1) plants can generate patches of closely related individuals due to restricted dispersal of pollen, seeds and vegetative clones (Born *et al.* 2008) and 2) the intensity of competition

for resources depends on genetic relatedness between competing individuals (Cheplick and Kane 2004).

In this paper we report results of an experiment designed to investigate patterns of biomass allocation to vegetative and reproductive plant modules in a perennial clonal species *Lamium maculatum* (L.) within experimental patches (treatments) composed of neighbors which genetic identity was controlled. In a glasshouse experiment, clonal replicates of 17 genotypes were planted into plastic pots (one plant per pot, 10 cm diameter and 400 cm³) filled with 1:1 sand/substrate mixture (Unitas Corporation, Čačak) and grown under three regimes: 1) control (C) – low level of competition, randomly distributed pots spaced approximately one pot diameter apart; 2) intraclonal (sibling) competition (S) – pots with clones of the same genotype in close proximity with no space between them; 3) interclonal (mixed) competition (M) – randomly distributed pots with plants of different genotypes with no space between them. The only limiting resource in competitive interactions was light. Six replicates of each genotype/treatment combination were placed in one of two blocks in glasshouse of Institute for Biological Research, Belgrade. After 30 weeks of growth, different plant modules (organs) were separated, dried in oven (60°C) and measured on Chyo JL-300 high precision weighing balance to the nearest 1 mg. Following traits were obtained from these measures: weight of flowers (FW), stem weight (SW), weight of leaves (LW), root weight (RW) and total dry weight (TWT). Flower weight was used as the approximation of plant biomass investment to sexual reproduction.

Prior to allometry analysis, measured traits were analyzed by mixed model ANCOVA with initial plant height (prior to experimental setup) as a covariate, competitive treatment (fixed), genotype and block (random factors) as main effects and genotype-treatment interaction, followed by Scheffe's test for multiple comparisons of treatments (SAS Institute 2003). Significant genetic variability was revealed for all traits (genotype effect, $P < 0.001$) and, with exception of SW, plastic responses to proximity of neighbors were detected as significant in ANCOVA

(treatment effect, $P < 0.001$). Since foliage shading reduces the quantity of light available for growth and photosynthesis (Schmitt and Wulff 1993), it was expected to find lower mean weights of plants in competition compared to control individuals. Additionally, comparisons of traits' mean values between treatments revealed that these plastic changes, except for FW and SW, were sensitive to genetic identity of competitors (FW: C>S, C>M; SW: C>M; LW: C>S>M; RW: C>S>M; TWT: C>S>M; Scheffe's test for $P < 0.05$). The lowest measures of vegetative modules in M treatment imply that the proximity of unrelated individuals was the most stressful environment for *L. maculatum* when the light was limiting factor. The only significant genotype-treatment interaction has been detected for FW ($P < 0.001$) which suggests that differences in plastic response to identity of competitors for final flower weight could be found mostly at the genotype level. Analysis of covariance reveals the overall effect of treatments on plant traits, but it tells us little about the relationship between traits, *i.e.* plant strategy of relative resource allocation within each environment, or if there are differences among treatments in growth allometry patterns.

Standardized major axis estimation (SMA, type II regression model) was employed to test for allometric functions between measured weights of all module types, using (S)MATR statistical software (Falster *et al.* 2003). SMA methodology is appropriate where there is error in both the x and y variables of the regression models (Sokal and Rohlf 1995) and when we are not interested in prediction but to estimate the line-of-best-fit relating two variables, which is the basic purpose of allometry estimates (Warton *et al.* 2006). A significant allometric relationship is indicated where the slope (b) of the relationship between logarithms of two variables (module measures) differs from isometry (Niklas 1994). An isometric relationship between modules ($b = 1$) indicates that relative biomass allocation is constant for all individual sizes. Using (S)MATR we have tested: 1) the hypothesis of growth isometry, 2) whether treatments differ in slopes of functional relationship between variables, and 3) whether groups are verti-

Table 1. Standardized major axis tests of the allometric relationship (log scaled variables) for reproductive effort (FW) and relative biomass investments to stems (SW), leaves (LW) and roots (RW) based on weight measures of *L. maculatum* plants grown in three experimental treatments (control - C, sibling - S and mixed - M competition). Scaling slope b , R^2 and P values for correlations within treatments are reported. Results of pairwise slope comparisons between treatments (based on 1000 iteration in permutation testing) are presented as letters in superscript. If differences among slopes are insignificant, pairwise tests of shift in elevation were performed and results are presented as letters in subscript [(e)-elevation]. Identical letters indicate insignificant difference of either slopes or elevation between treatments. Letter A point to the largest values. F statistics and P values of the test of differences between observed slope within treatments and $b = 1$ are reported as statistics of isometry testing.

Allometric relationship	Treatment	R^2	P	b	Test of isometry ($H_0: b = 1$)	
					F	P
logFW: log(SW+LW+RW)	C	0.00	0.720	2.52 ^A	105.36	0.000
	S	0.10	0.003	1.99 ^A	52.39	0.000
	M	0.24	0.000	1.59 ^B	22.77	0.000
logSW: log(FW+LW+RW)	C	0.80	0.000	1.18 ^A	14.02	0.000
	S	0.89	0.000	1.07 ^A	3.37	0.070
	M	0.89	0.000	0.99 ^B	0.04	0.841
logLW: log(FW+SW+RW)	C	0.64	0.000	0.82 _{A(e)}	10.92	0.001
	S	0.79	0.000	0.80 _{B(e)}	22.37	0.000
	M	0.83	0.000	0.78 _{C(e)}	33.75	0.000
logRW: log(FW+SW+LW)	C	0.68	0.000	1.41 _{B(e)}	37.39	0.000
	S	0.75	0.000	1.54 _{A(e)}	73.31	0.000
	M	0.86	0.000	1.53 _{A(e)}	119.84	0.000

cally separated (shift in elevation) if slopes are not different (Table 1). Within each treatment, allocation pattern between reproductive effort and vegetative growth was estimated as the relationship between FW and the sum of other measures. Similarly, to test allometry of biomass allocation to each type of vegetative organs we explored the relationships between one weight measure and the sum of three other weights. We have not used total plant weight as a variable in this model, since it could result in spurious correlations that may obscure the particular relationship (Bonser and Aarssen 2001, 2003). In general, relative biomass allocation to reproductive effort was greater for larger plants in all environments ($b > 1$, Table 1, Fig. 1). However, correlation between flower weight and vegetative weight was insignificant in control treatment ($P = 0.720$, Table 1), contrary to both S and M competitive environments. These results suggest that plant reproductive fitness in resource-rich environments, where

absolute fecundity is the highest, could be less determined by size than in competition. In close proximity of other plants it is expected for larger individuals to achieve higher reproductive outcome (Bonser and Aarssen 2003), since competitive interactions tend to be asymmetric, especially in competition for light (Thomas and Weiner 1990). Nevertheless, allocation patterns differed between S and M treatments. Significantly slighter slope in mixed genotype compared to sibling competition ($b = 1.59$, $P < 0.000$ for M; $b = 1.99$, $P < 0.003$ for S; Table 1, Fig. 1) indicates decreased difference in reproductive success between variable size individuals in interclonal interactions. In other words, when compete with unrelated plants small individuals tend to allocate larger proportion of acquired resources to reproductive organs compared to small plants in less intense competition (S treatment). The reverse is true for larger plants suggesting that successful individuals in severe asymmetric interaction (M

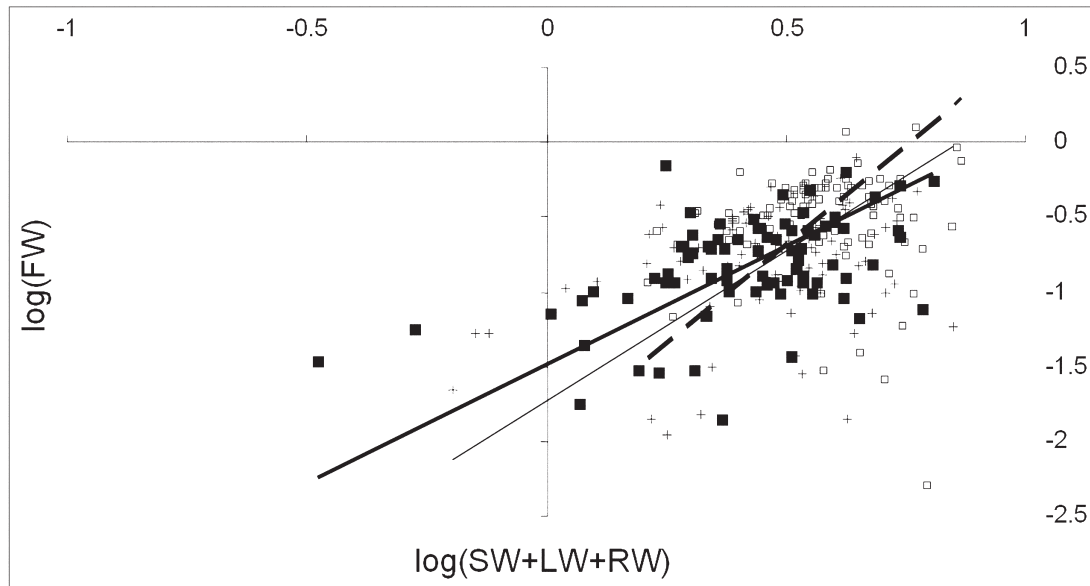


Fig. 1. Bivariate line fitting - the allometric relationship (log scaled variables) between reproductive effort (flower weight - FW) and allocation to vegetative modules for *L. maculatum* plants grown in three experimental treatments (control, sibling and mixed competition). The SMA allometric function plotted through the individuals in control treatment (dashed line), sibling competition (thin line) and mixed genotypes competition (thick line). Symbols for individuals in control (\square), sibling ($+$) and mixed (\blacksquare) competition.

treatment) can balance more productively between vegetative and reproductive fitness components than smaller plants.

Plants from mixed and same genotype experimental patches showed isometric relationship between stem weight and biomass proportion investment to other organs ($b = 1.07$, $P < 0.070$ for S; $b = 0.99$, $P = 0.841$ for M), but the slope of this allometric relationship was significantly lower in M than in other two treatments (Table 1). This result indicates that smaller plants in M treatment invested higher proportion of biomass into stems than within other environments. Although allocation to leaves was disproportionately lower for large individuals in all environments ($b < 1$, Table 1), plants with equal measures of other organs invested the smallest fraction of biomass into leaves when grown with unrelated genotypes (elevation shift, Table 1). According to Bonser and Aarssen (2001, 2003), allocation to branches and stems increases the potential production of new plant meristems which could be committed to reproductive function or to new branches and leaves. Following this conclusion in discussion of our results, we hypothesize that specific allometry

strategy of smaller plants in intense competition with unrelated individuals, compared to other treatments, could be the "allocation decision" (Stearns 1992) in favor to reproductive effort, both directly *via* allocation to flowers and indirectly into stems. Such investment to flowers may be beneficial for smaller individuals because allocation of limited acquired biomass to extensive vegetative growth (*e.g.* leaves) may leave insufficient time for successful reproduction before the end of growing season (Bonser and Aarssen 2001). It was previously reported that plants from competitive environments invested relatively more in sexual reproduction (Prati and Schmid 2000, Van Kleunen *et al.* 2002), but this study is among the first to show that this allocation pattern could be sensitive to the relatedness between competitors. Since plants can achieve similar mean reproductive outcome (approximated as FW) in different strength of competition (*i.e.* identity of competitors, Scheffé's test for FW) by modifying biomass allocation, analyses of growth allometry could be very important for understanding structured population dynamics in a competitive context.

It is interesting to note that the biomass allocation to roots was greater in both competitive treatments than in control (elevation shift, Table 1). Although in this experiment plants were exposed only to competition for light, plastic investment to roots could reveal past selection shapes of reaction norm for this trait in natural environments (Sultan 1995, Ghalambor *et al.* 2007) where closely positioned plants compete for all resources (including underground) and changed *R:FR* ratio in foliage shade is the first signal of future competitive interactions (Smith and Whitelam 1997).

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