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Patterns made of patterns: variation and covariation of leaf nutrient concentrations within and between populations of *Prunus mahaleb*

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Summary

• Patterns of within and between population variation in concentrations of nutrients (N, P, K, Ca, Na, Mg, Cu, Fe, Mn, Zn) are reported in mature leaves of individual *Prunus mahaleb* (Rosaceae) trees from five south-eastern Spanish populations.

• MANOVA and canonical discriminant analysis were used to identify the contribution of individual nutrients in explaining differences between populations in average nutrient composition. Common principal components analysis was used to test whether the structure of covariation between leaf macronutrients was maintained at different populations.

• Populations differed in average nutrient composition and were separated mostly by variation along an axis defined by Ca on the positive side, and Mg on the negative one. The sign and magnitude of the correlations between individual nutrients varied between populations. Multivariate patterns of nutrient covariation differed between populations, the variation being unrelated to population differences in mean nutrient composition.

• Results suggest that, at the regional scale, phenotypic integration of the foliage concentrations of different nutrients was weak, and highlights the importance of studying nutrient covariation structures.

Key words: Common Principal Components, individual variation, leaf nutrients covariation, mineral composition of leaves, phenotypic integration, *Prunus mahaleb*.

Introduction

Patterns of covariation of sets of phenotypic traits are equally or more relevant for phenotypic evolution as patterns of univariate variation, that is, variation of characters considered individually, without reference to the variation of other characters (Futuyma, 1998; Schlichting & Pigliucci, 1998). Plant traits that share the genetic control of their expression, have similar functional roles or a common resource base, or show a correlated response to microenvironment variability, will tend to covary among different conditions (Schlichting, 1989a). However, differences in the plastic responses to environmental variation of different traits can also modify the correlations between them (Schlichting, 1989a,b). Levels and patterns of phenotypic integration may be assessed by growing genotypes in different experimental conditions (e.g. Schlichting, 1989a; Pigliucci *et al.*, 1999) or by comparing the natural covariation observed in different mature populations (e.g. Pigliucci *et al.*, 1991). In this paper we adopt the second approach to study patterns of within- and between-population variation in leaf nutrient concentrations of *Prunus mahaleb* (Rosaceae), a small deciduous tree. We examine whether multivariate patterns of nutrient covariation across individual trees remain consistent between populations.

Patterns of variation in nutrient concentration in plants are ecologically and evolutionarily relevant for three main reasons. Firstly, because correlations between concentrations of elements have been long known in interspecific and geographical contexts (e.g. Garten, 1976, 1978; Garten *et al.*, 1977; Golley & Richardson, 1977; Herrera, 1987; Lebreton *et al.*, 1997; Thompson *et al.*, 1997), we also expected to find distinct patterns of nutrient covariation across individual plants at

the within-population level. Secondly, because studies on the magnitude, patterns and consistency of phenotypic correlations between plants traits have mainly focused on metric and morphological traits (e.g. Schlichting, 1989a; Pigliucci *et al.*, 1991; Waitt & Levin, 1993; Armbruster *et al.*, 1999). Phenotypic integration has been claimed to be possible also for physiological characters (Schlichting, 1986), but this expectancy remains essentially untested. And thirdly, because variation in nutrient concentrations is often related to patterns of host and habitat selection by herbivores (e.g. Tabashnik, 1982; Bergström & Danell, 1986; Athey & Connor, 1989; McNaughton, 1990), and herbivore consumption (e.g. Slansky & Wheeler, 1992). This provides biological support for the hypothesis that patterns of covariation between elements may be relevant to plant performance and thus deserve explicit consideration.

To our knowledge, variation between populations in between-individual covariance structures of leaf nutrients has not been investigated so far for any species. This is partly due to the fact that few analyses of plant nutrients have simultaneously considered within- and between-population levels of variation (but see Ohlson, 1995; Bauer *et al.*, 1997), but also to some methodological difficulties. Until relatively recently, analytical methods available for comparing covariance structures allowed only for tests of the relatively simple null hypotheses of identity or proportionality between sets of covariance matrices (e.g. Riska, 1985; Herrera, 1990), which represent a rather restricted subset of biological possibilities. Recent techniques aimed at the simultaneous analysis of the multivariate structure of different groups of biological entities (e.g. Common Principal Components analysis; Phillips & Arnold, 1999), provide useful tools for the study of between-population variation in the structure of between-plant trait covariation. The interest of the question increases when the traits studied are potentially relevant for the interaction between plants and animals, such as flower morphology for pollinators, fruit morphology for seed dispersers, and leaf nutrients for herbivores, since these interactions usually affect plant fitness (see e.g. Armbruster *et al.*, 1999 and references therein, for the significance of phenotypic correlations structure in the context of plant–pollinator interactions).

Rather than presenting evidence that patterns of covariation between nutrients are consequential for the interaction between plants and herbivores, the aim of this paper is to provide a case study where the analysis of such patterns of variation is explicitly addressed. We will analyse within and between population variation in the concentration of five macronutrients (N, P, K, Ca, Mg) and five microelements (Na, Cu, Fe, Mn, Zn) in the full-sized, mature leaves of individual *Prunus mahaleb* (Rosaceae) trees from five south-eastern Spanish populations. Specific questions addressed are (1) Do *Prunus mahaleb* populations differ in the average concentration of the different nutrients in the leaves of individual trees? (2) Are there significant bivariate and multivariate patterns of between-element covariation across individual trees at the within-population level? (3) If these actually exist, do they remain consistent between populations?; and (4) Is there some principal components structure in leaf nutrient composition that is shared by all populations, or alternatively, are there one or more location-specific trends of multivariate variation?

Materials and Methods

We studied leaf nutrient concentration from a total of 116 *P. mahaleb* trees from five different populations located between 1300 and 1700 m elevation in the Sierra de Cazorla, a limestone mountain range in Jaén province, south-eastern Spain. Populations for study were selected because of their accessibility and availability of trees. The two nearest populations were 2 km apart, and the two most distant were 7 km away. Names of localities and the abbreviations by which they are referred to in the text are shown in Table 1. For further details on sampling sites and relevant aspects of *P. mahaleb* natural history, see Alonso (1997, 1999).

Leaf samples were collected in July 1994, once leaves had completed development and reached their final size. The sampling method was specifically designed to avoid sampling biases and buffering potential variation in leaf features within the crown of individual plants. In each sampled tree, we randomly selected a major branch in each of the eight different positions determined by the combination of the four main compass directions and two different heights within the crown (under 2 m and above 4 m). Six leaves were selected per branch using a table of random numbers. When the eight sampling positions were not available on a given tree (either because it was not tall enough or had not well-developed branches in one or more compass directions), leaves were collected only from those positions available.

All leaves from the same tree were pooled into a single sample, air-dried, and ground in a coffee mill to prepare samples for chemical analyses, which were performed at the laboratories of Instituto de Recursos Naturales y Agrobiología de Sevilla, Consejo Superior de Investigaciones Científicas, Spain. Total N concentration was determined by the Kjeldahl method using a Technicon BD-40 Digestor Block for mineralization and Technicon AutoAnalyser II for determination. For the other elements, samples were incinerated before analysis. Potassium and Na concentration were determined by flame spectrophotometry, vanadate-molibdate spectrophotometry was used for P determination, and atomic spectrophotometry for the other elements (Pinta, 1973). A single set of nutrient concentration values was obtained for each sampled tree.

Data analysis

Multivariate covariation between nutrients at the within-population level was analysed by running for each population a principal components analysis (PCA) on the between-tree nutrient covariance matrix (SAS procedure FACTOR; unless

Table 1 Mean (SD) concentration of nutrients in mature *Prunus mahaleb* leaves from the five study populations. In parentheses, *N* = number of trees sampled, and the abbreviation used throughout to designate the population. *F* and *P*-values refer to univariate ANOVAs testing for differences among population means, and adjusted-R^2 represents the proportion of total variance explained by between-population differences. Different letters in the same row indicate a statistically significant difference among populations ($P < 0.05$, Student-Newman-Keuls test)

	Population							
Nutrient	Cabeza del Tejo (*N* = 26, CT)	Cañada de la Medianega (*N* = 21, LM)	Nava de las Correhuelas (*N* = 21, NC)	Poyo Manquillo (*N* = 28, PM)	Torcal del Cerecino (*N* = 20, TC)	$F_{4,111}$	*P*	R^2
N (%)	1.87(0.25)^{b}	2.08(0.25)^{a}	2.03(0.18)^{ab}	1.97(0.27)^{ab}	1.99(0.16)^{ab}	2.7	0.03	0.09
P (%)	0.16(0.03)^{c}	0.22(0.04)^{b}	0.18(0.05)^{c}	0.29(0.08)^{a}	0.18(0.02)^{c}	27.7	< 0.001	0.50
K (%)	1.40(0.40)^{b}	1.83(0.41)^{a}	1.82(0.35)^{a}	1.49(0.37)^{b}	1.26(0.50)^{b}	8.6	< 0.001	0.23
Ca (%)	1.93(0.35)^{c}	2.12(0.43)^{c}	1.93(0.35)^{c}	2.51(0.52)^{b}	3.16(0.62)^{a}	26.9	< 0.001	0.49
Mg (%)	0.88(0.16)^{a}	0.60(0.10)^{c}	0.75(0.13)^{b}	0.69(0.14)^{bc}	0.64(0.09)^{c}	16.4	< 0.001	0.37
Na (%)	0.04(0.01)^{a}	0.05(0.02)^{a}	0.05(0.01)^{a}	0.04(0.01)^{a}	0.05(0.01)^{a}	0.2	0.94	0.01
Cu (μg/g)	9.2(2.6)^{bc}	13.7(9.5)^{a}	10.6(5.2)^{b}	7.9(2.4)^{bc}	6.5(2.3)^{c}	6.5	< 0.001	0.19
Fe (μg/g)	103.5(15.3)^{ab}	98.2(16.6)^{bc}	112.5(22.2)^{a}	94.6(18.3)^{bc}	88.6(15.0)^{c}	5.7	0.0004	0.17
Mn (μg/g)	24.1(6.9)^{b}	36.0(11.0)^{a}	22.9(5.1)^{b}	22.1(6.6)^{b}	25.4(4.3)^{b}	13.9	< 0.001	0.33
Zn (μg/g)	12.7(10.7)^{a}	13.3(10.0)^{a}	15.0(5.8)^{a}	9.5(4.6)^{a}	9.3(6.3)^{a}	2.3	0.07	0.08

otherwise stated, all statistical analyses were conducted using the SAS statistical package, SAS Institute, 1996). The covariance matrices were calculated for all the variables expressed in the same units (0.1 mg g^{-1}). Only the first three PCs were considered in each analysis. This number was chosen because the number of 'explanatory' PCs (i.e. those individually explaining a higher proportion of variance than the average for single variables) was ≤ 3 at all populations. Variability of eigenvalues resulting from PCAs was used as a measure of the magnitude of multivariate covariation of nutrients between *P. mahaleb* trees at the within-population level (Wagner, 1984; Cheverud *et al.*, 1989). Tightly covarying elemental concentrations would produce highly variable eigenvalues, while a situation of very loosely covarying nutrients would be reflected by all eigenvalues being roughly similar. The statistical significance of differences between populations in the magnitude of covariation was assessed by using Levene's test for comparing the variability of eigenvalues (e.g. Schultz, 1983).

The null hypothesis that nutrient correlation matrices between *P. mahaleb* trees from the different populations were unrelated to each other was first tested by comparing them by means of all possible pairwise Mantel permutation tests (Manly, 1991). Variation between populations in the structure of covariation of nutrient concentrations was then examined in greater detail by means of Common Principal Components (CPC) analysis (Airoldi & Flury, 1988; Flury, 1988; Steppan, 1997; Phillips & Arnold, 1999). Only the five macronutrients (N, P, K, Ca, Mg) were included in the CPC analyses. Between-tree variation in microelements was relatively unimportant as determinant of explanatory PCs at any population (see Results section), and excluding them from the CPC analyses had the desirable consequence of reducing the number of parameters to be estimated in the model, thus enhancing the stability of results. CPC analysis allowed us to evaluate the extent and nature of differences between groups of subjects (populations in the present instance) in the covariance structure of macronutrient concentrations. The possible relationships between the covariance matrices include a hierarchically nested series of decreasing levels of relatedness, ranging from identity through proportionality, common principal components structure and partial common principal components structure, to complete independence (Flury, 1988). A CPC structure means that the orientation of the individual principal components is the same in all groups, although the variance associated with each one may vary. Under a *partial* CPC structure, in contrast, only some of the principal components have the same orientations in all groups, while other possible components either are absent from some groups or are group-specific. Comparisons between hypotheses will be performed in a stepwise, hierarchical fashion by means of likelihood ratio tests. The likelihood that a particular model is valid will be tested against the next lower model in the hierarchy, using the following 'step-up' sequence of hypotheses: unrelatedness-partial components (with increasing number of common components)-common components-proportionality-equality (Phillips & Arnold, 1999). Results obtained from this method will also be compared with those based on a 'model building' approach based on the Akaike Information Criterion (Phillips & Arnold, 1999). CPC analyses and associated tests were carried out with the program CPC, written by Patrick Phillips, University of Texas, Arlington, TX, USA.

We also addressed the question of whether the magnitude of differences between *P. malaheb* populations in mean nutrient concentrations was related to the magnitude of divergence in patterns of between-tree covariation. This was done by relating the matrix of Mahalanobis distances between populations with the between-population correlation matrix whose elements were the elementwise correlation coefficients between nutrient correlation matrices.

Results

Central tendency

Distributions of nutrient concentrations acceptably satisfied the normality assumption, as assessed by inspection of normal probability plots. There were significant differences between populations in the average nutrient concentration of the foliage of individual *P. mahaleb* trees, as illustrated by both univariate and multivariate ANOVA's. Considering nutrients individually, heterogeneity of population means was highly significant for 7 out of the 10 nutrients examined, marginally or barely significant for two nutrients (N and Zn), and far from significance for one nutrient (Na) (Table 1). Not unexpectedly therefore there was also a highly significant population effect on overall, multivariate nutrient composition of the leaves of individual *P. mahaleb* trees, as tested with MANOVA (Wilk's $\lambda = 0.04$, $F = 12.9$, d.f. = 40, 389, $P < 0.001$).

The relative importance of within- and between-population differences as determinants of total individual variation in nutrient concentration varied widely among nutrients, even for those nutrients that exhibited statistically significant variation between populations. Between-population differences were responsible for nearly 50% of total variance in P and Ca concentration, but accounted for < 20% in N, Na, Cu and Fe (Table 1). This indicates that individual variation in leaf composition tended to occur mostly at the within-population level for some nutrients, and at the between-population level for others.

A canonical discriminant analysis was used to identify the variables contributing most to explain differences between populations in average nutrient composition. The four possible canonical discriminant functions were statistically significant ($P \leq 0.001$), but the first two (CAN1 and CAN2) accounted for 83.7% of variance (Table 2). The distribution of individual trees over the space defined by CAN1 and CAN2 is shown in Fig. 1. Differences between populations were mainly related to variation along an axis defined by Ca on the positive side, and Mg on the negative one, which were the nutrients contributing most to the first discriminant function (Table 2). Although to a much lesser extent, variation in the concentrations of P, Cu and Mn accounted also for differences between populations (Fig. 1), as these nutrients contributed significantly to the second discriminant function (Table 2). Although CAN1 and CAN2 are, by definition, uncorrelated for the whole sample of trees, these two discriminant functions were significantly correlated in three of the populations, the relationship being positive in two cases (populations LM and TC) and

Table 2 Standardized coefficients of original variables on the four functions (CAN1 to CAN4) resulting from the canonical discriminant analysis of nutrient concentration data. These coefficients denote the unique (partial) contribution of each variable to the discriminant functions. See also Fig. 1

Nutrient	CAN1	CAN2	CAN3	CAN4
N	0.383	−0.205	0.021	0.276
P	0.414	−0.560	0.808	−0.169
K	−0.109	0.009	−0.252	0.609
Ca	1.141	0.473	−0.257	0.203
Mg	−1.083	0.217	0.264	−0.325
Na	0.071	−0.027	−0.012	0.166
Cu	−0.294	−0.493	−0.108	−0.017
Fe	−0.099	0.068	0.357	0.383
Mn	−0.157	−0.544	−0.486	−0.632
Zn	0.131	−0.100	−0.117	0.265
Eigenvalue	4.31	1.08	0.79	0.26
% variance	66.98	16.72	12.32	3.98

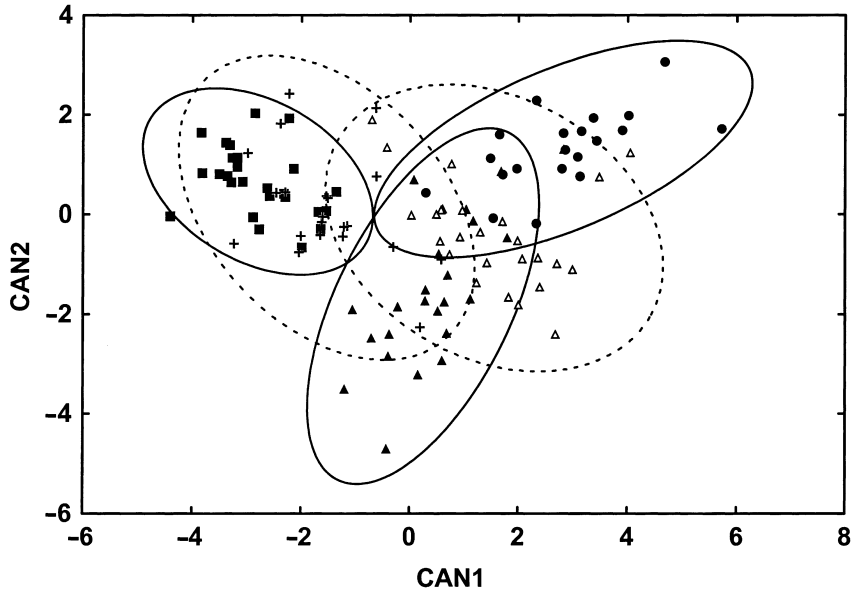

Fig. 1 Distribution of *Prunus mahaleb* trees studied on the plane defined by the first two discriminant functions (CAN1 and CAN2, see Table 2) based on the concentrations of 10 nutrients in mature leaves. Each data point corresponds to a different tree, and different populations are coded using different symbols. CT (closed squares); LM (closed triangles); NC (plus symbols); PM (open triangles); TC (closed circles). Abbreviations for populations are as in Table 1. Shown also are the 95% confidence area ellipses for each population. Those associated with statistically significant ($P < 0.05$) linear correlations between CAN1 and CAN2 are drawn with continuous lines, while dashed lines are used for those associated with marginally significant or nonsignificant correlations ($0.05 < P < 0.20$).

Table 3 Pearson product-moment correlation coefficients between nutrient concentrations in mature leaves of individual *Prunus mahaleb* trees at the five populations studied. In each correlation matrix, figures in bold type denote statistically significant correlations at a table-wide $\alpha = 0.05$, and underlined values are marginally significant ones (at $\alpha = 0.15$), tested using sequential Bonferroni tests (Rice, 1989). Abbreviations for populations as in Table 1

Population		N	P	K	Ca	Mg	Na	Cu	Fe	Mn
CT	P	0.549								
	K	−0.032	−0.143							
	Ca	**0.658**	0.150	0.110						
	Mg	0.594	0.380	−0.191	**0.670**					
	Na	0.039	0.192	−0.208	0.113	0.044				
	Cu	0.011	−0.243	0.141	0.010	−0.364	0.154			
	Fe	0.136	0.026	0.222	0.166	0.065	0.403	0.269		
	Mn	0.245	−0.140	0.410	0.392	0.023	0.109	0.169	0.376	
	Zn	−0.199	−0.102	0.083	−0.008	0.241	0.012	−0.084	0.254	−0.088
LM	P	−0.192								
	K	0.238	0.425							
	Ca	−0.226	−0.112	−0.183						
	Mg	−0.243	0.068	−0.171	**0.847**					
	Na	−0.291	0.291	0.142	0.340	0.423				
	Cu	0.085	0.008	−0.094	0.595	0.605	0.282			
	Fe	0.189	−0.105	0.017	−0.003	−0.005	−0.153	−0.095		
	Mn	0.024	0.174	0.162	0.260	0.489	−0.087	0.155	0.401	
	Zn	−0.449	0.282	−0.176	**0.686**	**0.716**	0.467	0.495	−0.033	0.133
NC	P	0.155								
	K	0.416	0.170							
	Ca	−0.250	−0.244	−0.142						
	Mg	−0.326	−0.060	−0.426	0.590					
	Na	−0.300	0.246	−0.356	−0.016	−0.080				
	Cu	0.123	−0.141	0.130	0.137	−0.060	−0.380			
	Fe	0.126	−0.142	0.125	0.549	0.368	−0.348	0.438		
	Mn	0.137	−0.111	0.041	−0.057	−0.368	0.311	−0.013	−0.001	
	Zn	−0.161	−0.169	0.227	0.047	−0.086	−0.188	0.429	0.357	−0.136
PM	P	−0.551								
	K	0.199	0.278							
	Ca	−0.226	0.176	−0.251						
	Mg	−0.071	0.057	−0.333	**0.609**					
	Na	0.308	−0.332	−0.125	0.084	0.211				
	Cu	0.043	−0.014	0.193	−0.095	−0.193	−0.123			
	Fe	0.428	−0.325	0.408	−0.263	−0.139	0.101	0.000		
	Mn	0.255	−0.131	0.046	0.086	−0.189	0.104	−0.453	0.126	
	Zn	0.180	−0.216	0.093	−0.243	−0.015	0.237	−0.094	0.492	0.190
TC	P	−0.315								
	K	0.225	0.426							
	Ca	−0.199	−0.326	**−0.840**						
	Mg	−0.052	0.083	−0.364	0.568					
	Na	0.094	0.290	0.101	−0.067	0.222				
	Cu	−0.099	−0.069	−0.432	0.557	**0.709**	0.279			
	Fe	0.114	−0.095	0.152	0.049	0.253	0.199	0.227		
	Mn	0.190	0.006	−0.036	0.174	−0.058	0.400	0.045	0.458	
	Zn	0.024	0.354	−0.182	0.356	0.386	0.261	0.654	0.178	0.353

negative in the other (population CT) (Fig. 1). This provides an indication that populations differ in the structure of nutrient covariation across trees, and that patterns of variation occurring across populations are not replicated among trees within populations. These aspects will be explicitly considered in the next section.

Nutrient covariation between trees

Between-tree correlation matrices for leaf nutrient concentrations are shown separately in Table 3 for the five populations studied. Although the power for detecting significant correlations with our sample sizes was limited to high correlation coefficients

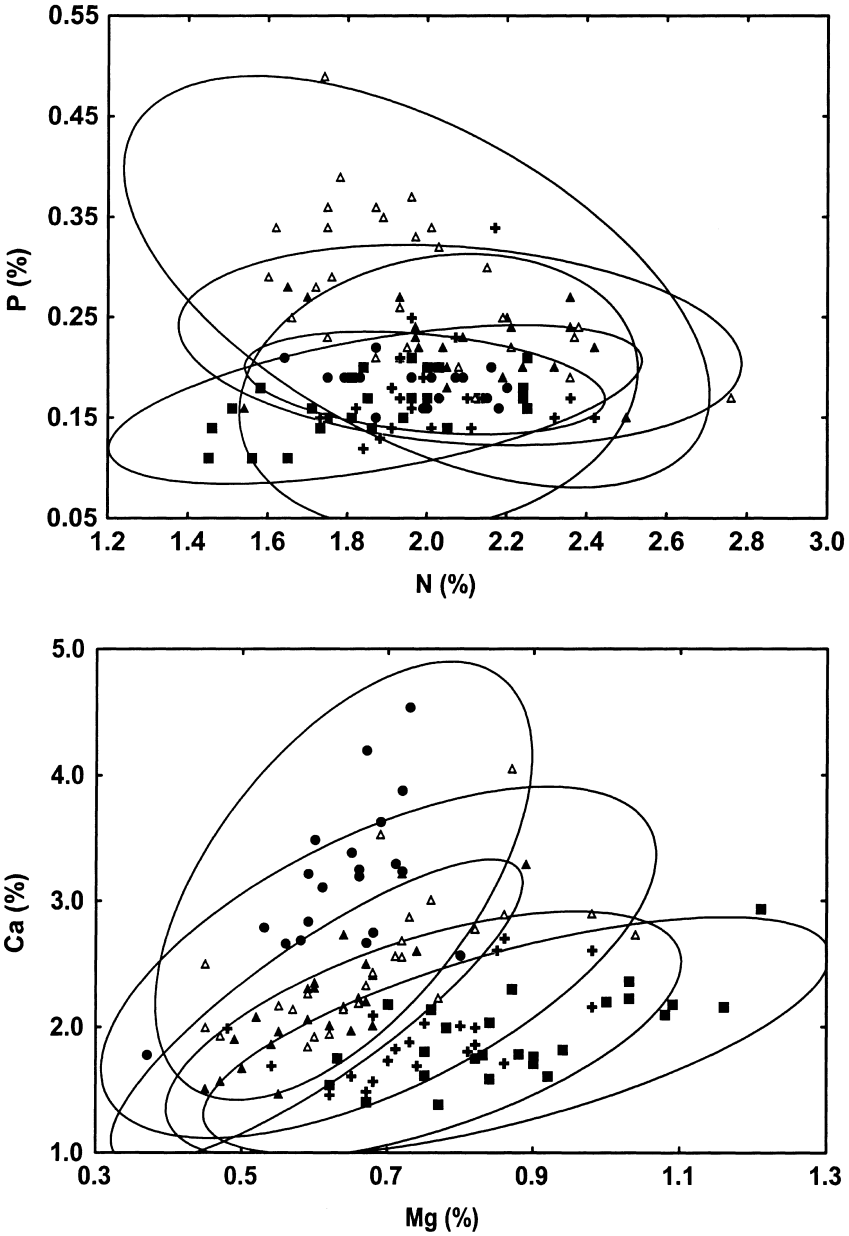

Fig. 2 Bivariate relationships between the concentrations (as percentage dry mass) of P and N (upper graph), and Ca and Mg (lower graph), in the leaves of *Prunus mahaleb* trees. Each data point corresponds to a different tree, and different populations are coded using different symbols (see Fig. 1 for population symbol codes). Shown also are the 95% confidence area ellipses for each population (see Table 3 for associated correlation coefficients).

(Phillips, 1998), some significant or nearly significant correlations between pairs of nutrients existed at all sites. Except for the positive correlation between Ca and Mg concentrations consistently occurring in all populations, statistically significant correlations tended to be site-specific, involving different pairs of nutrients in different populations. Nitrogen and Ca, for example, were significantly directly correlated at CT, and negatively but nonsignificantly at all the other localities. Nitrogen and P were positively related at one locality, negatively at other, and nonsignificantly at the other three. Taken together, these results provide strong evidence of both qualitative and quantitative variation between sites in patterns of pairwise correlations across individual *P. mahaleb* trees in nutrient concentrations. This is graphically illustrated in Fig. 2 for the relationships between P-N and Ca-Mg, where discordances in the shape and orientation of confidence ellipses are indicative of differences between populations in the sign (P-N) or magnitude (Ca-Mg) of nutrient correlations.

Principal component analyses conducted separately on the between-tree covariance matrices of nutrient concentrations corroborated the existence of patterns of multivariate covariation at all populations (Table 4). The number of explanatory PCs (i.e. those individually explaining a higher proportion of variance than the average for single variables) varied between populations, from one in TC through two at CT and NC, to three in LM and PM (Table 4). As would be expected from their higher concentration and larger absolute variance, macronutrients (N, P, K, Ca, Mg) consistently exhibited the

Table 4 Eigenvalues and eigenvectors corresponding to the first three principal components based on the covariance matrix of nutrient concentrations for the five study populations (abbreviations as in Table 1). Components explaining 'significant' proportions of variance in each population (i.e. those with associated eigenvalues higher than the average eigenvalue) are shown in bold type and, for these, correlations with individual nutrients ≥ 0.6 in absolute value are also highlighted to facilitate interpretation

Population	PC	Proportion of variance	Eigenvectors									
			N	P	K	Ca	Mg	Na	Cu	Fe	Mn	Zn
CT	**1**	**0.471**	**0.66**	0.18	0.55	**0.87**	0.53	−0.02	0.05	0.25	0.50	0.01
	2	**0.427**	0.49	0.31	**−0.84**	0.43	**0.60**	0.22	−0.15	−0.10	−0.17	−0.09
	3	0.072	0.56	0.53	0.03	−0.24	−0.02	−0.11	0.03	0.04	−0.04	−0.24
LM	**1**	**0.523**	−0.40	−0.26	**−0.63**	**0.87**	**0.76**	0.23	0.49	−0.03	0.13	**0.66**
	2	**0.337**	0.10	0.30	0.77	0.49	0.41	0.34	0.32	0.02	0.32	0.28
	3	**0.131**	**0.91**	−0.36	−0.10	0.08	0.01	−0.26	0.27	0.19	0.04	−0.24
NC	**1**	**0.526**	−0.52	−0.27	**−0.73**	**0.77**	**0.71**	0.22	< 0.01	0.28	−0.10	−0.09
	2	**0.358**	0.18	−0.04	**0.68**	**0.63**	0.11	−0.30	0.21	0.52	< 0.01	0.20
	3	0.081	0.83	0.02	−0.11	0.05	0.02	−0.15	0.10	0.21	0.11	−0.29
PM	**1**	**0.590**	−0.33	0.14	−0.46	**0.97**	**0.65**	0.08	−0.14	−0.36	0.04	−0.25
	2	**0.246**	0.18	0.32	**0.88**	0.23	−0.03	−0.05	0.15	0.31	0.11	−0.01
	3	**0.137**	**0.93**	**−0.63**	0.12	0.05	0.17	0.38	−0.03	0.29	0.26	0.12
TC	**1**	**0.881**	−0.23	−0.37	**−0.94**	**0.98**	0.51	−0.08	0.53	−0.03	0.12	0.30
	2	0.074	0.13	0.19	0.35	0.21	0.37	0.09	0.21	0.37	0.24	0.30
	3	0.037	0.97	−0.44	−0.03	< 0.01	0.03	0.07	< 0.01	0.07	0.19	0.05

higher correlations with PCs at all populations, thus denoting that they tended to be the nutrients most closely interrelated and responsible for the main gradients of multivariate variation across trees. Potassium, Ca and Mg consistently had the largest absolute correlations with PCs at all sites, while those for N and P tended to fluctuate somewhat erratically between populations. In all populations except CT, the main gradient of variation across trees in nutrient concentration (the first PC) was almost entirely related to an inverse association between trees of K, on one side, and Ca and Mg on the other (Table 4). At CT, covariation between these three nutrients was also responsible for the first component, but they were all positively associated at that site. At the four sites where it had a significant explanatory value, the association between nutrients reflected by the second PC was much less clearly defined, and varied between populations.

Variation between populations in the patterns of between-tree variation

The magnitude of the covariation of nutrients between individual trees differed significantly between populations. Levene's test on the locality-specific sets of eigenvalues from the nutrient covariance matrices indicated that differences between populations in the variability of eigenvalues were statistically significant ($\chi^2 = 10.7$, d.f. = 4, $P = 0.03$; Kruskal–Wallis test).

The pattern of bivariate and multivariate associations between elements, and the number of gradients that these associations determine, also varied between populations. Association between pairs of nutrients occurring across individuals can be represented by the population correlation matrices (Table 3). When each population matrix was compared with the matrices from the other 4 study populations by Mantel permutation tests, the null hypothesis that the associations between pairs of nutrients took place differently in different populations was rejected on all the possible pairwise comparisons ($N = 10$, $P \leq 0.01$; significance levels Bonferroni-corrected for multiplicity of simultaneous tests). Despite significance, however, Mantel correlation coefficients between matrices ranged widely ($r = 0.431–0.788$), which denotes a variable degree of matching between the interelement correlation matrices from different populations.

Results of CPC analysis are summarized in Table 5. At the lowermost level, the null hypothesis that nutrient covariance matrices from the different *P. mahaleb* populations studied shared a single principal component, PCP(1), was rejected. This finding indicates that nutrient covariance matrices were unrelated among themselves (i.e. mutually independent, or 'arbitrary', from a statistical viewpoint) or, at least, were not related in any of the ways tested by this approach. This result was also supported by the Akaike Information Criterion, which reached its minimum value (best fit) for the unrelated model (Table 5).

Composition vs covariation

As illustrated by Fig. 3, differences between *P. mahaleb* populations in the average foliage nutrient composition, represented by the Mahalanobis distances, were decoupled from variation in patterns of between-tree nutrient covariation, represented by Mantel correlation coefficients. A predictable relationship

Table 5 Summary of results of Common Principal Components analysis testing for the degree of relatedness of between-tree nutrient covariance matrices for the five *P. mahaleb* populations studied. Shown are results relevant to the 'step-up' (decomposition of the log-likelihood ratio) and 'best fitting' (based on the Akaike Information Criterion, AIC, which measures the lack of fit) testing approaches used. The 'Model' column indicates the two hypotheses under comparison by means of the likelihood ratio test. CPC denotes the full common principal components model, and CPC(*n*) are partial components hypotheses having *n* common components. The decomposition of the log-likelihood ratio shows the χ^2 values corresponding to the comparison between consecutive models in the hierarchy. Figures relevant to the interpretation of results are shown in bold type (see text for further details)

Model		Decomposition of log-likelihood ratio			
Higher (H0)	Lower (H1)	χ^2	df	*P*	AIC
Equality	Proportionality	13.94	4	0.0075	181.2
Proportionality	CPC	79.71	16	< 0.001	175.3
CPC	CPC(3)	3.25	4	0.52	127.6
CPC(3)	CPC(2)	5.78	8	0.67	132.3
CPC(2)	CPC(1)	34.51	12	0.0006	142.6
CPC(1)	Unrelated	**44.06**	**16**	**0.0002**	132.1
Unrelated					**120.0**

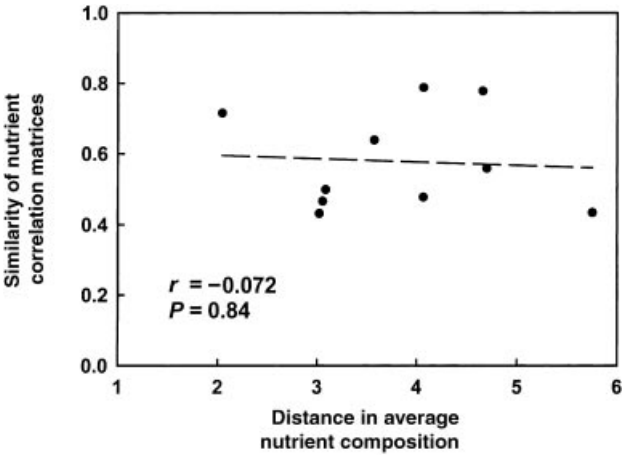

Fig. 3 Differences among *Prunus mahaleb* populations in mean nutrient composition are not significantly related to their differences in patterns of between-tree nutrient covariation. The vertical axis represents the pairwise similarity between populations in nutrient correlation matrices (Table 3), computed using elementwise correlation coefficients between matrices. The horizontal axis represents pairwise euclidean distances between populations on the multivariate space defined by canonical discriminant functions (Table 2). The dashed line is the least squares-fitted regression line. Each point corresponds to a pair of populations. Statistical significance of the relationship tested using Mantel permutation test.

between populations between mean nutrient composition and patterns of nutrient covariation would have led to a significant inverse relationship between the two variables shown in the graph. We also found no significant relationship between the matrix of geographical distances between populations and either their Mahalanobis distances on the nutrient multivariate space ($r = -0.366$, $P = 0.25$) or the similarity of their nutrient correlation matrices ($r = 0.050$, $P = 0.85$).

Discussion

Nutrient composition

Patterns of variation in elemental concentrations have been documented in considerable detail for a variety of plant parts, habitats, and geographical locations (e.g. Woodwell *et al.*, 1975; Garten, 1978; Auclair, 1979; Ricklefs & Matthew, 1982; Margaris *et al.*, 1984; Nordstrom, 1984; Hocking, 1986; Herrera, 1987; Thompson *et al.*, 1997, among many others). The vast majority of these studies, however, have focused on comparisons at the interspecific level, while intraspecific variation has received remarkably little attention (but see Garten *et al.*, 1977; Grimshaw & Allen, 1987; Ohlson, 1995; Bauer *et al.*, 1997; Thompson *et al.*, 1997). Apart from sampling and analytical effort considerations, there seems to be no sound ecological reason for this neglect, particularly if one considers that, in quantitative terms, intraspecific variation in the concentration of some elements often equals or even exceeds interspecific variation (e.g. Grimshaw & Allen, 1987; Garten *et al.*, 1977; Bauer *et al.*, 1997; Thompson *et al.*, 1997, but see Ohlson, 1988). In a study of 83 herbaceous species, Thompson *et al.* (1997: Table 3) found that within-species variation in leaf nutrient concentration accounted for 18.6% (Ca), 23.0% (K), 27.9% (N), 37.4% (Mg), and 44.4% (P) of total variance in the sample, as compared with corresponding values of 8.2%, 26.1%, 15.0%, 12.1% and 31.1% of variance accounted for by interspecific differences. The present study has also documented important levels of intraspecific variation in leaf nutrient concentrations for *P. mahaleb*, and the dissection of this intraspecific variance has further revealed that elements differ in the relative importance of between and within population variance components. Within-population (i.e. between individual trees) differences are responsible for most intraspecific variation in some elements (e.g. N, K, Cu, Fe), whereas the reverse was true in others (e.g. P, Ca). These findings further highlight the importance, when characterizing the nutrient contents profile of a given plant species, of explicitly considering patterns of variation in nutrient concentrations between individual plants.

Although the potential for individual variability in nutrient concentration is implicit in investigations that have used individual plants as sampling units (e.g. Ohlson, 1995; Thompson *et al.*, 1997), we are not aware of any previous study explicitly considering individual variation in leaf nutrient composition. Our data do not allow for a rigorous statistical demonstration of individual differences in nutrient concentrations in *P. mahaleb*, as we did not conduct replicated chemical analyses for individual trees. Two considerations suggest, however, that variation in our

tree-level estimates of nutrient concentrations largely reflect individual differences in the average chemical composition of their leaves, rather than analytical or sampling error. Firstly, differences in nutrient concentration values for samples from different trees were, except for Na, one to several orders of magnitude greater than analytical measuring error. And secondly, tree-level estimates were presumably little affected by sampling error and reflected accurately average values for the whole population of leaves, given the large number of leaves collected per tree and our stratified random sampling scheme, which accounted for possible within-crown variations due to height and orientation.

Although plants can regulate mineral nutrients intake and thus buffer differences in soil concentrations (Chapin, 1980; Sultan & Bazzaz, 1993), the differences in Ca and Mg concentrations, which underly the main gradient of between-population variation found in this study, most likely reflect local differences in the relative proportions of dolomite (calcium magnesium carbonate) and calcite (calcium carbonate) as major components of the limestone bedrock prevailing in the study region (e.g. González Parra *et al.*, 1985a,b). At the within-population level, differences between trees may likewise be attributable to variation in microenvironmental conditions such as soil features and light intensity (e.g. Somaggio *et al.*, 1995; Orians & Fritz, 1996; Phelan *et al.*, 1996), but also to individual plant genotypes (Hwang & Lindroth, 1997).

Nutrient covariation

Three main conclusions emerge from our analyses of nutrient covariation in *P. mahaleb*: (1) pairwise elemental correlations commonly found in interspecific contexts also occur, but only to a very limited extent, at the intraspecific level; (2) the nature and magnitude of the correlations between nutrients varied between populations; and (3) multivariate patterns of nutrient covariation from different populations were unrelated among themselves, and also to geographical distances between populations.

Concentrations of different nutrients frequently covary nonrandomly across species (Garten, 1976, 1978; Herrera, 1987; Duarte, 1992; Lebreton *et al.*, 1997; Thompson *et al.*, 1997). This probably stems from a combination of similarity between nutrients in intrinsic chemical properties and biochemical functionality in cell metabolism (see Garten, 1976, 1978; Garten *et al.*, 1977; Marschner, 1995; for further details). However the mechanistic bases of these correlations are not fully understood (see e.g. Gilroy & Jones, 2000) and even the existence within species has not been widely documented.

The present study found significant pairwise nutrient correlations between individual *P. mahaleb* trees living at the same location. Some of these intraspecific correlations between elements, like the consistent positive association between Ca and Mg occurring at all our study sites, are qualitatively similar to those reported for interspecific contexts (Garten, 1976, 1978; Herrera, 1987), but others are not. Among the latter, the most striking contrast involves the relationship between N and P, a pair of elements for which previous interspecific investigations have invariably found a positive correlation (Garten, 1976, 1978; Duarte, 1992; Cornelissen *et al.*, 1997; Thompson *et al.*, 1997). In this study, however, N and P were positively correlated across trees at only one population. Other correlations between pairs of elements commonly found in interspecific contexts either did not occur at any of our localities (Ca-Mn, Fe-Cu) or occurred only at some sites (Zn-Mg).

Further evidence that the nature and extent of interelement correlations may be strongly context-dependent comes from our finding that patterns of variation between populations did not mirror patterns of variation between trees within populations. The inverse association between Ca-Mg occurring between populations ran opposite to their significant and positive across-tree correlation occurring at all populations. Analogous context-dependence of element correlation patterns also seems to occur when within- and between-species patterns are compared (Garten *et al.*, 1977: Fig. 7). Contrasting elemental correlation patterns at different levels in the hierarchy from individuals to populations to species may lead to spurious results if the hierarchical structure of sampling units is not explicitly accounted for. In the present study, for example, we would have failed to detect any relationship between Ca and Mg concentrations if we had pooled data from all trees into a single sample ($r = 0.097$, $N = 116$, $P = 0.30$), just because the inverse between-site, and the direct within-site correlations between the two nutrients would have cancelled each other. The important practical corollary thus follows that statistical patterns of nutrient covariation may be heavily affected by both the nature of sampling units and how these are allocated among plant modules, individuals, populations and species.

The populations of *P. mahaleb* studied differed in patterns of covariation of nutrients, from both the bivariate and multivariate viewpoints. When pairs of populations were compared, the null hypothesis of unrelatedness was rejected for all pairs of between-tree interelement correlation matrices, although the degree of matching between matrices from different populations varied widely. This applies not only to correlations that occurred only at some populations (e.g. N-P, K-Ca), but also to those that occurred at most or all sites (e.g. Ca-Mg), whose degree of association varied between populations. It must be noted, however, that inconsistencies between the interelement correlation matrices of the kind found here for inter-population comparisons are also evident when results of different interspecific investigations are compared (compare, e.g., Table 4 in Garten, 1978 with Table 9 in Thompson *et al.*, 1997), and probably show that environmental or sampling effects often limit or override inherent physiological or chemical associations between elements.

As found in interspecific contexts (Garten *et al.*, 1977; Garten, 1978; Lebreton *et al.*, 1997), some nutrients tended to covary

in unison and defined independent gradients of variation in all the *P. mahaleb* populations studied. Nevertheless, populations differed in both the magnitude of multivariate association between nutrients, as judged from the different variabilities of eigenvalues from PC analyses, and in the number and biological meaning of the gradients involved. This suggests that phenotypic integration of nutrient concentrations in *P. mahaleb* leaves is weak, and differences in plasticity of intake and transport of nutrients override it. In fact, when the five populations were simultaneously compared by CPC analysis there was not any principal component common to all populations, and thus, we have to conclude that different populations of a single species did not share any pattern of covariation between nutrients, even when pairs of populations showed significant communalities. The same caveats expressed above in relation to pairwise nutrient correlations analyses that do not consider the individual-population-species hierarchical structure apply here as well, and will not be discussed further. Given the differences between populations in the multivariate nutrient covariance structures, pooled-sample principal components analyses will often become 'contaminated' to some extent by covariance components extraneous to the covariation between nutrients themselves, such as those due to variation between populations and species.

Composition, covariation and herbivory

This study was initially planned in relation to the hypothesis that the relationships between *P. mahaleb* and its invertebrate herbivores would be simultaneously influenced by within and between populations patterns of variation and covariation in leaf nutrient composition (Alonso, 1997, 1999). Patterns of host and habitat selection, and herbivores' fitness, may be related not only to variation in nutrient concentration (e.g. Tabashnik, 1982; Bergström & Danell, 1986; Athey & Connor, 1989; McNaughton, 1990), but also to the relative proportions of different nutrients in their food (e.g. Clancy, 1992a,b; Cates, 1996; Phelan *et al.*, 1996). In addition, nutrient imbalance can also affect plant resistance and tolerance (Marschner, 1995).

We expected to find at least a partial CPC structure of nutrient covariation shared by all populations, provided their proximity and homogeneity of the bedrock. The interactions between different nutrients at root intake (Marschner, 1995), and the capability of plants to buffer differences in soil concentrations (Chapin, 1980; Sultan & Bazzaz, 1993) suggest that maintenance of nutrient correlations could be achieved by either functional or physiological reasons. Furthermore, the expected common structure would characterize the species-specific balance of nutrients, and, from the viewpoint of herbivores, might be interpreted as indicative of predictability in food composition. However, since *P. mahaleb* populations differed with regard to both the composition and the covariation of nutrients, predictability of nutritive quality may be low even for its monophagous feeder, *Yponomeuta mahalebella*. The lability of the nutrient relationships precluded any further analysis of the relationships between the local degree of integration of macronutrients and the herbivory level experienced by tree individuals of different populations, an aspect that will require experimental tests. The results obtained, however, have broader implications for other plant-herbivore systems as well. The finding of this study, that differences between *P. mahaleb* populations in average foliage composition were unrelated to differences in patterns of covariation of nutrients between individual plants, implies that the latter may not be inferred from compositional data alone, and that adequate consideration should be given to both aspects when studying the response of herbivores to geographical variation in foliage plant composition. The absence of principal components common to all populations of a single plant species point out the inexistence of a 'common ground' for selective processes exerted by herbivores on plants, whereas locality-specific components provide possibilities for local adaptive adjustments of herbivores to their host plants. In this respect, an improved understanding of patterns of variation and covariation of plant nutritional traits within and between populations will be of immediate application to studies on plant–herbivore interactions, particularly in relation to tests of spatially explicit hypotheses on the evolution of plant–animal interactions (Thompson, 1994).

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