

## An approach to phenotypic analysis and environmental variability. The examples of the genera *Dianthus* L. and *Lotus* L. in the north of Portugal

ANTONIO L. CRESPI<sup>1\*</sup>, SONIA BERNARDOS<sup>2</sup>, JORGE PAIVA<sup>3</sup>, FRANCISCO AMICH<sup>2</sup>,  
CLAUDIA P. FERNANDES<sup>1</sup>, ADRIANO CASTRO<sup>1</sup>

<sup>1</sup> University of Trás-os-Montes and Alto Douro, Herbarium/Botanic Garden, D.E.B.A.,  
ap.1013, 5000-911 Vila Real, Portugal

<sup>2</sup> Faculty of Biology, University of Salamanca, Department of Botany, 37008  
Salamanca, Spain.

<sup>3</sup> University of Coimbra, Faculty of Science and Thecnology, Instituto Botânico »Júlio  
Henriques«, 3049 Coimbra, Portugal.

The analysis of the correlation between phenotypic plasticity and environmental variability has been very useful to describe the morphologic responses of individuals to environmental factors affecting them (in the present contribution the altitudinal variation), from a genecological perspective. However, the literature shows that the studies in this area have had a monospecific or monogeneric use. As a consequence, intergeneric comparative descriptions have not been possible. In the present work we propose an analytical method to compare the morphological expressivity of individuals included in the genera *Dianthus* and *Lotus* from the north of Portugal by means of a contingency matrix. This matrix was elaborated on the basis of amplitude parameters, in order to describe the variability present in the basic matrix of each genus studied. The results pointed out the existence of opposite phenotypic behaviours dependent on the altitudinal variation, thus indicating the importance of the analysis between different groups of taxa as a reference for the comparison of their morphological expressivities.

**Key words:** *Dianthus*, *Lotus*, phenotypic analysis, variability, Portugal.

### Introduction

Genecological science (TURESSON, 1922) became a very useful methodology to describe the correlations between the morphological expressions and the ecological factors, discussed in terms of »evolutionary taxonomy« (CONSTANCE 1953) or under ecotypic characterizations (HESLOP-HARRISON 1964). These studies became easier with the access to numerical taxonomic methodologies, especially the phylogenetic implications (RAVEN 1974;

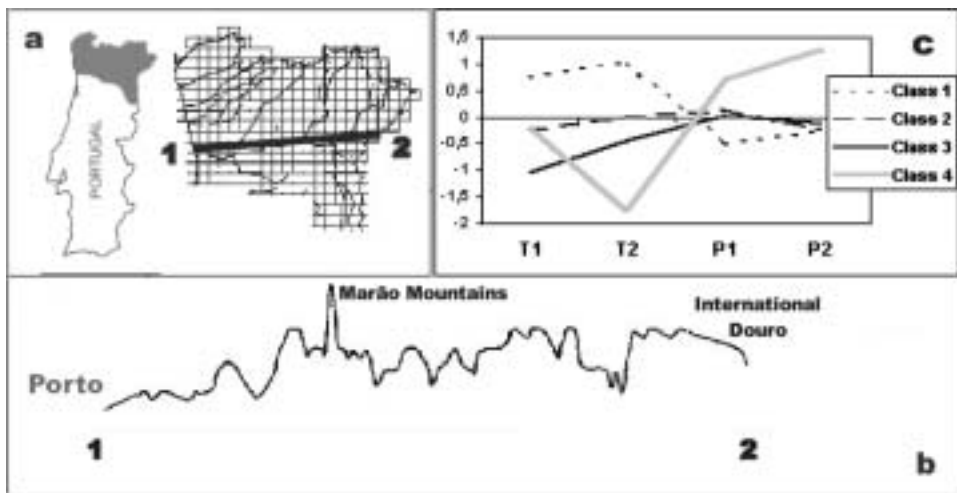
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\* Corresponding author: Fax: + (351) 259-350480, e-mail: [acrespi@utad.pt](mailto:acrespi@utad.pt)

DUNCAN and BAUM 1981), usually described for monospecific or monogeneric taxa grown in a series of environments (SCHLICHTING 1986, HINZ 1988, WAITT and LEVIN 1993, DELESALLE and MAZER 1995, GILBERT et al. 1996, LORETI and OESTERHELD 1996, COLUNGA-GARCÍA and MAY-POT 1997, VENEABLE et al. 1998, CASAS et al. 1999, SCHOETTLE and ROCHELLE 2000, RYSER and EEK 2000, LEISS and MÜLLER-SCHÄRER 2001).

The present work is focused on the genecological analysis perspective, in order to quantify the apparent morphological plasticity of the individuals (BRADSHAW 1965, 1972; SCHLICHTING 1986; SULTAN 1987; WEST-EBERHARD 1989) using a multivariate methodology. Based on this assumption, the separate responses of the individuals will be correlated with the variability of environmental factors (*biogeocoenose*, from the point of view of SCHMALHAUSEN 1960). In this study we will consider the altitudinal variation factor.

With the goal of testing the methodology proposed, two different genera with occurrence in the north of Portugal (Fig.1) will be examined: the perennial species of the genus *Dianthus* and the species of the genus *Lotus* (with the exception of *L. creticus* L., which is very rare in the area mentioned). The taxonomic, biologic and ecological characteristics are clearly different for the species of these two genera: the species of the genus *Lotus* are more usual in *Festuco-Brometea* Br.-Bl. and Tüxen 1953, *Helianthemetea guttati* (Br.-Bl. in Br.-Bl. and col. 1952) Rivas Goday and Rivas-Martínez 1963 and *Molinio-Arrhenatheretea* Tüxen 1937; whereas the species of the genus *Dianthus* frequently appear in *Phagnalio-Rumicetea indurati* (Rivas Goday and esteve 1972) Rivas-Martínez, Izco and Costa 1973.



**Fig. 1.** (A) Location of the area (North of Portugal) and indication of the geomorphological West-East profile; (B) graphic representation of the West-East profile, from Porto to the International Douro, through the Marão Mountains (1400 m); (C) the termopluiometric transition between the four classes of altitude is represented, according to the standardised values of average temperatures (T1 = average of the lowest annual temperatures, T2 = average of the highest annual temperatures) and precipitations (P1 = average of the lowest annual precipitations, P2 = average of the highest annual precipitations).

Through the utilization of a contingency matrix, the morphological expressions of the taxa indicated above will be correlated with the altitudinal variation of the area. This methodology implies the examination of the morphologic expressivity of defined areas using different taxonomic groups of individuals, as a multivariate description of the genecological characterization proposed by Turesson.

### Material and methods

The present work was elaborated with the goal of characterizing the phenotypic plasticity of species of the genera *Dianthus* L. and *Lotus* L. in the north of Portugal (Fig. 1A). In this work plasticity is considered the adaptation of the genotypic expressivity to the environmental variations (SULTAN 1987), in accordance with the concept introduced by BRADSHAW (1965; 1972). From the analytical standpoint, the characterization of the phenotypic plasticity will be the result of the morphological expressivity of each individual.

The methodology employed involved a multivariate analysis initially supported by basic matrices and afterwards by a »contingency« matrix, in the light of a numerical phenetic methodology (DUNCAN and BAUM 1981). The basic matrices were elaborated from OTUs –Operational Taxonomic Units– (SOKAL and SNEATH 1963) of the specimens analysed per genus.

The analytical methodology proposed here was applied to taxa of the same genus and for different genera, in order to establish differentiation between their respective phenotypic plasticities according to the environmental variations. Taxa of the genera *Dianthus* and *Lotus* were phenotypically characterized: 30 characters (OTUs, in terms of the analytical process) were selected for the genus *Dianthus* and 26 for the genus *Lotus* (Tab. 1). The selection of the morphological characters was established on the basis of the morphologic description of these taxa (WILLKOM and LANGE 1880, COUTINHO 1939, ROZEIRA 1944, BALL 1968, FRANCO 1971, BOLÒS and VIGO 1984, GALLEGO 1987, RUÍZ DE CLAVIJO 1987, SAMPAIO 1988, BOLÒS and VIGO 1990, BERNAL et al. 1990, TUTIN and WALTERS 1993, CRESPI 1999, VALDÉS 2000).

**Tab. 1.** Relation of OTUs per genus (with indication of the abbreviations used).

Ref.	OTUs ( <i>Lotus</i> )
Ll	Highest length of the central leaflet in the inflorescence-basal leaf
Lw	Highest width of the central leaflet in the inflorescence-basal leaf
Lp	Highest length of the pedicel of the inflorescence-basal leaf
Lel	Highest length of the stipule-basal leaf
Lew	Highest width of the central leaflet in the inflorescence-basal leaf
Cl	Highest length of the space between the two first inflorescences-nodes
Fcl	Highest length of the calyx
Fal	Highest length of the corolla
Fcd1	Smallest length of the calyx-teeth
Fcd2	Highest length of the calyx-teeth
Fcdw	Highest width of the calyx-teeth
Fct	Highest width of the calyx-tube
Il	Highest diameter of the inflorescence

**Tab. 1.** – continued

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Ifcl	Highest lenght of the first inflorescences-node
Ipl	Highest lenght of the peduncles per head in the fructification
Irn	Highest number of the inflorescences-nodes
Ln1	Smallest number of flowers per inflorescence (=head)
Ln2	Highest number of flowers per head
Ipl	Highest lenght of the central leaflet of the head-basal leaf
Ilw	Highest width of the central leaflet of the head-basal leaf
Ill	Highest lenght of the pedicel per head
Rl	Hihgest lenght of the legume
Rw	Highest width ot the legume
Rsn	Highest number of seeds per legume
Flp	Highest lenght of the pedicel per legume
Sd	Highest diameter of the seeds
S11	Smallest lenght of the annual stem
S12	Highest lenght of the annual stem
L1M	Highest lenght of the leaves
LwM	Highest width of the leaves
Fsl1	Smallest lenght of the calyx
Fsl2	Highest lenght of the calyx
Fsd11	Smallest lenght of the calyx-teeth
Fsd12	Highest lenght of the calyx-teeth
Fsda1	Smallest width of the calyx-teeth
Fsda2	Highest width of the calyx-teeth
Fsad1	Smallest width of the calyx at the base of teeth
Fsad2	Highest width of the calyx at the base of teeth
Fsab1	Smallest width of the calyx at the top of the epicalyx
Fsab2	Highest width of the calyx at the top of the epicalyx
Fge	Highest lenght of the style and stigate
Ful1	Smallest lenght of the corolla out of the calyx (claw)
Ful2	Highest lenght of the corolla out of the calyx (claw)
Flac	Lenght ot the corolla-lobes
H	Presence/absence of hairs in the throat of the corolla
Fel1	Smallest lenght of the epicalyx
Fel2	Highest lenght of the epicalyx
Fsa1	Smallest lenght of the calyx-teeth awn
Fsa2	Highest lenght of the calyx-teeth awn
Fbia1	Smallest lenght of the awn of the internal epicalyx-bracts
Fbia2	Highest lenght of the awn of the internal epicalyx-bracts
IfL	Highest lenght of the inflorescence
Fn1	Smallest number of flowers of the inflorescence
Fn2	Highest number of flowers of the inflorescence
Lbif1	Smallest lenght of the basal inflorescence-bracts
Lbif2	Highest lenght of the basal inflorescence-bracts

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The choice of the number of morphologic characters has been a polemic issue, especially considering the criteria established by SOKAL and SNEATH (1963). However, according to HILL (1980) and THIÉBAUT (2000), the selection of the morphological characters depends on the quality of information they may offer. The morphological characters selected here are quantitative and qualitative (only three of them –one for *Dianthus* and two for *Lotus*-). With the aim of excluding the artificiality of the analysis (LUBISCHEW 1963), morphology-related parameters were not considered in this work.

In the area studied four perennial species of the genus *Dianthus* (i. e., *D. hyssopifolius* L., *D. langeanus* Willk., *D. laricifolius* Boiss. and Reut. and *D. lusitanus* Brot.) and seven of the genus *Lotus* (i. e., *L. angustifolius* L., *L. corniculatus* L., *L. conimbricensis* Brot., *L. glaber*, *L. parviflorus* Desf., *L. subbiflorus* and *L. uliginosus*) were found (CRESPI 1999; CRESPI *et al.* 2000). Regarding the species of the genus *Lotus*, it must be noted that *L. castellanus* has not been detected in the herbaria of the country or in any expedition of the authors, in contrast with the reference of VALDÉS (2000).

The contingency matrix was constructed from the information included in the basic matrices of the two genera under analysis. To calculate the parameters of the contingency matrix, the Pearson correlation matrix and the city-block distance matrix must be created. The information contained in the contingency matrix must be the summary of that contained in basic matrices and in the correlation and distance matrices. This allows the collection of all the morphologic information of each taxon in a single matrix. The variables considered were established on the basis of the largest difference between the smallest and the highest value (highest amplitude) and the average value for the three different types of matrices (basic, correlation and distance) per genus and per species examined: HMA (highest morphologic amplitude), AvV (average variability), HMASp (highest morphologic amplitude per species), HMAGe (highest morphologic amplitude per genus), HMAT (highest morphologic amplitude per totality), AvVSp (average variability per specie), AvVGe (average variability per genus), AvVT (average variability per totality), HDA (highest distance amplitude), AvVD (average variability of distances), HDASp (highest distance amplitude per species), HDAGe (highest distance amplitude per genus), HDAT (highest distance amplitude per totality), HCA (highest correlation amplitude), AvVC (correlation variability of distances), HCAGe (highest correlation amplitude per genus), HCAT (highest correlation amplitude per totality).

The calculation of the highest amplitudes involved the use of the following formula:

$$HEA = \sum_{i=n}^{i=1} (X_i - x_i)$$

$X_i$  The highest value for the set of highest values per variable

$x_i$  The smallest value for the set of smallest values per variable

The morphological, distance or correlation amplitude was obtained from the highest difference between the highest of the highest values per morphologic parameter or individual and the smallest ones.

In this work we examined the correlation between the altitude and the morphological expressivity. Based on the geomorphological variability of the area (Fig. 1B), four classes of altitude are proposed to account for the correlation with the basic matrices and with the

contingency matrix:  $x < 400$  m (class 1),  $400 \leq x < 700$  m (class 2),  $700 \leq x < 950$  m (class 3),  $x \geq 950$  m (class 4). These four classes are established taking into account to the thermo-pluviometric variation of the area. Thus the highest average temperatures and lowest average precipitations will be found in the first class. In classes 2, 3 and 4 the average temperatures become gradually lower and average precipitation higher. This behaviour is observed in Fig. 1C, where standardized average temperatures are represented by the lowest (T1) and highest (T2) values per month, and precipitation figures by the lowest (P1) and the highest (P2) value per month.

Each matrix (basic or contingency) was first standardized to equalize phenotypic variances (FELSENSTEIN 1988) prior to proceeding to the analytical process.

The statistical analysis was elaborated according to the phenetic characterizations (SOKAL 1986). Discriminant Canonical Analyses (DCAs) were elaborated, with a stepwise method of statistical significance (HAIR et al. 1995), and applied on basic matrices to observe the capacity of discrimination between the species analysed and between the classes of altitude. This allowed us to determine the capacity of discrimination per species or per altitudinal class, according to the F value for statistical significance of the  $R^2$  (Rao's approximation).

The genealogical analysis from the contingency matrix was also developed through a similarity characterization by a cluster analysis of the average values per group of analysis according to UPGMA linkage distance and city-block distances, based on the previous lack of correlation between variables (HAIR et al. 1995) and a correlative description by a multifactorial analysis (principal component analysis –PCA–) taking into account the first three factors.

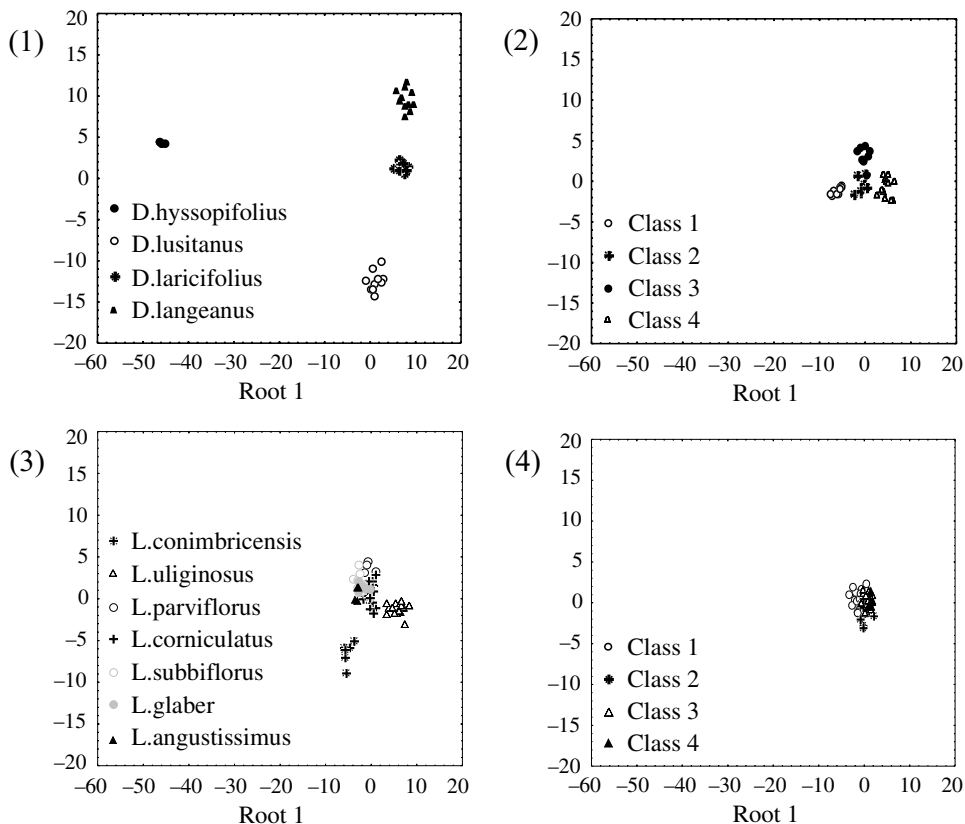
Finally, and with the objective of describing the whole capacity of morphological expression per genus, according to the altitudinal variation, the Highest Expressivity Amplitude (HEA) is calculated. This parameter is based on the largest difference between the smallest and the highest value, in this case per the totality of OTUs analysed in the contingency matrix.

## Results

The graphic results obtained for the DCA per species and per altitudinal class from the basic matrices, for the genera *Dianthus* and *Lotus* are illustrated in figure 2.

The DCA results per species for the two genera investigated show a high discrimination:  $F = 136,5$  for the species of *Dianthus* and  $F = 73,5$  for the species of *Lotus*. In contrast, the capacity of discrimination per altitudinal class is very low in the two cases examined: for the *Dianthus* species the discrimination is higher than for *Lotus* ( $F = 13,5$  and  $F = 7,4$ , respectively). This is probably due to their tendency of adopting preferential altitudinal distributions, not so significant as the taxonomic behaviour: the species of *Dianthus* have a preferential distribution in higher classes than the *Lotus* species. However the general distribution is more equilibrated for the species of *Dianthus*, since an accumulation of diversity in the lowest class for the species of *Lotus* is evident (Fig. 3).

It is noteworthy that the correlational behaviour for the genus *Lotus* reveals larger variation in the morphological expression for the lowest class of altitude (class 1), in contrast



**Fig. 2.** DCAs for the species of *Dianthus* (1) and for their altitudinal distribution per classes (2); DCAs for the species of *Lotus* (3) and for their altitudinal distribution per classes (4).

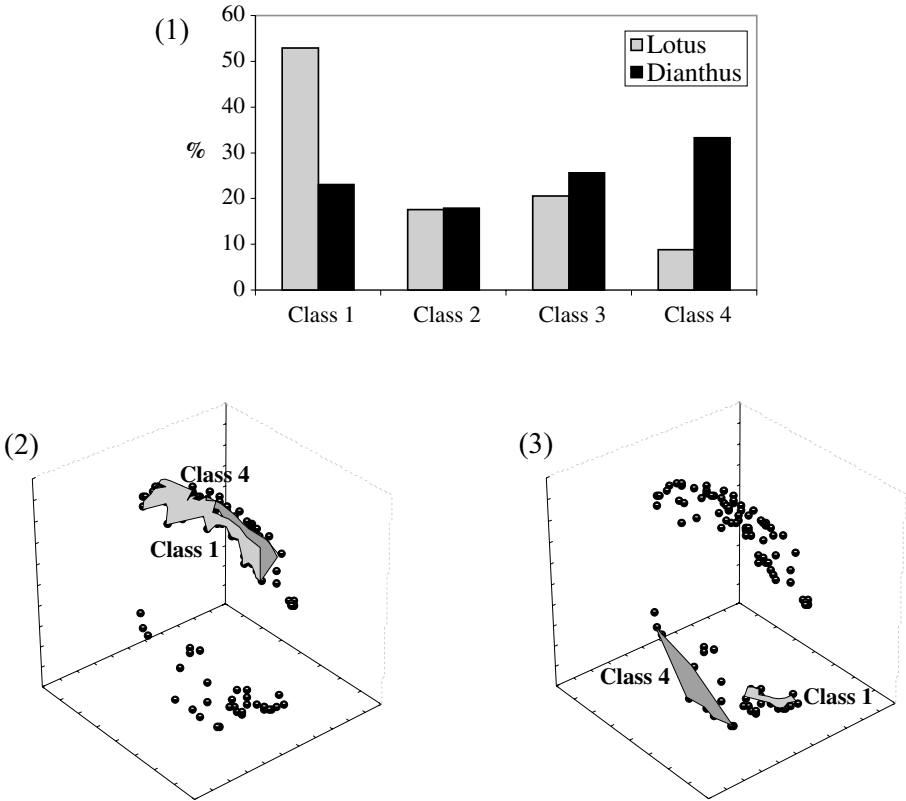
with the variation observed for the genus *Dianthus* which develops larger correlational variation in the highest class (class 4).

The correlation group analysis (CRESPI et al. 2001) allowed us to determine eight groups which were grouped in four classes, according to the UPGA distance analysis (Fig. 4)

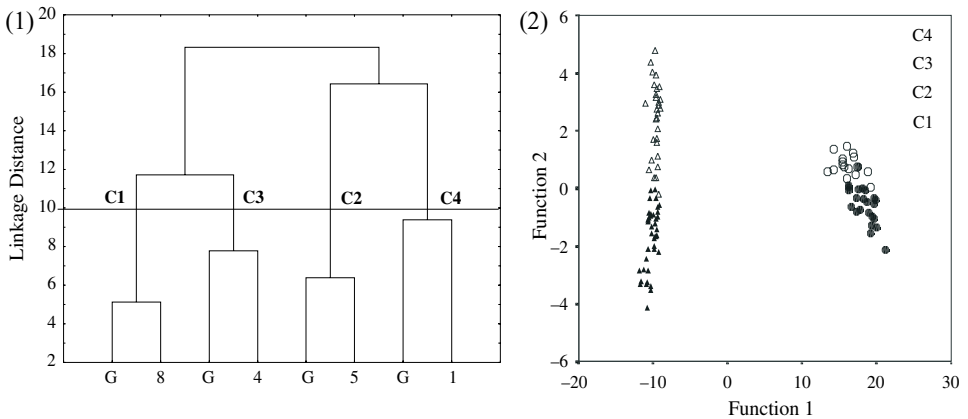
The DCA regarding these four classes suggests two different tendencies: the tendency of groups 7, 3, 6 and 2, and the tendency of the remaining correlation groups.

Table 2 distributes the individuals per tendency: the first tendency (groups 7, 3, 6 and 2) comprises the specimens of *Lotus*, whereas the second tendency (the remaining groups) contains the specimens of *Dianthus*.

The HEA analysis showed the largest values in the two highest classes (classes 3 and 4): The comparison between the HEA per class and the average values for the sum of the average variables per class is given in Fig. 5A and the average of the four altitudinal intervals per genus and class is represented in Fig. 5B. Based on these results we may confirm that the HEA for *Lotus* is lower than for *Dianthus* and their expressivities are altitudinally opposed.



**Fig. 3.** Histogram for the percentage of presence of individuals of the genus *Lotus* and *Dianthus* per class of altitude (1); PCAs for the contingency matrix (first three factors) with the indication of the correlation area for the classes of altitude 1 and 4 in the genus *Lotus* (2) and *Dianthus* (3).

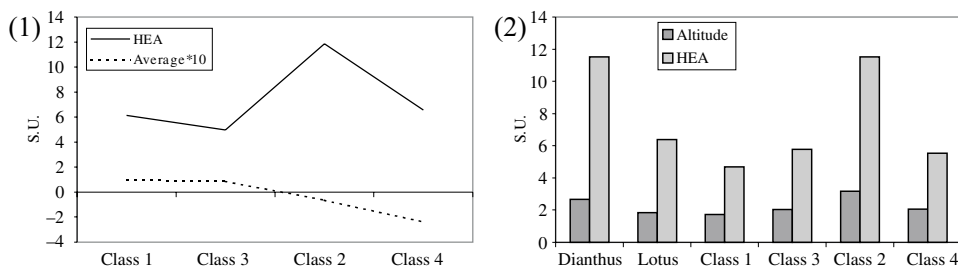


**Fig. 4.** Dendrogrammatic representation of the eight correlation groups obtained from the PCA regarding the contingency matrix (1); DCA representation of the four classes deduced by the similarity analysis (2).



**Tab. 2.** Relation of groups of correlation (Gr.) and individues (Indiv.) included in each group (H = *Dianthus hyssopifolius*; L = *D.lusitanus*; LR = *D.laricifolius*; LN = *D.langeanus*; C = *Lotus corniculatus*; U = *L.uliginosus*; S = *L.subbiflorus*; G = *L.glaber*; A = *L.angustifolius*; P = *L.parviflorus*; N = *L.conimbricensis*).

Gr.	Indiv.	Gr.	Indiv.	Gr.	Indiv.	Gr.	Indiv.	Gr.	Indiv.	Gr.	Indiv.	Gr.	Indiv.	Gr.	Indiv.
1	H1	4	L3	5	H2	8	L10	2	C4	3	C1	6	U3	7	P2
1	H3	4	L8	5	L11	8	LN11	2	U1	3	C2	6	S2	7	G1
1	H4	4	LN1	5	L2	8	LN4	2	C7	3	C3	6	A1	7	G2
1	L1	4	LN10	5	L4	8	LN9	2	U2	3	C5	6	P1	7	S1
1	L5	4	LN2	5	LR6	8	LR3	2	U5	3	C6	6	C11	7	C21
1	L6	4	LN3	5	LR8	8	LR9	2	S3	3	U4	6	C19	7	N1
1	L7	4	LN5					2	U6	3	C8	6	S7	7	C10
1	L9	4	LN6					2	U7	3	S9	6	U13	7	S5
1	LR11	4	LN7					2	U8	3	N2	6	N6	7	N3
1	LR12	4	LN8					2	C13	3	G3			7	N4
		4	LR1					2	C17	3	S4			7	S6
		4	LR10					2	C18	3	C9			7	P3
		4	LR13					2	U10	3	C12			7	S10
		4	LR2					2	G4	3	C14			7	A6
		4	LR4					2	U11	3	C15			7	N5
		4	LR5					2	U12	3	C16				
		4	LR7					2	U14	3	U9				
								2	S8	3	A2				
								2	U15	3	A3				
								2	C22	3	A4				
										3	C20				
										3	A5				
										3	P4				
										3	S11				



**Fig. 5.** Variation of the HEA per classes of correlation groups and of the average of the sum of the average values of each variable of the contingency matrix (1) –S.U. = Structural Units-; histogram of the variation of HEA and average of the altitudinal classes per genus (left side) and classes of correlation groups (right side) (2).

## Discussions and conclusions

The methodology proposed has been used to compare the phenotypical plasticity (or morphological expressivity of each individual examined) of different groups of individuals subjected to different environmental factors. In this work the environmental factors were analysed in terms of altitudinal variations, because of the climatic variations associated with the latter.

The phenotypical plasticity analysis proposed here is based on the comparison between the behaviour of the taxa included in a defined group (genus in this case) and the phenotypic plasticity of the groups studied. The description of the intra-group and inter-group taxa is fundamental to obtain a wider perspective of the morphological variation for the taxa analysed. The most difficult task was to combine the morphologic information contained in the basic matrices for two very different groups of species (the species of the genus *Dianthus* and of the genus *Lotus*). Our study suggests that the contingency matrix elaborated with amplitude and average parameters, retrieved from the respective basic matrices, contributes to the genecological characterization and comparison. In fact, the multivariate approach applied showed the opposite behaviour of the species of *Dianthus* and *Lotus* with respect to the morphological expressivity, in the present case as a function of their altitudinal occurrence. These data fully confirm the morphological apparent effects pointed out previously by our group (CRESPI 1999, CRESPI et al. 2000) on the basis of chorological data, in particular the tendency of the *Dianthus* diversity for the average and highest altitudinal classes, as opposed to the tendency for the lowest classes detected in the case of the *Lotus* species.

The comparison of the specific and altitudinal DCA differentiation for the two genera is clearly more discriminant in the *Dianthus* case: the specific classification of the individuals reached high values of F in both genera, although the latter were more elevated for the genus *Dianthus*. Consequently, the morphological-altitudinal correlation will be just considered under the perspective of tendential behaviour.

The genecological approach to the taxa based on the altitudinal variation is described from the application of one contingency matrix. In fact, the application of contingency matrices has been very successful in ecological characterizations of the structure of vegetation (CRESPI et al. 2001a, b, c), where very different types of vegetal communities were compared. In this case, the amplitude parameters may be useful types of variables to characterize the effects of the environmental factors on the morphological expressivity, and ultimately to describe the phenotypic plasticity of the groups of individuals under analysis. A larger morphological variability was detected for the highest classes of altitude, based on the values of HEA, whereas it was reduced for the lower classes. The variations observed in the correlation areas per genus, or by the discriminant analysis per altitude class, show a characteristic polarity in the morphologic response of the groups of individuals to the variation of altitude: the morphologic expressivity in the lowest class (class 1) and that in the highest class (class 4). Therefore, the amplitude parameters used provided extremely valuable information to describe the observed morphologic expressivity of the individuals.

The use of the amplitude of morphologic variation parameters to characterize phenotypic behaviour has not been mentioned in the literature. The transformation of the data into amplitude parameters was essentially elaborated with quantitative characters (just 5% of them were qualitative), without any related parameters. In the present case, the application

of a contingency matrix implied the use of amplitude parameters, which allowed the determination of the degree of conservatism, according to FARRIS 1966) of the characters analysed. In terms of phenotypic correlation, the application of amplitude parameters is thus very useful for the comparison of the reaction norms of the individuals (SCHMALHAUSEN in WAITT and LEVIN 1993) under different environmental conditions, according to the profile of phenotypes produced among them. The integration between the conservatism of the morphological characters and the reaction norms of the individuals determines the amplitudes of their phenotypic profiles for each environmental class and, consequently, the behavioral adaptability of the groups analysed to the influence of external environmental heterogeneity (WCISLO 1989) or, under the point of view of SCHMALHAUSEN (1960), the phenotypes evolved in the biogeocoenose – environmental information – analysed.

The differences of amplitude observed in the lowest and the highest classes of altitude may be explained in terms of adaptability or nonadaptability plasticity (SCHEINER 1993). Higher values of HEA may involve more instability (BRADSHAW 1965) than lower values. In this work the classes of altitude 3 and 4, with more restrictive climatological conditions, must be considered as agents of development (WEST-EBERHARD 1989) in the stimulation of a polymorphism reaction for the individuals of the genus *Dianthus*, in contrast with individuals of the genus *Lotus*, with more limited morphological expressions in these altitudinal classes.

The comparison of the morphological expressivities from individuals of different groups of genera will be a very useful approach for the description of their genecological behaviour according to environmental factors. In terms of future descriptions, proposed by RAVEN (1974) or KAPLAN (2001), the application of numerical methodologies in the morphological characterizations suggests the possibility of applying several types of parameters and analytical matrices which may provide valuable knowledge regarding the phenotypic plasticity of the individuals. The methodology proposed describes the morphological expressivity (i.e., genecological behaviour) of the individuals examined according to the environmental factor selected (the altitude in the present case): the species of the genus *Lotus* concentrate their expressivities at low altitude, in contrast with the species of the genus *Dianthus*, expressivities of which are mainly revealed in higher intervals of altitude. Consequently, the use of contingency matrices appears to be very useful in a comparison of the morphologic variability of the individuals of different taxa with respect to environmental variations, independently of the genetic variation.

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