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Univariate and Multivariate Analysis on Phenotypic Divergence in *Phleum*

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Summary. An analysis of phenotypic and genetic variability was performed on *Phleum ambiguum* populations obtained by vegetative propagation and grown in different environments. The investigation on a single character and the canonical analysis on several plant traits indicate that, while genetic variability (h^2) is essentially constant, phenotypic variance and covariance are affected by different environments and successive clonations. The observed changes seem not to be reversible when plants, transferred to a different environment, are returned to the original one. Within-clones variability seems to be affected by environmental conditions without being directly related to them. The results reported seem to be in good agreement with the phenotypic divergence hypothesis.

Key words: *Phleum ambiguum* – Variability – Divergence hypothesis

Introduction

Phenotypic modifications of perennial grasses following environmental changes, are usually attributed either to phenotypic plasticity or to intrasomatic selection.

The presence of intrasomatic selection was shown in *Lolium* (Breese et al. 1965) and was supposed to result in divergence among clones (Hayward 1970; Shimamoto and Hayward 1975). On the other hand, intrasomatic selection was found not to be a sufficient explanation for the results observed in *Phleum ambiguum* involving changes of both 'between and within clones' phenotypic variability (Palenzona and Micardi 1967; Palenzona, Cavicchi and Micardi 1973). In particular the intrasomatic selection hypothesis was not consistent with the estimates of phenotypic covariance between propagules obtained from the same plant by clonation. An hypothesis was proposed on the basis of these results assuming that irreversible phenotypic changes may result from both clonation and environmental change that are not attributable to genotypic changes: a phenomenon similar to that of phenotypic plasticity or phenotypic convergence (Baldwin 1896; Bradshaw 1965).

An experiment was designed to test whether the change of phenotypic variability is consistent with the phenotypic convergence hypothesis.

An estimate of the phenotypic changes was attempted by a multivariate canonical analysis to ascertain whether additional information could be obtained by considering correlations among several traits.

Material and Methods

Seeds of a wild population of *Phleum ambiguum* were collected and allowed to grow on Monte Terminillo, near Rieti in central Italy, in single plant culture in the experimental fields of the Centro Appenninico di Genetica C. Jucci, at 1000 m. altitude.

By vegetative propagation, two experimental plots were established in 1965, one at 1000 m and another at 300 m altitude; in each plot 5 propagules from each of 80 mother plants were completely randomized; the distance between propagules was 50 cm.

In the next two years two successive vegetative propagations were performed on these populations to produce all possible combinations of three-step environmental sequences of the two locations (1000 m = B; 300 m = A) considered.

The experimental design may be summarized as follows:

1965	1966	1967	1968	1969	1970	1971	1972	1973
A	AA	AAA						
		AAB						
	AB	ABA						
		ABB						
B	BA	BAB	data collection			Cor-		data
		BAA				niolo		col-
	BB	BBA				trans-		lection
		BBB				plant		

After the first, clonations were performed by halving every propagule, one half to be transplanted at the same altitude, the other one at the alternative location. Three clonations were performed at Terminillo, then the plants were allowed to grow with only

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rearing care. Measurements were collected in 1967, 1968, 1969, 1970.

In autumn 1971, 5 years after the last clonation performed at Terminillo, 10 clones were chosen in each of two populations BBB and BAB, 5 clones showed the highest 'within clones' variance estimates, the remaining 5, the lowest ones. Each propagule from these clones was subdivided into 12 parts which were all transplanted in experimental fields at Corniolo, near Forli, at 900 m altitude, 300 miles north from the original location. The propagules were completely randomized in the field.

Four traits were measured on all the populations considered:

x_1 – spike length
x_2 – 1st internode
x_3 – plant height
x_4 – stem number

Four additional traits were added for the populations located at Terminillo:

x_5 – diameter of the tuft at the base
x_6 – diameter of the tuft at mid-height
x_7 – 2nd internode
x_8 – green weight

Characters 1, 2 and 7 are given as the average of five measurements taken on 5 stems for each propagule.

A canonical analysis was performed using a computer program proposed by Davies (1971), partially modified. The analysis of data collected on populations grown at Corniolo was performed by reduced eigen vectors to obtain unit 'within-groups' variance, by means of Seal's algorithm.

Results

a) Analysis of plant height

'Between and within clones' variance estimates are shown in Table 1 for each subpopulation obtained in successive vegetative propagations. The heritability estimates (h^2) are quite similar for the eight subpopulations, in agreement with the fact that the subpopulations were obtained by cloning.

On the other hand phenotypic variance shows a certain degree of differentiation between subpopulations that may be ranked according to the 'dosage' of the two environments A and B; indeed it increases markedly from AAA to BBB regardless of the actual location. Populations located in the same environment, A or B, at the end of the clonation and transplanting processes, were each compared with AAA or BBB populations respectively (Table 2). It may be observed that interaction variances plant x clonation, contributed by the differences among clones from the same mother plant in two different subpopulations, are smaller in comparisons 1 and 4 than in comparisons 2, 3, 5, 6.

To evaluate these differences of phenotypic variability correctly it should be noticed that mean values are quite similar for the eight populations considered, and that some of the different phenotypic variances are found in populations differing only for the sequences of environmental change.

Mean values and standard errors are reported in Table 3 for the populations transplanted into Corniolo (D) experimental fields, together with the corresponding values of the populations grown at Terminillo (F).

Each population was subdivided into two subpopulations (High and Low variability) according to the estimates of variance between propagules within each clone, on plant height data collected at Terminillo.

It appears that mean values are not affected by the

Table 1. Estimates of phenotypic variances and heritability (h^2) relative to the eight populations grown at Terminillo

	total	between clones	within clones	h^2
AAA	32.352	79.213^{++}	11.850	0.636
AAB	35.541	89.046^{++}	10.826	0.596
ABA	43.658	90.285^{++}	16.963	0.613
BAA	50.126	135.926^{++}	16.664	0.619
ABB	45.049	105.419^{++}	21.852	0.548
BAB	58.965	130.561^{++}	25.571	0.660
BBA	55.319	100.151^{++}	21.693	0.609
BBB	63.084	154.194^{++}	23.222	0.634

$^{++}$ $P < 0.01$

Table 2. Comparisons between populations (clones) maintained in the same environment (AAA and BBB) and those which are actually in the same environment after various environmental changes

comparisons	between clones	between clonations	plants X clonation	within clones
1. AAA – ABA	96.496^{++}	25.905	16.014^{+}	7.498
2. – BAA	128.595^{++}	52.509^{+}	75.196^{++}	10.442
3. – BBA	102.361^{++}	0.921	78.642^{++}	15.419
4. BBB – BAB	405.409^{++}	10.453	31.747	21.157
5. – ABB	175.632^{++}	25.716	133.575^{++}	25.308
6. – AAB	160.179^{++}	3.0	155.149^{++}	18.079

$^{+}$ $P < 0.05$
$^{++}$ $P < 0.01$

Table 3. Mean values ± s.e. in the four subpopulations transplanted from Terminillo to Corniolo

		Terminillo	Corniolo
BBB	H	64.00 ± 1.89	46.40 ± 0.66
	L	67.15 ± 0.89	45.00 ± 0.45
BAB	H	64.20 ± 1.64	46.84 ± 0.59
	L	65.30 ± 1.13	45.69 ± 0.48

High and Low variability classification. Differences are instead observed between F and D environmental conditions, which are shown to be more effective in changing the mean values than the two localities considered at Terminillo.

Estimates of phenotypic variability, either total or within clones variance (Table 4), show a disappearance of the differences between High and Low variability subpopulations when transplanted in D environment; there is a decrease of High and an increase of the Low variability subpopulations variance values. Total variance values in BBB and BAB, regardless of the High and Low classifications, appear to be reduced in D compared with F environment.

b) Canonical analysis

Mean values of the measurements collected on populations grown at Terminillo are shown in Table 5, while Table 6 shows the mean values for the four subpopulations (BBB, High and Low variability; BAB, High and Low variability) grown at Terminillo and at Corniolo. It may be observed that the environment where the plants are actually located has a generally larger effect on mean values than the previous environmental changes and clonations.

The results from the canonical analysis for data collected on Terminillo populations are given in Table 7 (eigen values and vectors) and in Fig. 1. (relative positions of the populations considered obtained by plotting the two canonical variates). Populations in the A environment are discriminated from those in B by the first axis of variation (λ_1); populations which have been subjected to different environmental sequences are mainly distinguishable by the second (λ_2), but also by the abscissa values, especially those populations located in the A environment.

Table 8 and Figs. 2 and 3 show the results from the canonical analysis performed on subpopulations High and Low variability located at Corniolo and at Terminillo. Discrimination seems to be consistent compared with both first (λ_1) and second (λ_2) direction of variation at Terminillo: High and Low variability populations have different λ_1 values while the BBB and BAB sequences exhibit distinct ordinates.

When the same subpopulations are grown at Corniolo they show a greater overlapping as to both axes (Fig. 3).

Table 4. Estimates of phenotypic variance relative to the four subpopulations transplanted from Terminillo (F) to Corniolo (D)

		BBB			BAB		
		total	between clones	within clones	total	between clones	within clones
High	F	68.210	160.875^{++}	43.500	51.082	136.325^{++}	28.350
	D	36.685	255.179^{++}	25.760	36.046	482.096^{++}	18.203
Low	F	14.905	34.175^{++}	9.766	24.326	95.175^{++}	5.433
	D	19.991	47.871^{+}	18.793	27.025	183.516^{++}	21.436

$^{+}$ $P < 0.05$
$^{++}$ $P < 0.01$

Table 5. Mean values ± s.e. for the traits observed on the eight populations at Terminillo

	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8
BBB	7.37 ± 0.09	33.86 ± 0.24	63.44 ± 0.49	94.11 ± 3.58	19.82 ± 0.24	35.05 ± 0.47	14.93 ± 0.19	106.49 ± 4.16
BAB	7.67 ± 0.08	33.99 ± 0.27	64.05 ± 0.53	108.42 ± 3.87	20.27 ± 0.23	36.03 ± 0.52	15.41 ± 0.19	122.95 ± 5.19
ABB	7.85 ± 0.08	33.49 ± 0.22	63.69 ± 0.41	118.75 ± 4.27	21.34 ± 0.22	38.27 ± 0.48	14.75 ± 0.17	152.07 ± 6.39
AAB	7.70 ± 0.09	34.02 ± 0.26	64.65 ± 0.55	97.87 ± 4.25	21.56 ± 0.26	37.09 ± 0.56	14.85 ± 0.19	124.93 ± 6.01
AAA	7.39 ± 0.07	31.45 ± 0.22	62.46 ± 0.37	131.74 ± 3.52	24.35 ± 0.26	40.94 ± 0.45	15.15 ± 0.14	218.60 ± 7.03
ABA	7.27 ± 0.08	32.32 ± 0.25	61.02 ± 0.45	116.97 ± 3.44	24.73 ± 0.25	38.01 ± 0.42	15.42 ± 0.16	200.63 ± 6.49
BAA	7.29 ± 0.09	31.38 ± 0.26	59.81 ± 0.61	125.89 ± 5.44	23.42 ± 0.32	39.26 ± 0.59	14.51 ± 0.19	189.33 ± 8.77
BBA	7.72 ± 0.10	31.52 ± 0.33	59.29 ± 0.57	131.67 ± 4.71	22.64 ± 0.29	40.19 ± 0.54	14.84 ± 0.20	177.70 ± 7.76

Table 6. Mean values ± s.e. for the traits observed on the four subpopulations transferred from Terminillo to Corniolo

	Terminillo				Corniolo			
	x_1	x_2	x_3	x_4	x_1	x_2	x_3	x_4
High BBB	6.95 ± 0.41	34.54 ± 0.76	64.00 ± 1.89	103.45 ± 16.07	6.65 ± 0.38	28.49 ± 0.45	46.40 ± 0.66	27.39 ± 3.65
Low	7.39 ± 0.33	35.56 ± 0.73	67.15 ± 0.89	131.10 ± 13.64	6.89 ± 0.32	27.68 ± 0.51	45.00 ± 0.45	25.71 ± 3.04
High BAB	7.88 ± 0.25	33.28 ± 0.69	64.20 ± 1.64	125.35 ± 13.89	6.81 ± 0.33	29.00 ± 0.67	46.84 ± 0.59	23.83 ± 1.87
Low	8.15 ± 0.19	34.06 ± 0.72	65.30 ± 1.13	128.90 ± 17.29	7.14 ± 0.25	27.65 ± 0.79	45.69 ± 0.48	26.28 ± 3.54

Table 7. Eigen values (λ_i) and vectors (Z_i) from the canonical analysis performed on the Terminillo populations

	1	2						
λ_i	0.469	0.145						
% discr.	76.37	23.63						
χ^2	928.25(14)	237.11(12)						
P	0.005	0.005						
	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8
Z_1	−0.1275	−0.1874	−0.1567	−0.0590	0.7951	−0.1360	0.5176	0.0449
Z_2	−0.3088	−0.2745	0.5461	−0.0439	0.1211	−0.0191	−0.7168	0.0177

Table 8. Eigen values (λ_i) and vectors (Z_i) from the canonical analysis performed on the four subpopulations transferred from Terminillo to Corniolo

	Terminillo			Corniolo		
	1	2	3	1	2	3
λ_i	0.1429	0.0492	0.0096	0.0849	0.0446	0.0095
% discr.	70.8	23.4	4.8	61.1	32.1	6.8
χ^2	10.02(6)	3.60(4)	0.72(2)	5.87(6)	3.14(4)	0.68(2)
P	0.10	0.50	0.75	0.50	0.50	0.75
	Z_1	Z_2	Z_3	Z_1	Z_2	Z_3
x_1	0.6927	0.1707	0.5599	0.6187	0.0890	0.2665
x_2	−0.1014	0.1296	0.4061	−0.0319	0.4406	−0.0996
x_3	−0.0335	0.0632	−0.1823	−0.2025	−0.1986	0.0993
x_4	−0.0015	0.0056	−0.0069	−0.0014	−0.0246	−0.0653

Discussion

Previous results had shown that phenotypic changes arising in *Phleum* populations following vegetative propagation are scarcely detectable on the basis of mean values and heritability estimates, while they are quite evident when covariance estimates are considered.

The persistence of the observed changes, their amount and the fact that they show no simple relationships with the environmental changes may suggest a genetic continuity of the factors involved; the fact that the observed changes have been obtained following vegetative propagation could indicate that intrasomatic selection may be responsible for the phenotypic modifications observed. Even considering the evident stability of heritability estimates, intrasomatic selection is quite difficult to rule out: directional intrasomatic selection seems however not to be consistent with the above results since it is expected to reduce genetic variability.

Estimates of phenotypic variability for plant height seem to be consistent neither with a disruptive intrasomatic selection hypothesis, nor with a stabilizing selection effect: no clear cut trend is detectable toward an increased or decreased phenotypic variance, while covariance has been shown to be consistently affected (Palenzona, Cavicchi and Micardi 1973).

Changes of phenotypic variability for plant height as well as the results of the canonical analysis observed in Low and High variability populations seem to oppose the intrasomatic selection hypothesis and, instead, to be in agreement with the concept of phenotypic convergence (Bradshaw 1965). Following this, the obtained results may be satisfactorily explained as follows:

i) Cloning and/or environmental changes result in a loss

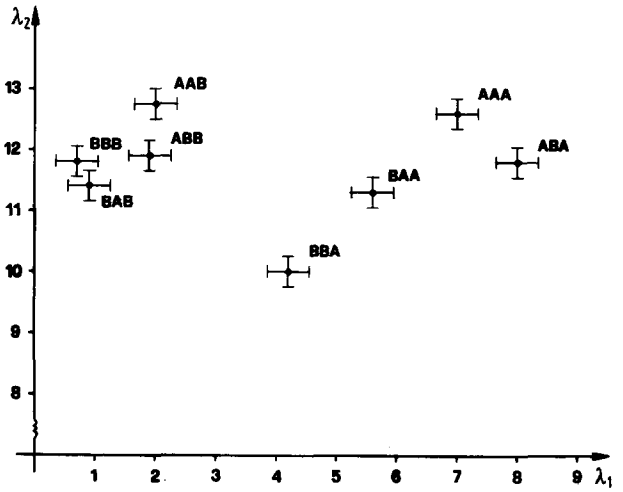

Fig. 1. Graphical representation of the results obtained from the canonical analysis performed on the eight populations at Terminillo

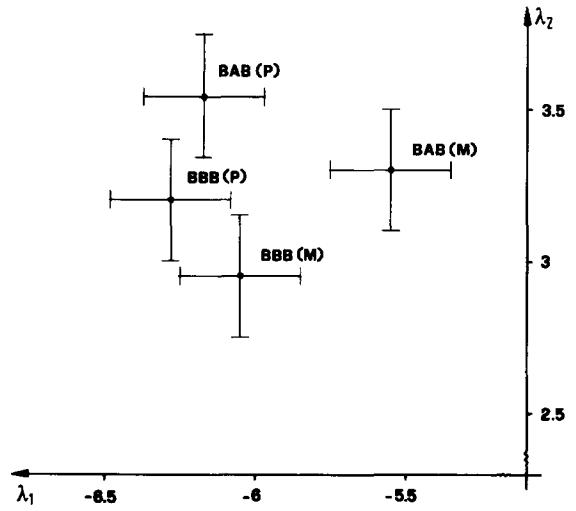

Fig. 3. Graphical representation of the results obtained from the canonical analysis performed on the four subpopulations at Corniolo

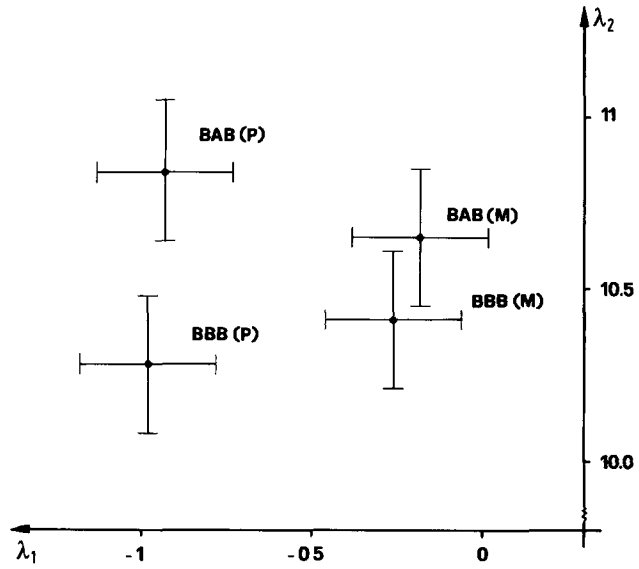

Fig. 2. Graphical representation of the results obtained from the canonical analysis performed on the four subpopulations at Terminillo

of phenotypic stability (Mather 1953; Waddington 1957) determined by random fluctuations independent of the agents which have produced them; an increase of 'between propagules' variance may be observed, which is partially under genetic control (genotype x environment interactions become significantly higher than error variance).

ii) A new phenotypic equilibrium is reached in the new environment after 2-3 years from the last clonation (h^2 estimates increase and stabilize around a common value); clonation and environmental changes result in lower phenotypic variability than clonation alone (BBB higher than AAA).

iii) Comparisons between populations cloned and maintained in the same locality (AAA and BBB) with those transplanted in different environment (Table 2) show that 'plants by clonations' variances differ strikingly according to the sequence of environmental changes (AAA-ABA vs. AAA-BAA; BBB-BAB vs. BBB-ABB), while the 'between clones' variance differs significantly from error variance. This strongly suggests that changes of phenotypic variability are attributable to plant-by-environment interactions.

iv) High and Low variability subpopulations show an increased overlapping, both in terms of plant height variance and of discrimination by canonical analysis, when transferred from Terminillo to Corniolo; this suggests that phenotypic variability, having reached an equilibrium point in the Terminillo environment, can be rearranged at random when in the Corniolo fields.

v) Previous environmental sequences and actual growing locations are detected by canonical analysis as different sources of variability (Fig. 1), suggesting that plant x environment interactions are a complex phenomenon involving different factors, some of whose effects appear to be memorized by the plant.

As a whole the reported results seem to indicate that the phenotypic variability of a population in which genetic variability changes are not detectable may be under different types of controlling factor, whose effects seem to be partially memorized by the plants.

The data seem to fit reasonably well with the phenotypic convergence concept. It seems also that both variation and covariation have to be taken into account to obtain a more complete representation of the mechanisms involved in phenotypic changes.

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