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Association of protein amount polymorphism (PAP) among maize lines with performances of their hybrids

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Summary. It has been suggested that molecular foundations of phenotypic diversity reside in the variability of genome expression. This variability can be appraised through the polymorphism of individual protein amounts (PAP: protein amount polymorphism). Eight maize inbred lines and ten of their single-cross hybrids were analyzed by two-dimensional polyacrylamide gel electrophoresis in order to examine the potential of PAP for predicting hybrid vigor. The 28 possible pairs of lines were characterized for: (i) the number, H of expected heterozygous structural loci in their hybrid, in the sample of loci revealed by 2D-PAGE; (ii) four distance indices based on PAP; (iii) the hybrid values for five agromorphological characters measured in four different year/locations. For the subset of ten hybrids analyzed by 2D-PAGE, the number of cases of nonadditive inheritance (NA) was also counted. Whereas H appeared to be related neither to the PAP indices, nor to NA, nor to hybrid performances, PAP indices were correlated to NA, and both were positively associated to hybrid performances. The possibility that PAP is responsible for quantitative trait variation is discussed. This could result in the definition of biological predictors of heterosis.

Key words: *Zea mays* L. – Two-dimensional electrophoresis – Protein amount – Genetic variability – Heterosis

Introduction

A permanent challenge of maize (*Zea mays* L.) breeders is to identify inbred lines that produce hybrids exhibiting high levels of heterosis. In many crops, predicting heterosis from agromorphological performances of parental lines is very unreliable (Siddiqui et al. 1976; Shifriss and Sacks 1980; Ghaderi et al. 1984; Smith and Smith 1989 a). Predictors computed from testcross data (lines × testers) may be much more efficient (Marchais 1978; Charcosset et al. 1990). However, morphological data are affected by environmental factors and their measurement is expensive and time-consuming.

Allozyme and restriction fragment length polymorphism (RFLP) techniques provide a virtually unlimited number of neutral markers distributed throughout the genome. Given the theoretical relationship between heterozygosity and heterosis, such markers can substitute for kinship coefficients to predict heterosis between related lines (Smith and Smith 1989 b). However, the power of neutral markers to predict heterosis in the range of genetic distances explored by maize breeders seems rather low (Frei et al. 1986; Lee et al. 1989; Melchinger and Lee 1990). Actually, the only relevant loci in terms of phenotypic expression of heterosis are the QTLs (quantitative trait loci). Since the number of QTLs with significant effect for a given character is limited, the prediction could be improved by selecting markers in linkage disequilibrium with QTLs, which are not likely to be common among unrelated genotypes. Increasing the number of random markers will necessarily result in decreasing the correlation by increasing the number of noninformative markers (Charcosset et al. 1991).

Parameters derived from the variability of genome expression could provide a source of nonneutral markers. Some authors have proposed that the evolution of patterns of gene expression during development lies at the heart of organismal evolution (for a recent review, see Atchley 1989). The question then arises as to whether or not the variability of genetic expression also plays a role

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in the phenotypic diversity at the intraspecific level. This variability can be assessed through the polymorphism of individual protein amounts (PAP), which is consistently detected when comparing genotypes by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of denatured proteins (Klose 1982; Zivy et al. 1983, 1984; Bahrman et al. 1985). PAP is not significantly affected by environmental fluctuations (de Vienne et al. 1988) and the relationships between genotypes do not depend on the organs or developmental stages analyzed (de Vienne et al. 1988; Seguin 1990). Moreover, the genetic determinism of PAP can be merely monogenic (Bahrman and Damerval 1989). Finally, PAP has been shown to reflect pleiotropic phenomena at the protein level that corresponded to major morphological changes (Gottlieb and de Vienne 1988).

A study by Damerval et al. (1987a) showed that PAP between five maize lines was related to a Mahalanobis distance computed from general combining abilities of 14 morphological and developmental traits. More recently, we found nonadditive inheritance for protein amounts in hybrids of maize (de Vienne et al. 1988; Leonardi et al. 1988; Leonardi 1989).

In the present study, 28 maize single-cross hybrids of a diallel design between eight parental lines were characterized for agromorphological performances in four different environments. PAP analysis was made from coleoptiles of the parental lines. Furthermore, for the ten hybrids between five of the parental lines, the cases of nonadditive inheritance of the protein amount variations were counted. The aim of this study was to evaluate the extent to which the variability of genetic factors involved in genetic expression and their interactions are relevant to the phenomenon of heterosis.

Materials and methods

Plant material

Eight maize lines (*Zea mays* L.) involved in a diallel cross were studied: F2, F1254, F1772 (all flint type), F188 (flint-dent type), F252, W117, F220, and F154 (all dent type). These lines were chosen for their different endosperm types and because they belong to different groups of combining ability. For the lines F188, F220, and F154, the coleoptiles of 8-day-old etiolated seedlings were analyzed by 2D-PAGE. For the other five lines (F2, F1254, F1772, F252, and W117) and their ten single-cross hybrids, the coleoptiles of 8-day-old etiolated and green seedlings were analyzed by 2D-PAGE.

Kernels were grown either in the dark at 24 °C (etiolated seedlings) or under a 16 h photoperiod with a thermoperiod of 16 h at 25 °C light/8 h at 22 °C dark (green seedlings). In both cases, coleoptiles were harvested from 8-day-old seedlings. Each sample consisted of one individual coleoptile. At least three samples were analyzed for each genotype.

The eight lines and their 28 single-cross hybrids were evaluated for five agromorphological characters under four environmental conditions, as described below.

Two-dimensional electrophoresis

The procedure used involved various modifications and simplifications of the original O'Farrell technique (1975), which resulted in high resolution 2-D gels with improved reproducibility.

The protein extraction procedure, avoiding protease action, was as described in Damerval et al. (1986). Seventy microliters of resolubilization solution was used to resuspend 1 mg of protein pellet. Isoelectric focusing (IEF) was as described in Leonardi et al. (1987). Protein concentration in the extract was determined according to Scopes (1974) to ensure similar total protein amount on each 2-D gel. Thirty microliters of solubilized extract was layered on top of each IEF gel, which corresponded to about 60 µg of total proteins. The second (SDS) dimension and the silver staining were performed as in Damerval et al. (1987b), with 20 2-D gels bound to GelBond PAG films simultaneously treated (Granier and de Vienne 1986).

Scoring procedure

The gels were compared visually by superimposition, using a lightbox. The assessment of spot position was checked by coelectrophoreses 1:1 of the line samples for each of the 28 line pairs. Only the major reproducible differences in relative spot intensities were considered. Moreover, we retained as quantitative variation only those cases in which the spot intensities grouped into well-separated classes. Depending upon the polypeptides, two, three, or four classes of intensities were found. Thus, our visual analysis underestimated the actual quantitative variation, since the continuous variations over the lines were not considered.

For the ten hybrids analyzed by 2D-PAGE, the cases of nonadditive inheritance found in coleoptiles of etiolated and green seedlings were counted. Hybrid patterns were compared to parental line patterns and to coelectrophoreses of the parental samples, these latter representing the expected hybrid patterns under the hypothesis of additivity of protein amounts. The inheritance was considered as nonadditive only when the hybrid spot differed from the coelectrophoresis spot, and was either similar to one of the parental spots, or more intense than the most intense, or fainter than the faintest parental spot (Leonardi et al. 1988).

Molecular parameters

All the molecular parameters defined below were computed from 2D-PAGE data of coleoptiles of 8-day-old etiolated seedlings.

When two inbred lines are compared by 2D-PAGE, the protein variation can appear as two forms: quantitative – the spot intensity groups into two different classes; qualitative – two close polypeptide spots with similar staining, and differing usually in their isoelectric point, are mutually exclusive between the two lines.

The latter case may correspond either (i) to posttranslational modifications controlled by a polymorphic modifier locus (or loci): the action of such modifiers had been put forward to explain occurrence of allozymes, but it seems to represent a quite exceptional mechanism to account for biochemical polymorphism; or (ii) to allelic variation of the gene coding for the polypeptide. Allelic variations of structural genes are detectable by 2D-PAGE when amino acid substitutions change the protein conformation, which results in a difference in apparent molecular mass or, more frequently, when substitutions modify the charge of the polypeptide, which results in a difference in isoelectric point (Rosenblum et al. 1983). In these cases, the visual criterion for allelism is a shift in the position of the spot. Moreover, in a set of inbred lines each line displays one and only one polypeptide of an allelic series. These two criteria allowed us to detect 32 polymorphic putative structural loci. In order to verify this genetic determinism, we further analyzed three F_2 progenies

[between F1254 and W117, between F2 and F252, and between F2 and an additional line, MBS847 (dent type)]. The allelic nature of the variations was fully confirmed for the 20 loci found to be polymorphic in these progenies (data not shown). This is consistent with Bahrman and Damerval's (1989) results in maritime pine and Zivy et al.'s (1991) results in barky which showed that the visual criterion for allelism is reliable. Considering the 32 polymorphic loci, we computed for each of the 28 pairs of lines the number, H, of loci that displayed different alleles in the two lines. Therefore, H is also the number of heterozygous structural loci in the hybrid between two lines, in the sample of loci revealed by 2D-PAGE.

On the other hand, the quantitative variation reveals the variability of any genetic factors affecting the abundance of gene products. We defined an index, PAD (protein amount dissimilarity), equal to the ratio of the number of spots displaying quantitative variation to the number of common spots between two lines (called dQ in Damerval et al. 1987a). Moreover, three other indices were derived from PAD:

– Fourteen groups of polypeptides were defined, containing 2 to 15 polypeptides per group: the quantitative variations of the polypeptides within a group were correlated over the eight lines. Due to the partition of quantitative variation into discrete classes, the correlations between polypeptides of a given group were equal to 1 or −1. An index, PAD$\overline{\text{cov}}$, was computed as PAD, except that only one spot per group was taken into account.

– The complementary index, PADcov, was computed as PAD by using the subset of covariable spots alone. The resulting number of variable spots between two lines could have been reduced to less than ten, which makes PADcov less accurate than other indices.

– PAD$\overline{\text{na}}$ was computed as PAD, except that the polypeptides displaying nonadditive inheritance in any of the ten hybrids analyzed by 2D-PAGE were excluded.

The angular transformation ($\sin^{-1}$ (i)$^{1/2}$) was made for all the indices accounting for PAP, in order to eliminate the mean-variance relationship, and they were computed for the 28 pairs of lines.

Agromorphological characters

The eight lines and their 28 single-cross hybrids were studied under field conditions in Gif-sur-Yvette (France, Essonne, latitude 49°5 N) in 1983 and 1984 and in Lusignan (France, Vienne, latitude 46°4 N) in 1981 and 1984. The design was an 8 × 9 lattice. The replications usually used consisted of single-row plots, 0.8 m apart with internal references, described in Table 1. Lines and F_1 hybrids were separately tested to avoid competition effects between the two generations.

Four characters were measured: the total plant height (TH, cm), the ear insertion height (EH, cm), the dry matter percentage when harvesting (DMP, %), and the fresh forage yield (FFY, Mg ha^{-1}). The fresh forage yield was measured by harvesting the aerial part of all the plants in a row at the ensilage stage. A sample of 800 g was then dried (48 h at 80 °C) and the ratio of the dry to the fresh weights determined the dry matter percentage. The dry forage yield (DFY, Mg ha^{-1}) was calculated from the fresh forage yield and the dry matter percentage.

Agromorphological data consisted of the values of hybrids and heterosis, calculated as the difference between the hybrid value and the mean of the two parents. Heterosis was calculated only for the data of Lusignan 1981 and Gif-sur-Yvette 1983, because data of some lines were missing in Lusignan 1984 and Gif-sur-Yvette 1984. For the two former sets of data, the correlations between the hybrid values and the heterosis values are shown in Table 2. Since both types of data gave similar information, we further considered only the hybrid values. Agromorphological data are not shown here, but are available upon request.

Table 1. Description of the trials of the diallel design

Environment		No. of replications	Length of one plot	No. of plants/plot
Lusignan 1981	Inbred lines,	5	4.5 m	35
	F_1 hybrids	5	4.5 m	35
Lusignan 1984	Inbred lines,	3	4.5 m	35
	F_1 hybrids	3	4.5 m	35
Gif-sur-Yvette 1983	Inbred lines,	2	5.0 m	38
	F_1 hybrids	2	3.0 m	28
Gif-sur-Yvette 1984	Inbred lines,	3	5.0 m	38
	F_1 hybrids	3	5.0 m	38

Table 2. Correlations, for every character, between hybrid values and heterosis, for the data from Lusignan 1981 and Gif-sur-Yvette 1983

	Lusignan 1981	Gif-sur-Yvette 1983
Total plant height	0.86**	0.79**
Ear insertion height	0.76**	0.81**
Fresh forage yield	0.89**	0.98**
Dry forage yield	0.92**	0.98**
Dry matter percentage	0.62*	0.78**

*, ** Significant at the 5 and 1% levels, respectively

Relationships between the different data

For each molecular parameter, an 8 × 8 symmetrical matrix was constructed, in which the element (i, j) corresponded to the parameter value for the pair of lines i and j. Principal coordinate analysis (PCA) (Gower 1966) was performed on each matrix in order to compare the relationships between the lines on the basis of the first discriminating plane.

All the correlations between the protein indices were computed and the significance of the correlation coefficients was tested by using permutation tests, since values of such matrices are not independent (Damerval et al. 1987a).

Correlations were also computed between the protein indices and hybrid values for each of the five agromorphological characters. The values were not available for 2 of the 28 hybrids in Lusignan 1981, for seven hybrids in Gif-sur-Yvette 1983, and for one hybrid in Gif-sur-Yvette 1984. Thus, the permutation test could be used only for the data of Lusignan 1984. Since the number of degrees of freedom lies between 6 and 24 (Lusignan 1981), between 6 and 19 (Gif-sur-Yvette 1983), and between 6 and 25 (Gif-sur-Yvette 1984), we chose 0.53 ($\alpha = 5\%$) or 0.66 ($\alpha = 1\%$) (12 df), 0.58 ($\alpha = 5\%$) or 0.70 ($\alpha = 1\%$) (10 df), and 0.51 ($\alpha = 5\%$) or 0.64 ($\alpha = 1\%$) (13 df) for the significance threshold of the correlation coefficients of Lusignan 1981, Gif-sur-Yvette 1983, and Gif-sur-Yvette 1984, respectively.

In addition, the number of nonadditivity situations, NA, scored in the ten hybrids was compared to the other protein indices computed for their five parental lines and to the same agromorphological data as above. For the former comparison, the permutation test was used to test the significance of the correlation coefficient. It was also used for comparison with data of Lusignan 1981 and 1984 and data of Gif-sur-Yvette 1984. For the comparison with data of Gif-sur-Yvette 1983 where two of

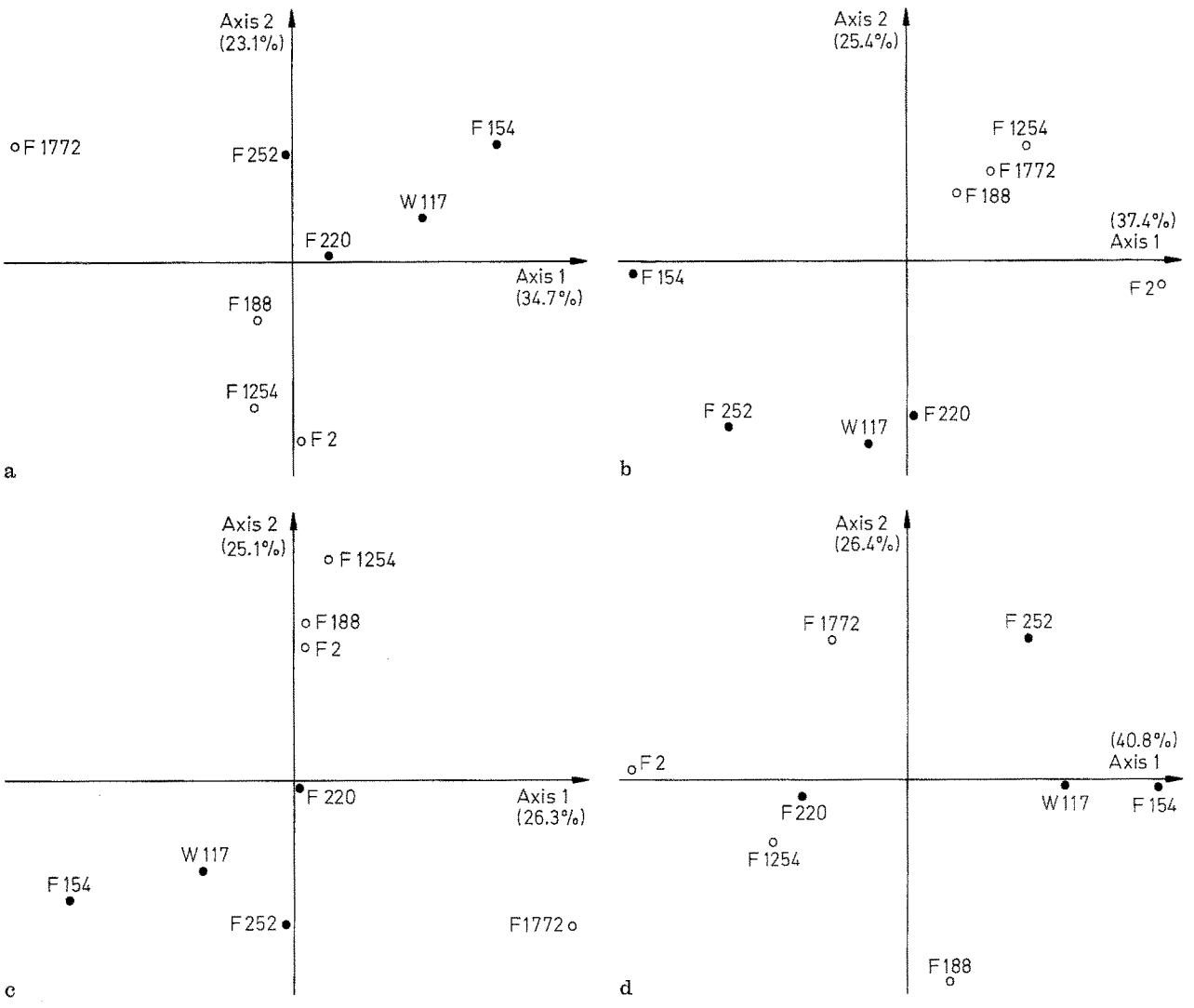

Fig. 1 a–d. Molecular parameters. First planes of principal coordinate analyses of PAD (**a**), PAD$\overline{\text{cov}}$ (**b**), PAD$\overline{\text{na}}$ (**c**), and $\bar{H}$ (**d**) matrices. The percentages of variance explained by the two first axes (1 and 2) are given in parentheses. ●: dent lines; ○: flint lines

the ten hybrids were missing, we chose 0.7 for the significance threshold of the correlation coefficient ($\alpha = 5\%$ for 6 *df*).

Results

Among the 500 polypeptide spots scored over the eight lines, 77 were quantitatively variable between at least two lines, while 75 polypeptides appeared as the products of 32 polymorphic structural loci (22 biallelic loci, nine triallelic loci, and one quadriallelic locus) (see 'Materials and methods'). Between two lines, the number, H, of such loci with different alleles varied from 8 to 24.

For the quantitative variations, we found two classes of intensity for 82 polypeptides, three classes for 22 polypeptides, and four classes for 6 polypeptides. The values of the molecular indices are shown in Table 3.

Relationships between indices based on PAP

Table 4 shows that while PAD (protein amount dissimilarity) and PAD$\overline{\text{na}}$ (with no polypeptides displaying nonadditivity) were very close to each other ($r = 0.96$), the correlation between PAD (or PAD$\overline{\text{na}}$) and PAD$\overline{\text{cov}}$ (relieved of redundancy of the variation) was not as high ($r = 0.69$ or 0.63). The relative discrepancy between PAD$\overline{\text{cov}}$ and PAD could hardly be due to a sampling problem, since the calculation of both PAD$\overline{\text{cov}}$ and PAD$\overline{\text{na}}$ resulted in the same reduction in the number of variations taken into account (about 30%).

The first planes of the principal coordinate analyses of PAD (Fig. 1 a), PAD$\overline{\text{cov}}$ (Fig. 1 b), and PAD$\overline{\text{na}}$ (Fig. 1 c) matrices confirmed that result. The most remarkable difference between PAD (or PAD$\overline{\text{na}}$) and PAD$\overline{\text{cov}}$ was in the

Table 3. Values of the molecular indices H (number of heterozygous structural loci in the hybrid between two lines), PAD (protein amount dissimilarity), PAD$\overline{cov}$ (relieved of redundancy of the variation), PADcov (covariations alone), and PAD$\overline{na}$ (with no polypeptides displaying nonadditivity) computed for the 28 pairs of lines. Angular transformation was made for all indices except H

Pair of lines	H	PAD	PAD$\overline{cov}$	PADcov	PAD$\overline{na}$
F 2 & F 188	19	17.56	15.16	8.68	14.57
F 2 & F 1254	16	17.36	14.65	9.20	15.23
F 188 & F 1254	19	16.64	13.76	9.23	14.50
F 2 & F 1772	11	20.53	15.52	13.16	17.59
F 188 & F 1772	21	19.19	14.05	12.75	17.17
F 1254 & F 1772	16	20.00	14.63	13.38	17.64
F 2 & F 252	21	18.43	16.08	8.73	15.23
F 188 & F 252	20	17.15	15.21	7.68	15.21
F 1254 & F 252	21	17.56	15.04	8.78	16.19
F 1772 & F 252	12	18.24	13.91	11.57	15.93
F 2 & W 117	20	17.46	15.00	8.76	13.78
F 188 & W 117	15	18.05	14.85	10.02	15.70
F 1254 & W 117	17	18.53	15.32	10.14	15.89
F 1772 & W 117	13	20.44	14.59	13.98	17.08
F 252 & W 117	14	17.36	15.50	7.68	14.33
F 2 & F 220	13	15.89	14.21	7.05	12.62
F 188 & F 220	15	16.74	14.50	8.14	14.50
F 1254 & F 220	17	16.85	14.90	7.67	13.69
F 1772 & F 220	13	18.43	13.58	12.26	15.08
F 252 & F 220	16	17.05	15.70	6.45	13.63
W 117 & F 220	17	12.66	10.87	6.47	10.05
F 2 & F 154	24	19.73	16.48	10.59	15.23
F 188 & F 154	17	18.34	14.59	10.86	15.74
F 1254 & F 154	20	19.37	15.30	11.72	16.96
F 1772 & F 154	17	22.14	16.31	14.61	19.55
F 252 & F 154	15	16.32	13.69	8.70	14.00
W 117 & F 154	8	15.34	13.53	7.02	12.56
F 220 & F 154	18	16.43	13.91	8.65	13.91

Table 4. Correlations between H and the transformed indices PAD, PAD$\overline{cov}$, PADcov, and PAD$\overline{na}$

	H	PAD	PAD$\overline{cov}$	PADcov
PAD	0.12			
PAD$\overline{cov}$	0.32	0.69*		
PADcov	−0.03	0.87**	0.25	
PAD$\overline{na}$	0.12	0.96**	0.63*	0.87**

*, ** Significant at the 5 and 1% levels, respectively, from the permutation test

case of lines F1772 and F2. For PAD (or PAD$\overline{na}$), F2 was closer to the other flint-type lines than F1772, while for PAD$\overline{cov}$, F1772 was closer to these lines than F2 (Fig. 1).

Relationships between the expected number of heterozygous loci (H) and the indices based on PAP

No correlation was found between H and the PAD index ($r=0.12$) (Fig. 2). No other PAP indices were correlated with H (Table 4). The first plane of the principal coordinate analysis on the matrix of H displayed striking differences with that of PAD (Fig. 1 d). In particular, PAD distinguished much better between the flint and dent types.

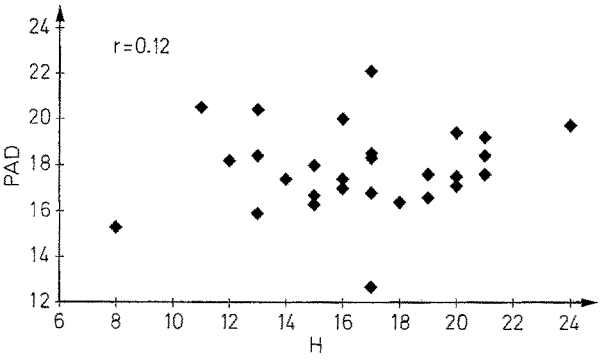

Fig. 2. Correlation between PAD and H computed for the 28 pairs of lines

Relationships with agromorphological data

The values of the correlations between molecular parameters and agromorphological data are given in Table 5. None of the correlations between H and agromorphological data was significant (Fig. 3 a).

On the contrary, a positive relationship appeared between the PAP indices, except PAD$\overline{cov}$, and the hybrid values for three year/locations of experimentation: Lusignan 1981 and 1984, and Gif-sur-Yvette 1983. Even though the dry matter percentage, which is the only nonheterotic trait, was not correlated to any of the PAP indices, the 36 possible correlations between the hybrid values for the four other agromorphological characters and PAD (Fig. 3 b, c), PADcov (Fig. 3 d, e), and PAD$\overline{na}$ were all positive, 29 of them being significant or highly significant (Table 5).

On the other hand, it is worth noting that PAD$\overline{cov}$, which corresponded to PAD relieved of variation redundancy, presented no correlation with any agromorphological data (Fig. 3 f).

Data of Gif-sur-Yvette 1984 gave different results, since all the correlations, either with H or the PAP indices, were close to zero (Table 5). Actually, a strong genotype × environment interaction appeared comparing these data to the three other data sets: for the five agromorphological characters, correlations between Lusignan 1981, Lusignan 1984, and Gif-sur-Yvette 1983 were all positive and all but two were significant, whereas correlations between these data and data of Gif-sur-Yvette 1984 were close to zero (Table 6). Given the atypical results obtained for this year/location compared to the three others, the absence of correlation between these data and molecular parameters is consistent.

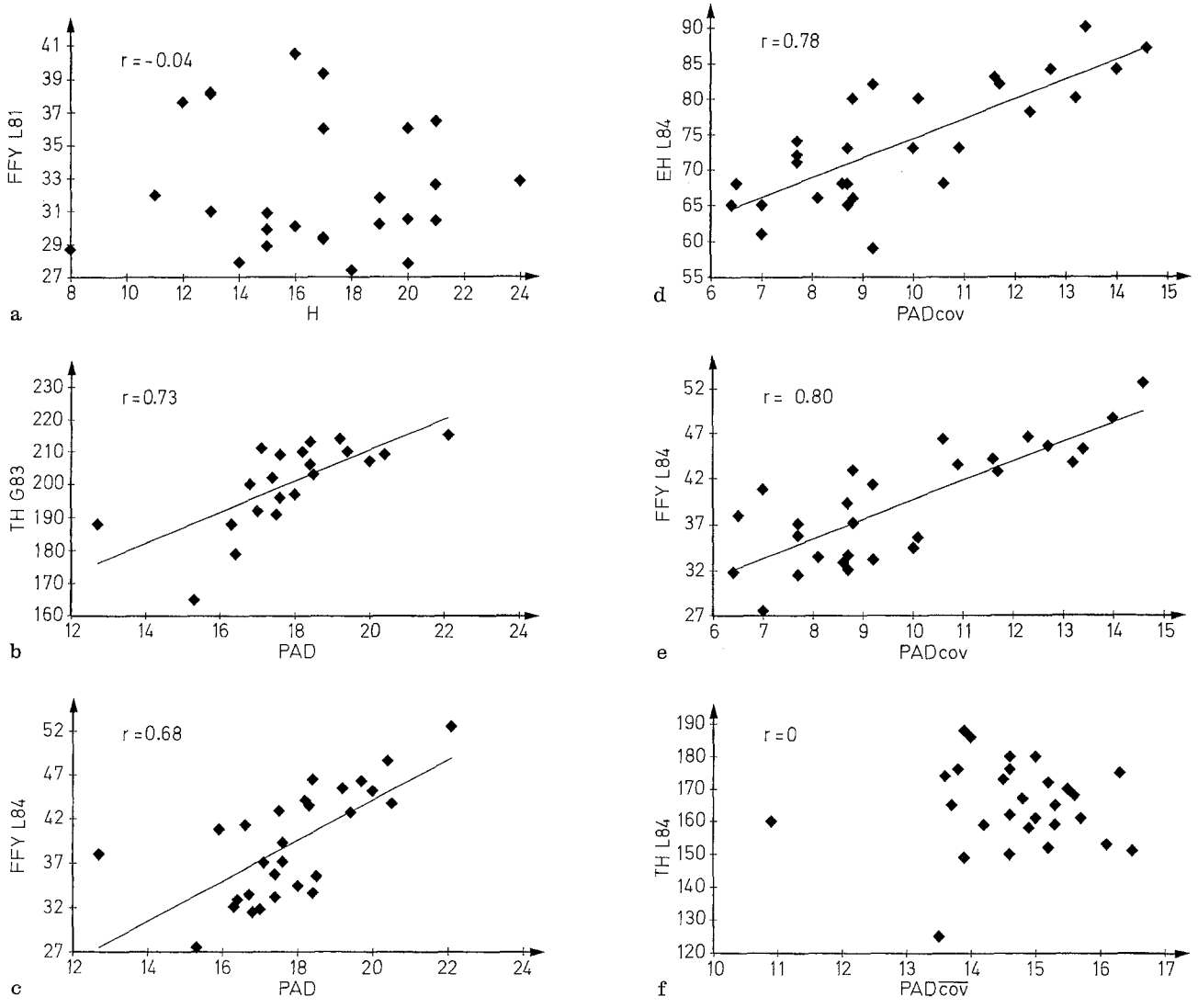

Fig. 3 a–f. Correlation between hybrid fresh forage yield measured in Lusignan 1981 (FFY L81, Mg ha^{-1}) and H (**a**). Correlations between PAD and hybrid total plant height measured in Gif-sur-Yvette 1983 (TH G83, cm) (**b**), and fresh forage yield measured in Lusignan 1984 (FFY L84, Mg ha^{-1}) (**c**). Correlations between PADcov and hybrid ear insertion height measured in Lusignan 1984 (EH L84, cm) (**d**), and fresh forage yield measured in Lusignan 1984 (FFY L84, Mg ha^{-1}) (**e**). Correlation between hybrid total plant height measured in Lusignan 1984 (TH L84, cm) and PAD$\overline{\text{cov}}$ (**f**)

Relationships between nonadditivity and molecular or agromorphological data

The number of nonadditivity (NA) cases in the ten hybrids analyzed by 2D-PAGE appeared significantly correlated to the PAD index computed for the ten pairs of parental lines ($r=0.61$ and $\alpha=1\%$, according to the permutation test), whereas it was not correlated to H.

All the correlations between NA and the hybrid values at the various environments for the heterotic characters (i.e., TH, EH, FFY, and DFY) were positive (not shown), one being significant at the 5% level ($r=0.72$ between NA and hybrid values of EH measured in 1983 in Gif-sur-Yvette).

Discussion

Within the set of 28 hybrids of maize studied, the heterozygosity of the sample of structural loci revealed by 2D-PAGE (H) failed to be predictive of hybrid values for any agromorphological trait. Such a negative result shows that: (i) the polymorphism of the structural genes studied does not have any effect on quantitative trait variation; and (ii) in our material, the level of linkage disequilibrium between structural loci and loci controlling the agromorphological characters under study (QTLs) was limited, if present at all. It is worth noting that our sample of polymorphic structural loci could well cover a large part of the genome, since only loose linkage

Table 5. Correlations between molecular parameters and hybrid values for the agromorphological characters

		H	PAD	Pad$\overline{cov}$	PADcov	PAD$\overline{na}$
Lusignan 1981	Total height	0.26	0.53*	0.26	0.52	0.56*
	Ear insertion height	0.26	0.75**	0.45	0.69**	0.73**
	Fresh forage yield	−0.04	0.71**	0.15	0.83**	0.69**
	Dry forage yield	0.17	0.58*	0.29	0.57*	0.56*
	Dry matter percentage	0.34	−0.27	0.19	−0.48	−0.28
Lusignan 1984	Total height	0.10	0.40	0.00	0.52*	0.49*
	Ear insertion height	0.04	0.63*	0.08	0.78**	0.69**
	Fresh forage yield	0.15	0.68**	0.16	0.80**	0.63**
	Dry forage yield	0.29	0.52**	0.21	0.56**	0.50**
	Dry matter percentage	0.22	−0.35	0.10	−0.55*	−0.34
Gif-sur-Yvette 1983	Total height	0.30	0.73**	0.40	0.65*	0.73**
	Ear insertion height	0.20	0.65	0.34	0.63*	0.71**
	Fresh forage yield	0.05	0.56	0.02	0.69*	0.57
	Dry forage yield	0.04	0.26	−0.08	0.37	0.29
	Dry matter percentage	0.01	−0.47	−0.10	−0.56	−0.46
Gif-sur-Yvette 1984	Total height	−0.28	0.11	−0.14	0.28	0.11
	Ear insertion height	−0.14	0.09	−0.17	0.27	0.10
	Fresh forage yield	−0.28	−0.02	−0.11	0.08	0.02
	Dry forage yield	−0.23	0.02	−0.11	0.14	0.05
	Dry matter percentage	0.10	0.11	−0.02	0.17	0.09

*, ** Significant at the 5 and 1% levels, respectively

Table 6. Correlations, for each character, between the data from Lusignan in 1981 (L81), Lusignan in 1984 (L84), Gif-sur-Yvette in 1983 (G83), and Gif-sur-Yvette in 1984 (G84)

	L81/L84	L81/G83	L81/G84	L84/G83	L84/G84	G83/G84
Total plant height	0.82**	0.85**	−0.02	0.76**	0.00	0.05
Ear insertion height	0.75**	0.78**	−0.28	0.73**	0.05	−0.13
Fresh forage yield	0.74**	0.61*	−0.04	0.66*	0.08	−0.15
Dry forage yield	0.62*	0.33	−0.07	0.36	0.00	−0.23
Dry matter percentage	0.65*	0.64*	−0.03	0.59*	−0.39	−0.23

*, ** Significant at the 5 and 1% levels, respectively

appeared between some of the 20 structural loci variable in the three F_2 progenies analyzed (data not shown) [the maize genome is about 1,400 cM long (Murray et al. 1988)]. Nevertheless it could be argued that the number of markers was not sufficient to be representative of the heterozygosity of the QTLs. More likely, our results illustrate a point underlined in the "Introduction": random, neutral markers would not be the most relevant markers to predict phenotypic trait variation.

The absence of relation between H and any of the indices based upon the protein amount polymorphism (PAP) leads to the same type of conclusions, applied to loci controlling protein abundances instead of QTLs.

On the other hand, we found numerous significant correlations between PAP indices and hybrid vigor for agronomical traits. The correlations were mostly significant or highly significant for the height and yield characters for experiments in three year/locations. The relation vanished with data of Gif-sur-Yvette 1984, which gave a classification of the genotypes different from that of the three other sets of data, and different from what is known about this material (A. Gallais, unpublished data). Climatic conditions were unfavorable in this year/location: compared to Gif-sur-Yvette in 1983, during the whole phase of maize germination and emergence, the global radiation was reduced by more than 20%, the sum of temperatures over 10 °C was 30% lower, associated with excessive rainfall (40% higher). Therefore, particular environmental constraints may have altered the genome expression.

The correlations between PAP and performances of the hybrids observed in three different experiments mean that protein amount loci either (i) are in strong linkage disequilibrium with the QTLs, or (ii) are themselves QTLs.

Concerning the first hypothesis, it should be emphasized that the correlation between PAP indices and hybrid values could well be due to a limited number of genes. The index PAD$\overline{\text{cov}}$, which corresponds to PAD relieved of redundancy of variation, is not related to hybrid values, while PADcov is. The covariable polypeptides, within a group, can be modified products of a single structural gene, or the products of several structural genes under common control (e.g. hierarchical, pleiotropic ...). As the polypeptides of a given group are usually distributed throughout the gel, the second hypothesis is the most likely. Anyway in both cases a few genes seem to a large extent to be responsible for the high value of the correlations. In an extreme situation, if each group of covariable polypeptides were controlled by only one locus, the correlations would be due to as few as 14 loci. Thus, under the first hypothesis, the force that would maintain such a specific disequilibrium remains to be explained, since we did not find linkage disequilibrium with structural loci.

The significant correlation between PADcov and hybrid values is actually consistent with the latter hypothesis, namely, protein amount loci would themselves be QTLs. Moreover, the PAD index is correlated to the number of nonadditivity situations, which appeared also to be related to hybrid values. This correlation cannot, in any case, be explained in terms of linkage disequilibrium.

The last argument in favor of the second hypothesis ensues from a comparison of our findings with recent RFLP studies in maize. The major conclusions are that RFLP analyses may be of value in allocating maize inbreds to heterotic groups (Lee et al. 1989), but that no relationship between RFLP-based genetic distance and hybrid performance is apparent (Godshalk et al. 1990; Melchinger and Lee 1990). It is furthermore interesting to compare our graphs of correlations with the one published by Lee et al. (1989), which also involved eight inbred lines covering a similar range of diversity. Clearly, for the upper half of their RFLP-based distance estimates, no relation exists with F_1 yield: high RFLP-based distance could correspond either to low or high F_1 yield, while in our case, high values of PAD corresponded exclusively to high F_1 yield. This result is actually expected if loci controlling protein amounts are not QTL-linked markers but are themselves QTLs.

Thus, our results suggest that genes controlling protein amounts, and particularly those with multiple effects, directly affect the expression of hybrid vigor. Basically, such a conclusion is almost a necessary consequence of the role of the regulation of gene expression in development: the genetic variations in the profile of gene expression can hardly have no phenotypic effect, and certainly constitute a nonneutral polymorphism. In this connection, it is interesting to note that the high correlation value found by Damerval et al. (1987a) between PAD and phenotypic Mahalanobis distance between maize lines involved, in the majority, variables accounting for developmental processes.

In conclusion, one can imagine that the more two genotypes differ in their profile of genetic expression during development, the more numerous the possibilities of interactions at the molecular level will be: nonadditivity would be the expression of these interactions, and therefore it is quite logical that it is related to PAP. From this point of view, heterosis could be considered as a measure of these interactions. Thus PAP, as well as frequency of nonadditivity, could allow the definition of predictors of heterosis based on molecular processes, and hence be of general value.

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References

Atchley WR (1989) Introduction to the symposium "Evolution of developmental systems." Am Nat 134:437–512

Bahrman N, Damerval C (1989) Linkage relationships of loci controlling protein amounts in maritime pine (*Pinus pinaster* Ait.). Heredity 63:267–274

Bahrman N, Vienne D de, Thiellement H, Hofmann JP (1985) Two-dimensional gel electrophoresis of proteins for genetic studies in Douglas Fir (*Pseudotsuga menziesii*). Biochem Genet 23:247–255

Charcosset A, Lefort-Buson M, Gallais A (1990) Use of topcross designs for predicting performance of maize single-cross hybrids. Maydica 35:23–27

Charcosset A, Lefort-Buson M, Gallais A (1991) Relationship between heterosis and heterozygosity at marker loci: a theoretical computation. Theor Appl Genet 81:571–575

Damerval C, Vienne D de, Zivy M, Thiellement H (1986) Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. Electrophoresis 7:52–54

Damerval C, Hébert Y, Vienne D de (1987a) Is the polymorphism of protein amounts related to phenotypic variability? A comparison of two-dimensional electrophoresis data with morphological traits in maize. Theor Appl Genet 74:194–202

Damerval C, Le Guilloux M, Blaisonneau J, Vienne D de (1987b) A simplification of Heukeshoven and Dernick's silver staining of proteins. Electrophoresis 8:158–159

Frei OM, Stuber CW, Goodman MM (1986) Use of allozymes as genetic markers for predicting performance in maize single cross hybrids. Crop Sci 26:37–42

Ghaderi A, Adams MW, Nassib AM (1984) Relationship between genetic distance and heterosis for yield and morphological traits in dry edible bean and faba bean. Crop Sci 24:37–42

Godshalk EB, Lee M, Lamkey KR (1990) Relationship of restriction fragment length polymorphisms to single-cross hybrid performance of maize. Theor Appl Genet 80:273–280

Gottlieb LD, Vienne D de (1988) Assessment of pleiotropic effects of a gene substitution in pea by two-dimensional polyacrylamide gel electrophoresis. Genetics 119:705–710

Gower JC (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrica 53:325–338

Granier F, Vienne D de (1986) Silver staining of proteins: standardized procedure for two-dimensional gels bound to polyester sheets. Anal Biochem 155:45–50

Klose J (1982) Genetic variability of soluble proteins studied by two-dimensional electrophoresis on different inbred mouse strains and on different mouse organs. J Mol Evol 18:315–328

Lee M, Godshalk EB, Lamkey KR, Woodman WW (1989) Association of restriction fragment length polymorphism among maize inbreds with agronomic performance of their crosses. Crop Sci 29:1067–1071

Leonardi A (1989) Variabilité et hérédité des quantités de protéines chez le maïs: analyse par électrophorèse bidimensionnelle et relations avec la variabilité agromorphologique. PhD thesis, University of Paris VII, 166 pp

Leonardi A, Damerval C, Vienne D de (1987) Inheritance of protein amounts: comparison of two-dimensional electrophoresis patterns of leaf sheath of two maize lines (*Zea mays* L.) and their hybrids. Genet Res 50:1–5

Leonardi A, Damerval C, Vienne D de (1988) Organ-specific variability and inheritance of maize proteins revealed by two-dimensional electrophoresis. Genet Res 52:97–103

Marchais L (1978) Une analyse génétique chez le petit mil pénicilliaire à l'aide de la distance R de Hanson et Casas. Ann Amel Plant 28:165–193

Melchinger AE, Lee M (1990) Use of RFLPs for investigating relationships among inbreds and predicting heterosis in maize. In: Proc 15th Congress of Eucarpia, June 4–8, 1990, Baden, Austria

Murray M, Cramer J, Ma Y, West D, Romero-Severson J, Pitas J, DeMars S, Vilbrandt L, Kirshman J, McLeester R, Schilz J, Lotzer J (1988) Agrigenetics maize RFLP linkage map. Maize Genet Coop Lett 62:89–91

O'Farrell P (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

Rosenblum BB, Neel JV, Hanash SM (1983) Two-dimensional electrophoresis of plasma polypeptides reveals "high" heterozygosity indices. Proc Natl Acad Sci USA 80:5002–5006

Scopes RK (1974) Measurement of protein by spectrophotometry at 205 nm. Anal Biochem 59:277–282

Seguin M (1990) Etude de l'hétérozygotie chez le blé tendre par électrophorèse bidimensionnelle des protéines totales et relation avec des caractères agronomiques. PhD thesis, University of Paris-Sud, 211 pp

Shifriss C, Sacks JM (1980) The effect of distance between parents on the yield of sweet pepper × hot pepper hybrids, *Capsicum annuum* L., in a single harvest. Theor Appl Genet 58:253–256

Siddiqui JA, Prasad RC, Mehrar B (1976) Hybrid performance in relation to genetic divergence in some varieties of *Gossypium hirsutum*. Z Pflanzenzuecht 77:215–221

Smith JSC, Smith OS (1989a) The description and assessment of distance between inbred lines of maize. I. The use of morphological traits as descriptors. Maydica 34:141–150

Smith JSC, Smith OS (1989b) The use of morphological, biochemical, and genetic characteristics to measure distance and to test for minimum distance between inbred lines of maize (*Zea mays* L.). In: Proc UPOV Workshop, Versailles, October 1989

Vienne D de, Leonardi A, Damerval C (1988) Genetic aspects of variation of protein amounts in maize and pea. Electrophoresis 88 11:742–750

Zivy M, Thiellement H, Vienne D de, Hofmann JP (1983) Study on nuclear and cytoplasmic genome expression in wheat by two-dimensional gel electrophoresis. 1. First results on 18 alloplasmic lines. Theor Appl Genet 66:1–7

Zivy M, Thiellement H, Vienne D de, Hofmann JP (1984) Study on nuclear and cytoplasmic genome expression in wheat by two-dimensional gel electrophoresis. 2. Genetic differences between two lines and two groups of cytoplasms at five developmental stages or organs. Theor Appl Genet 68:335–345

Zivy M, Devaux P, Blaisonneau J, Jean R, Thiellement H (1991) Segregation distorsion and genetic linkage studies in microspore derived doubled haploid lines of *Hordeum vulgare* L. Theor Appl Genet (in press)