*Genet. Res., Camb.* (1987), **50**, *pp.* 1–5 *With 2 text-figures Printed in Great Britain* 

# Inheritance of protein amounts: comparison of two-dimensional electrophoresis patterns of leaf sheaths of two maize lines (*Zea mays* L.) and their hybrids

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*(Received 9 December 1986 and in revised form 28 January 1987)*

**Summary**

Denatured total proteins of two maize lines and their reciprocal $F_1$ hybrids were analysed by two-dimensional electrophoresis. Quantitative (spot more or less intense) and qualitative (presence or absence of spots) differences exist between the lines, and correspond to 11% of the total reproducible spots. Non-additive effects on spot intensities were found in the hybrids, which display spots similar to the more intense parental spot for 11% of varying spots. This may correspond to *dominance for regulatory systems* controlling the protein amounts. Such interactions contrast with additivity classically described for enzymes or DNA.

## 1. Introduction

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of denatured proteins (O'Farrell, 1975; Garrels, 1979) is a powerful tool to reveal numerous gene products and to analyse their polymorphism and/or their behaviour during development. Moreover, variations in protein amounts between genotypes can be scored, allowing genetic studies of regulatory factors controlling the gene product level (Klose, 1982; Anderson *et al.* 1985; Zivy *et al.* 1983, 1984, Bahrman *et al.* 1985).

Using this technique we have found numerous reproducible qualitative (presence or absence) and quantitative (spots more or less intense) differences between maize lines at the same morphological stage of seedling. Distances between lines based on quantitative variation of proteins appeared to be correlated with Mahalanobis distances computed from the general combining abilities of fourteen heritable, morphological or agronomical characters (Damerval *et al.* in the press).

In this paper we initiate the study of the inheritance of protein amounts by comparing two maize lines and their $F_1$ hybrids. The inheritance of the amount of individual proteins is only described in terms of spot intensities: so (i) 'additivity' or 'codominance' corresponds to a hybrid spot intensity intermediate between the parental ones; (ii) 'dominance' and 'overdominance' mean that the hybrid spot is similar to one of the parental spots or more intense than the two parental spots, respectively; and (iii) 'semidominance' means that the intensity of the hybrid spot is different from the midparental value, but lies between the parental values. The use of these terms is justified by the genetic control of protein quantities. Protein quantities show additive inheritance in 89% of the cases, while 11% of the variable spots are affected by dominance effects. This result is discussed in connection with other results on enzyme regulation.

## 2. Material and Methods

Two maize lines $W_{117}$ (dent type) and $F_{1254}$ (flint type) and their two reciprocal hybrids ($\varphi W_{117} \times \sigma F_{1254}$ and $\varphi F_{1254} \times \sigma W_{117}$) were studied. Plants were grown under controlled conditions in a climatic chamber. Kernels were allowed to germinate in Petri dishes on water-imbibed filter paper in the dark at 20 °C. When the coleoptiles were about 2 cm long, the seedlings were transferred to small pots filled with vermiculite and supplied each day with nutrient solution (de Bildering & Lourtioux, 1976). The temperature was 24 °C and the photoperiod 16 h; the basal etiolated part of the sheath of the second leaf was removed 14 days after planting out; the morphological stage was 'four visible leaves' for all the genotypes. Three individuals were taken per line and $F_1$ hybrid, and constituted replicates of the genotypes. The controlled growth conditions, the well-defined morphological stage and organ ascertain the genetical basis of any variation observed between the protein patterns of the different genotypes. If some physiological differences exist between genotypes, they are the direct result of genetical differences, and so they need to be considered in our study.

(i) *Protein extraction*

The extraction procedure was as described in Damerval *et al.* (1986) and Zivy (1986) except that 40 $\mu$l of the resolubilization solution was used to resuspend 1 mg of pellet.

(ii) *Isoelectric focusing (IEF)*

Rod gels 24 cm long and 1 mm in diameter were used. The IEF solution was 9·2 M urea, 3·78% acrylamide, 0·22% bisacrylamide, 2% Triton X 100, 4% ampholyte mixture (3% Pharmalyte pH 5–8, 1% Pharmalyte pH 5–6). The catholyte was a 50 mM degassed sodium hydroxide solution and the anolyte was 50 mM orthophosphoric acid. Twenty-five microlitres of extract were layered on the top of each IEF gel and the run was performed for 33800 Vh. The gels were equilibrated 15 min in 62·5 mM Tris, 2·3% sodium dodecyl sulphate (SDS), 10% saccharose pH 8·8.

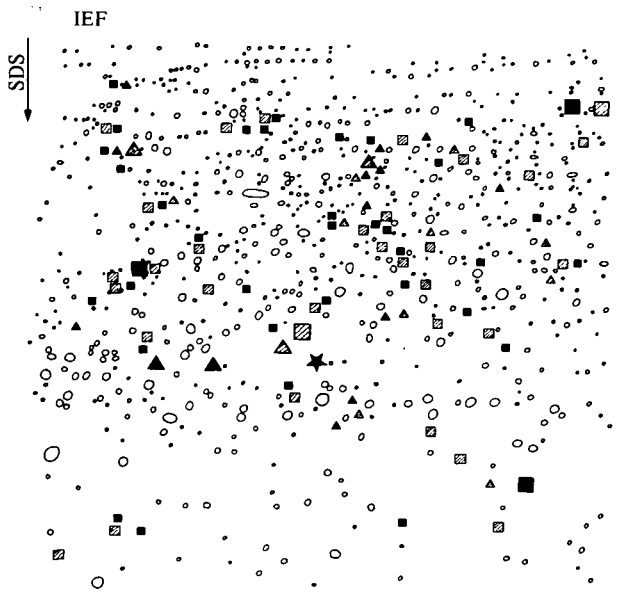


Fig. 1 Map of all the reproducible spots found in $F_{1254}$, $W_{117}$ and their hybrids. The squares represent the qualitatively varying spots: ▨, present in $W_{117}$ but absent in $F_{1254}$; ■, present in $F_{1254}$ but absent in $W_{117}$. The triangles represent the quantitatively varying spots: △, more intense in $W_{117}$ than in $F_{1254}$; ▲, more intense in $F_{1254}$ than in $W_{117}$. The large symbols are for dominance cases and the small ones are for codominance cases; the star is for the superdominant spot.

(iii) *Second dimension*

The second dimension with SDS was performed as in Damerval *et al.* (1986). The 2D gels were bound to Gelbond PAG film from Marine Colloids (20 × 17 cm) to make them easier to handle in the subsequent steps.

(iv) *Staining procedure*

The 2D gels were silver stained, eight gels being simultaneously treated as described by Granier & de Vienne (1986). The silver staining was modified from Heukeshoven & Dernick (1985): Farmer's reducer was applied for 1·30 min with subsequent rinsings before the $AgNO_3$ step; after this latter step, the gels were soaked in 2·5% sodium carbonate for 5 min before the three developer baths. This technique allows 2D gels with high contrast to be obtained without the need for a second destaining–restaining cycle (Damerval *et al.* in the press).

(v) *Scoring procedure*

Three gels per genotype (one per individual plant), which represented the three replicates, were visually scored by three different observers. Co-migrations between the parental lines (mixture of each extract in equal proportions) were performed.

Table 1. *Inheritance of the variant spots in the hybrids between $W_{117}$ and $F_{1254}$.*

| $W_{117}$ | $F_{1254}$ | Hybrids | Number | Total |
|---|---|---|---|---|
| Qualitative variants[a] | | | | |
| + | − | Codominance | 32 | 66 |
| − | + | | 34 | |
| + | − | Dominance | 2 | 5 |
| − | + | | 3 | |
| Quantitative variants[b] | | | | |
| ++ | + | Codominance | 6 | 17 |
| + | ++ | | 11 | |
| ++ | + | Dominance | 3 | 5 |
| + | ++ | | 2 | |
| | | Superdominance | 1 | 1 |

[a] + and − respectively represent presence and absence of the spots.
[b] ++ and + respectively represent high and low intensity of the spots.

https://doi.org/10.1017/S0016672300023260 Published online by Cambridge University Press

### 3. Results

A combined map of all the reproducible spots found by two-dimensional electrophoresis of $F_{1254}$, $W_{117}$, $F_{1254} \times W_{117}$ and $W_{117} \times F_{1254}$ is shown in Fig. 1. About 20% of the spots were discarded as non-reproducible, most of them being faint and not always clearly visible in the three replicates. A few cases of residual heterozygosity were suspected in the lines, where one of the three replicates of a parental line displayed two 'allele' spots like the $F_1$ hybrids. Nevertheless, intragenotypic variability was very low. Spots showing qualitative and quantitative variations were chosen for further study only if they were clearly visible in the three replicates of each genotype and varied little, if at all, in amount. Among the 816 reproducible spots numbered, 93 (11·4%) were clearly variant between the parental lines; seventy-one spots (8·7%) showed qualitative (presence or absence) variation, and 22 (2·7%) were more or less intense according to the lines.

The inheritance of spot intensities was carefully determined by comparing the hybrid pattern with a 1:1 co-migration of the parental lines, which represents the 'theoretical' hybrid pattern under the hypothesis of additivity of protein amounts. Neither of the hybrids had any protein spots that were not found in one or both of the parents. Sixty-six of the 71 qualitatively varying spots and 17 of the 22 quantitatively varying ones showed additive inheritance (i.e. there was no intensity difference between

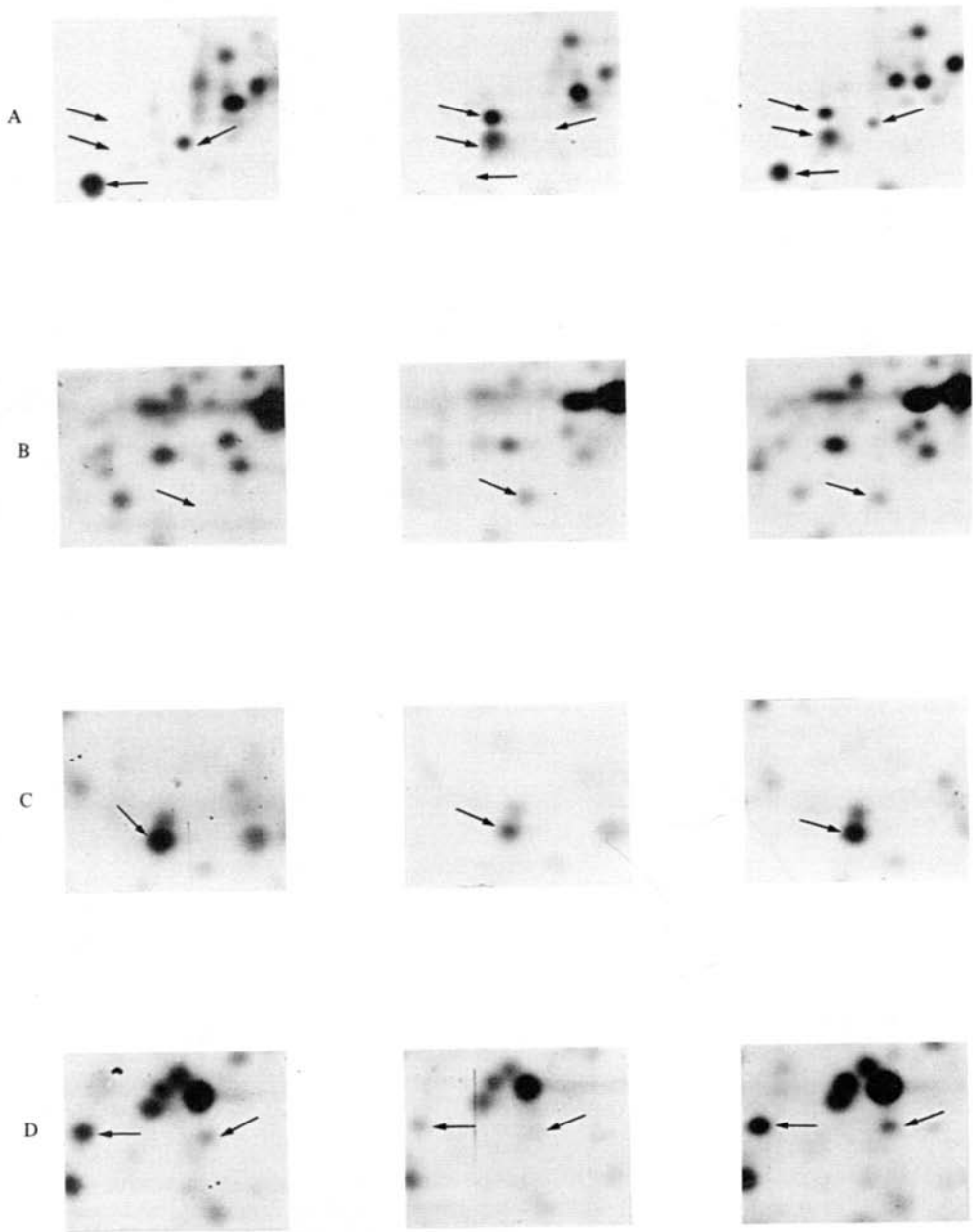


Fig. 2. Examples of variations between the lines and their inheritance in the hybrids. First, second and third columns respectively show patterns of $F_{1254}$, $W_{117}$ and hybrid. A, qualitative variants with codominant inheritance. B, qualitative variant with dominant inheritance. C, quantitative variant with codominant inheritance. D, quantitative variant with dominant inheritance.



https://doi.org/10.1017/S0016672300023260 Published online by Cambridge University Press

the hybrid and the co-migration spots), and 10 spots showed very clear complete dominance effects (Table 1 and Fig. 2). Only one case of overdominance was found, where the hybrid $W_{117} \times F_{1254}$ spot was more intense than both $W_{117}$ and $F_{1254}$ spots, these two being visually identical; in hybrid $F_{1254} \times W_{117}$, the intensity of this spot was that found in the parents; this was the only reciprocal effect observed. Some cases of semidominance were suspected, but we preferred to pool them with codominance ones. Thus, non-additive effects affected 11 spots and 10·8% of the variable spots. In *all* cases where dominance was retained, high intensity (or presence) was dominant over low intensity (or absence).

## 4. Discussion

In recent years, numerous studies have been devoted to the identification of regulatory factors controlling the timing and/or the level of expression of various structural genes in Eukaryotes (reviewed by Paigen, 1979; Scandalios & Baum, 1982; McIntyre, 1982). It has become apparent that the structural genes can be influenced by several regulatory loci, either closely or distantly located. The closely located elements, which can even be inside the structural gene, are *cis*-acting and exhibit additive inheritance (Lalley & Shows, 1977; Allendorf, Knudsen & Phelps, 1982; Chandlee & Scandalios, 1984); in all cases examined, the proteins were affected at the synthesis level (Bernstine & Koh, 1980; Shaffer & Bewley, 1983; Sano, 1984). The distantly located elements seem to be numerous, and have been found whenever looked for (Rawls & Lucchesi, 1974; Laurie-Ahlberg *et al.* 1980, 1982; Colas des Francs & Thiellement, 1985; Thiellement *et al.* 1986). When studied, their inheritance is mostly non-additive (Abraham & Doane, 1978; McDonald & Ayala, 1978; King & McDonald, 1983; Maroni & Laurie-Ahlberg, 1983; Gibson *et al.* 1986, but see Scandalios *et al.* 1980). They probably act in *trans*, by producing diffusible molecules and especially affect the degradation of the gene product under regulation (Bewley & Laurie-Ahlberg, 1984).

Most of these studies concern enzyme activity and/or quantity. Some cases of dominance/recessive inheritance of protein quantities revealed by 2D-PAGE have already been mentioned by Klose & Feller (1981) in the mouse, but the main study is by Anderson *et al.* (1985), who precisely quantified the protein amounts in two mouse strains and their hybrids using their TYCHO software system. They also found dominance/recessive situations (complete or partial), but unlike our result, these situations were symmetrically distributed around the mean of both parental values. In our case, we cannot imagine any systematic artifactual bias being responsible for the disymmetry observed. The same thing was observed in another plant species (*Brassica napus*) in our laboratory (Feugier & Bordon, unpublished results).

In our interpretation scheme, the so-called qualitative variants encompass allelic polymorphism of structural genes for which additive inheritance may be currently expected in the absence of any regulatory variation. Quantitative variants and a fraction of the qualitative ones – where the faintest spot is below the detection level and when the protein synthesis is inhibited whatever the mechanism – correspond to the variability of regulatory elements controlling protein quantities; in these cases the inheritance of the differences between lines could not be predicted without genetic hypotheses. If the proteins revealed by 2D-PAGE are submitted to the same kind of regulatory mechanisms as enzymes, the disymmetry of dominance effects could merely reveal the action of distant *trans*-acting regulatory elements. The large proportion of additive effects could be due to an important polymorphism of *cis*-acting, closely linked elements, relative to the unlinked *trans*-acting elements, or to a broader range of quantity variation induced by the former than by the latter. For enzymes, experimental evidence to support these assumptions is lacking. Another possibility is that, unlike enzymes, numerous distantly located regulatory elements exhibit additive inheritance (Scandalios *et al.* 1980) and/or are *cis*-acting.

More precise genetic studies are needed to further interpret these first results. Particularly, it will be of great interest to determine the relative extent to which regulatory elements with additive and dominant inheritance control protein quantity. Analysis of the genetic determination of protein amounts is now under way in our laboratory to answer this question.

We are grateful to J. Blaisonneau, M. P. Digard and M. Le Guilloux for expert technical assistance. We thank our colleagues for critical reading of the manuscript.

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