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Genetics of gliadins coded by the group 1 chromosomes in the high-quality bread wheat cultivar Neepawa

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Summary. The inheritance and biochemical properties of gliadins controlled by the group 1 chromosomes of the high-quality bread wheat cultivar Neepawa were studied in the progeny of the cross Neepawa × Costantino by six different electrophoretic procedures. Chromosome 1B of Neepawa contains two gliadin loci, one (*Gli-B1*) coding for at least six ω- or γ-gliadins, the other (*Gli-B3*) controlling the synthesis of gliadin N6 only. The map distance between these loci was calculated as 22.1 cM. Amongst the chromosome 1A gliadins, three proteins are encoded at the *Gli-A1* locus whereas polypeptides N14-N15-N16 are controlled by a remote locus which recombines with *Gli-A1*. Six other gliadins are controlled by a gene cluster at *Gli-D1* on chromosome 1D. Canadian wheat cultivars sharing the *Gli-B1* allele of Neepawa were found to differ in the presence or absence of gliadin N6. The electrophoretic mobilities of proteins N6 and N14-N15-N16 were unaffected by the addition of a reducing agent during two-dimensional sodium dodecyl sulphate polyacrylamid-gel electrophoresis, suggesting the absence of intra-chain disulphide bonds in their structure.

Key words: Gliadin loci – Group 1 chromosomes – Recombination – Electrophoresis – *Triticum aestivum*

Introduction

Gliadins and glutenins, the storage proteins of the wheat kernel, are each a mixture of different polypeptide species (Wall 1979). Gliadins consist of monomeric, alcohol-soluble polypeptides which can be classified into four groups on the basis of their mobilities after acidic polyacrylamide-gel electrophoresis (A-PAGE). The so-called α-, β- and γ-gliadins have molecular weights of 30,000 to 50,000 and show very similar amino-acid compositions, whereas ω-gliadins have higher molecular weights (45,000 to 75,000) and differ from α-, β- and γ-gliadins in that they lack cysteine and methionine (Shewry et al. 1986).

Glutenins occur as large heterogeneous aggregates under non-reducing conditions (Huebner and Wall 1976). Upon the addition of a reducing agent, they break down to component subunits which can be classified into A, B, C and D groups on the basis of their mobilities in sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE) and their isoelectric points (Payne and Corfield 1979; Jackson et al. 1983). The B, C and D groups have molecular weights of 35,000–50,000 and are collectively called low-molecular-weight (LMW) subunits, whereas the A group includes larger polypeptides (80,000–150,000) termed high-molecular-weight (HMW) subunits. Both types of subunits can form polymers by association amongst themselves through disulphide bonds (Huebner and Wall 1976; Payne and Corfield 1979).

The genes coding for most γ- and ω-gliadins have been mapped on the short arms of chromosomes 1A, 1B and 1D at the *Gli-A1*, *Gli-B1* and *Gli-D1* loci respectively, whereas the genes coding for most α- and β-gliadins occur on the short arms of group 6 chromosomes at the *Gli-A2*, *Gli-B2* and *Gli-D2* loci (see Payne 1987 for review). Each gliadin locus was found to control the synthesis of several jointly inherited polypeptides, and 12–25 alleles have been recently assigned to each locus (Metakovsky 1991). Intra-locus recombination is very

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rare (Metakovsky et al. 1986; Pogna et al. 1990). Evidence has also accumulated for the occurrence of gliadin genes located at a distance from the *Gli-1* clusters. Metakovsky et al. (1986) described two gliadin genes which are located on both sides of the *Gli-A1* locus, at a recombination distance of 5% and 13% from it, respectively.

Another family of remote genes coding for ω-gliadins was located on the short arms of chromosomes 1A and 1B between the *Glu-1* and *Gli-1* loci. In the line CS(Thatcher-1B) Galili and Feldman (1984) observed one protein, termed B30, whose synthesis is controlled by the *Gld-B6* locus 28.1 cM from *Gli-B1*, whereas Metakovsky et al. (1986) calculated a frequency of recombination of 29% between *Gli-B1* and a gene coding for one ω-gliadin band in the variety Bezenchukskaya 98. Jackson et al. (1985) described an additional locus, named *Glu-B2*, about 22 cM from *Gli-B1* on chromosome 1B. Later, Payne et al. (1988) found that *Gld-B6* and *Glu-B2* are allelic and renamed these loci as *Gli-B3*. Genes coding for ω-type gliadins were also found on chromosome 1A at a comparable position to *Gli-B3* on chromosome 1B (Sobko 1984; Metakovsky et al. 1986).

HMW glutenin subunits (A subunits) are encoded at the *Glu-1* loci on the long arms of group 1 chromosomes whereas LMW subunits (B, C and D subunits) are encoded by genes on the short arms of the same chromosomes (Payne 1987). In particular, genes coding for B and C subunits occur at the *Glu-3* loci. The *Glu-B3* locus was found to be 1.8–2.0 cM from *Gli-B1*, and proximal to it on the short arm of chromosome 1B (Singh and Shepherd 1988; Pogna et al. 1990). In contrast, no recombination was found between *Glu-A3* and *Gli-A1*, and between *Glu-D3* and *Gli-D1* (Singh and Shepherd 1988).

There are several references on the relation of gliadin alleles, or particular gliadin bands, to dough quality (Sozinov and Poperelya 1980, 1982; Wrigley et al. 1981; Pogna et al. 1982). Canadian wheats have outstanding dough quality characteristics and unique gliadin electrophoretic patterns (Ng et al. 1988); therefore we set up a research programme to ascertain the relationship between dough strength and *Gli-1* allele composition in the well-known, high-quality wheat cultivar Neepawa. Neepawa was chosen because it is the legal standard of quality for the Canadian Western Red Spring (CWRS) class of wheat. Its gliadin pattern occurs in several cultivars of this class, but is sporadic or absent in the other classes of Canadian wheat as well as in world wheat germplasms (Ng et al. 1988, 1989; Metakovsky et al. in press). This pattern is rather complex, containing many weak ω-gliadins not represented in the published patterns of gliadins of common wheat (Metakovsky et al. 1984; Metakovsky 1991).

The present paper describes the application of six different electrophoretic procedures to the genetic and biochemical analysis of gliadins encoded by group 1 chromosomes in the progeny of the cross between Neepawa and the Italian cultivar Costantino.

Materials and methods

Plant material

Crosses between the spring bread wheat cultivar Neepawa and the spring variety Costantino were made by standard procedures in the glasshouse. More than 120 F_2 grains were grown in the glasshouse in large pots to obtain as much F_3 grain as possible. Seeds from single F_3 or F_4 spikes were also used in this study.

Gliadin extraction

About 60 mg of flour obtained from eight-to-ten crushed grains of each F_2, F_3 or F_4 spike was extracted for 2 h at 50 °C with 200 µl of aqueous 70% (v/v) ethanol. After centrifugation for 5 min at 20,000 *g*, the gliadin extract was divided into 30 µl aliquots.

Acid, polyacrylamide-gel electrophoresis (A-PAGE)

One gliadin aliquot was mixed with 30 µl of a solution containing 60% (w/v) of glycerol and 0.05% (w/v) of pyronin G. After centrifugation, the clarified supernatant (25 µl) was fractionated at pH 3.1 as described previously (Pogna et al 1986).

Sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE)

One gliadin aliquot was mixed with 60 µl of solution A containing 16.5% (v/v) glycerol, 0.1 M Tris-HCl, pH 6.8, 3.5% (w/v) SDS and 0.016% (w/v) pyronin Y. After centrifugation, 35 µl of the supernatant was fractionated according to Laemmli (1970) with the following modifications. The gels consisted of a 15% separating gel, pH 8.4, beneath a 3% stacking gel, pH 6.8. Electrophoresis was carried out at room temperature using a home-made vertical electrophoresis apparatus (Pogna et al. 1989) and was continued for 2 h after the tracking dye (pyronin Y) had eluted from the bottom of the gel.

Two-dimensional electrophoresis

Four types of two-dimensional electrophoresis were used. The first was A-PAGE followed by SDS-PAGE (A-PAGE × SDS-PAGE) as described by Payne et al. (1984), except that the second dimension SDS-PAGE was carried out as described above. The second type was two-dimensional SDS-PAGE. One aliquot of unreduced gliadin extract was mixed with 60 µl of solution A and centrifuged. Forty microliters of supernatant were loaded onto a 10 × 5 cm, 1.5-mm thick gel and fractionated by SDS-PAGE at 100 V for 3 h using the mini-Protean apparatus (Bio-Rad). Separating and stacking gels were 15% acrylamide, pH 8.4, and 4.5% acrylamide, pH 6.8, respectively. Following electrophoresis, the gel was cut into thin strips parallel to the direction of electrophoresis and individual strips were incubated for 30 min in an equilibration solution containing 2.4% (w/v) SDS; 0.07 M Tris-HCl, pH 6.8, and 5% (w/v) 2-mercaptoethanol. Each strip was placed on top of a new gel and SDS-PAGE was carried out as above.

The third type of two-dimensional electrophoresis was A-PAGE followed by electrophoresis at pH 9.2 (A-PAGE × B-PAGE), according to Lafiandra et al. (1984). Finally, gliadins were fractionated by isoelectrofocussing in the first dimension and SDS-PAGE in the second (IEF × SDS-PAGE). One aliquot

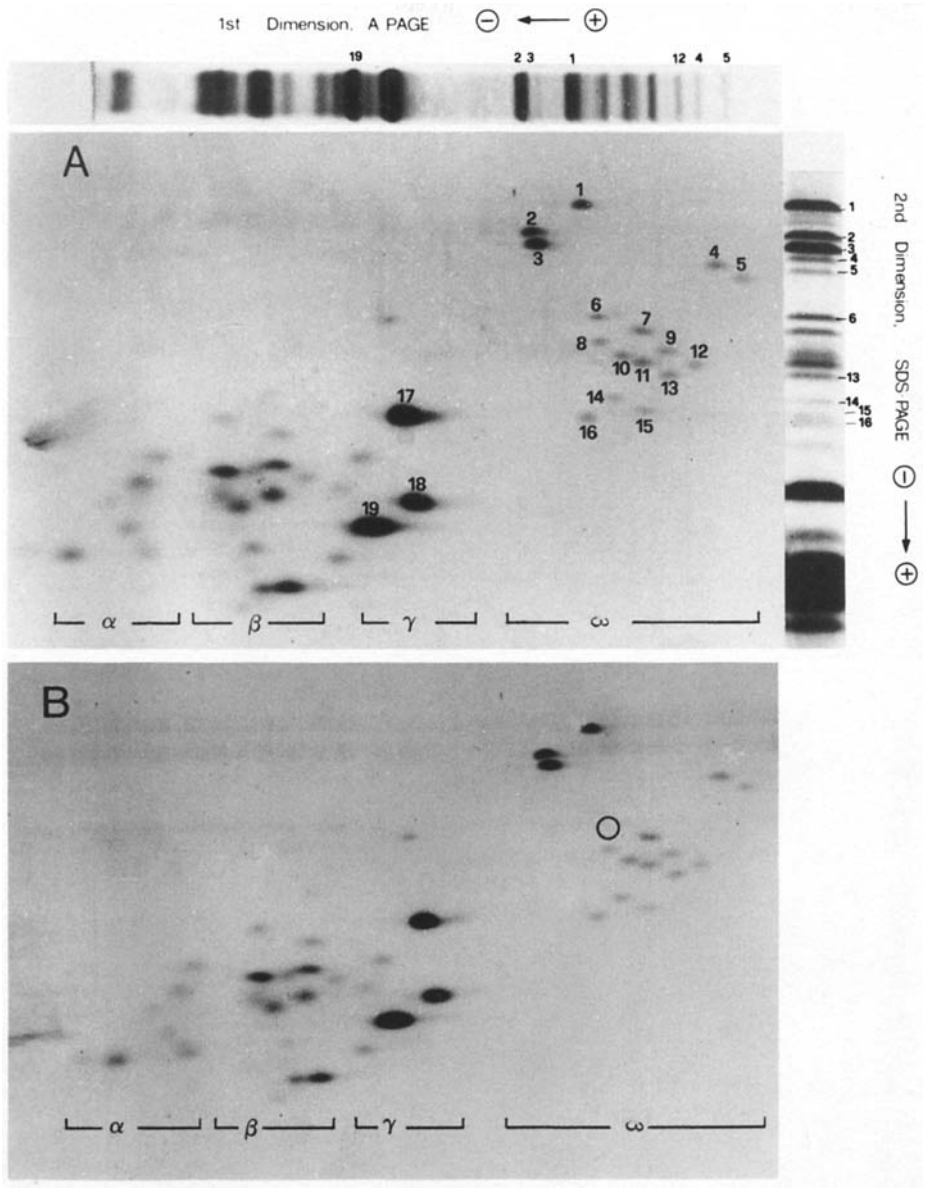

Fig. 1. Two-dimensional A-PAGE × SDS-PAGE fractionation of gliadins from bread wheat cultivar Neepawa (**A**) and one F_5 line from the cross Neepawa × Costantino (**B**). One-dimensional A-PAGE (on the top) and SDS-PAGE (on the right) of unreduced gliadins are also shown. Gliadins analysed by recombination mapping are *numbered*. The position of gliadin N6 is marked by *open circle* in **B**

Table 1. Segregation data for progeny from the cross Neepawa (N) × Costantino (C)

Gliadin bands		No. of progeny				χ^2 (2:1:1)	χ^2 (9:3:3:1)	Conclusion
a	b	ab	a–	–b	––			
F_2 generation								
N1-N2-N3	C1-C2-C10	45	36	27	–	4.5, ns		Allelism
N4-N5-N12	C3-C4-C5-C6	45	26	37	–	5.2, ns		Allelism
N1-N2-N3	C3-C4-C5-C6	63	17	18	10		2.5, ns	No linkage
F_3 generation								
N13-N19	C11	67	41	45	–	2,6, ns		Allelism
N1-N2-N3	C11	81	30	26	14		2.7, ns	No linkage
N4-N5-N12	C11	80	35	28	9		2.7, ns	No linkage

ns, not significant

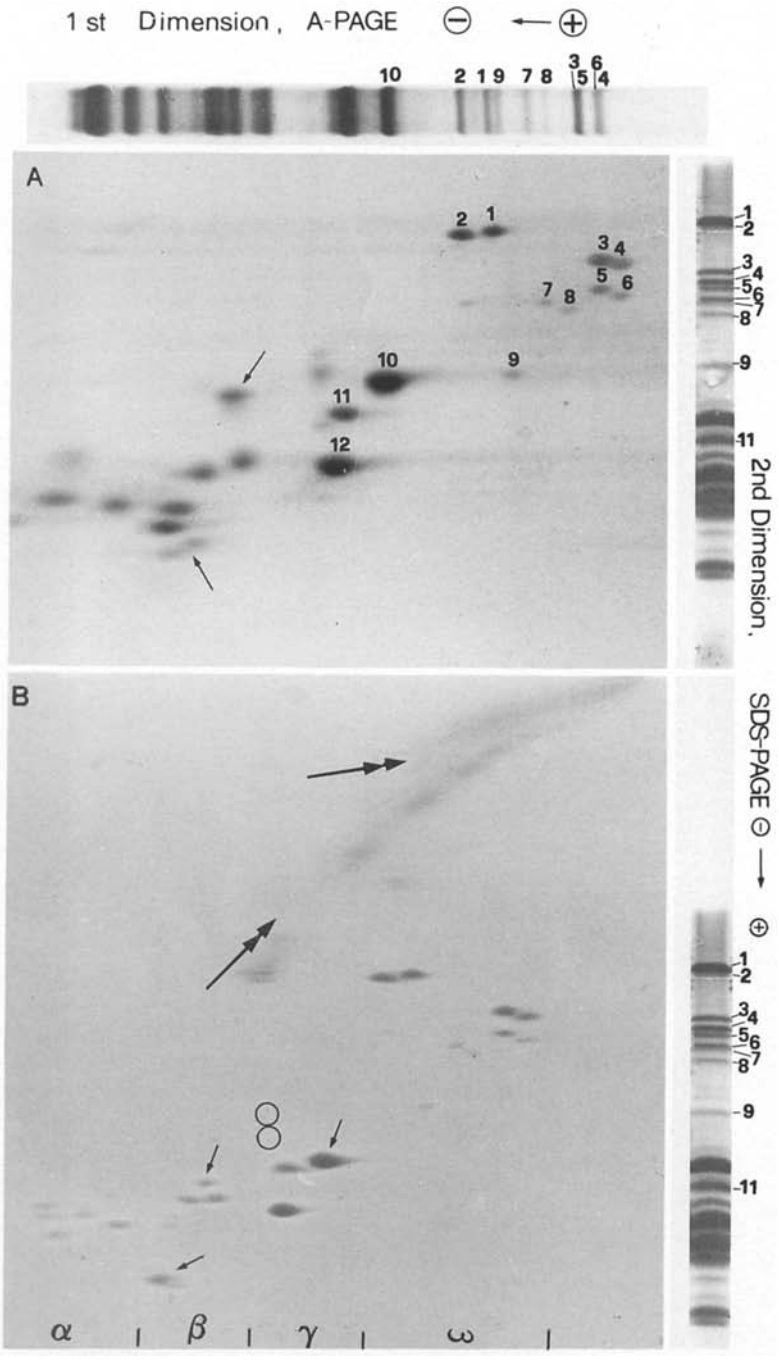

Fig. 2. Two-dimensional A-PAGE × SDS-PAGE fractionation of gliadins from cv Costantino. The second dimension separation was carried out on reduced (**A**) or unreduced (**B**) gliadins. One-dimensional A-PAGE (on the top) and SDS-PAGE (on the right) of unreduced gliadins are also shown. Gliadins analysed by recombination mapping are *numbered*. *Single-headed arrows* indicate gliadins having very different positions in **A** and **B**. *Double-headed arrows* indicate aggregated proteins. Proteins absent in the unreduced gel are marked by *open circles*

of gliadin extract was mixed with 30 µl of a solution containing 60% (w/v) glycerol and 2% (w/v) SDS. After centrifugation, 40 µl of the supernatant were loaded onto a 3.5% rod gel and fractionated using the procedure of Holt et al. (1981). The concentration of ampholines was 5%, pH range 3.5–10. In the second dimension, 15% gels, pH 8.4, were used. After the first dimension separation, the gels were treated with an equilibration buffer without a reducing agent.

Genetic analysis

The frequency of recombination between the genes was calculated using the method of maximum likelihood (Allard 1956). The Kosambi function (Kosambi 1944) was used to calculate the genetic distances in centi Morgan (cM) units.

Results

Electrophoretic analysis of the parental cultivars

Gliadins from the grain of Neepawa were fractionated by one-dimensional A-PAGE or SDS-PAGE under unreduced conditions, and two-dimensional A-PAGE × SDS-PAGE after reduction with 2-mercaptoethanol

(Fig. 1 A). About 45 proteins were resolved by two-dimensional electrophoresis. The ω- and γ-gliadins were numbered N1 to N19. Several bands obtained by one-dimensional fractionations consisted of more than one protein and so only seven and 11 gliadins could be identified by A-PAGE and SDS-PAGE, respectively (Fig. 1 A). The concentration of acrylamide (15%) and the pH value of the separation gel (pH 8.4) during SDS-PAGE gave increased resolution of gliadins, particularly of ω-gliadins, compared with the conventional 10% acrylamide, pH 8.8 system. Also, fractionation by SDS-PAGE under non-reducing conditions allows us to investigate gliadin patterns in the absence of other proteins such as albumins and LMW glutenin subunits.

The two-dimensional map of gliadins from Costantino was much simpler and showed about 35 proteins (Fig. 2 A). Amongst the 12 gliadins numbered C1 to C12 only ten could be identified during A-PAGE or SDS-PAGE. Gliadins from Costantino were also fractionated by A-PAGE × SDS-PAGE under non-reducing conditions (Fig. 2 B). Prior to electrophoresis in the second dimension, the gel rod was incubated in an equilibration solution in which the reducing agent 2-mercaptoethanol was omitted. The two dimensional map of unreduced gliadins showed the presence of aggregated proteins which appeared as two undifferentiated smears over the upper half of the gel and disappeared under reducing conditions. Two proteins, which were observed in the reduced gel (Fig. 2 A), were absent from the unreduced gliadin extracts (Fig. 2 B, open circles). Moreover, most α-, β- and γ-gliadins, including band C10 and two β-gliadins arrowed in Figs. 2A and B, were not present at the same relative positions in the reduced gel, showing lower relative mobilities when reduced. However, 2-mercaptoethanol did not affect the relative mobilities of ω-gliadins. This is consistent with the absence of cysteine residues in this group of gliadins (Shewry et al. 1986). A-PAGE × SDS-PAGE fractionation of unreduced gliadins of Neepawa gave similar results (data not shown).

Genetics of gliadins

A selection of seed progeny of selfed F_2 plants of the cross Neepawa × Costantino analysed by A-PAGE is shown in Fig. 3. The segregation of gliadins N1 to N5, N12 and N19 from Neepawa, and C1 to C6 plus C10 from Costantino could be easily followed. However, some ω-gliadins had very similar mobilities in A-PAGE and, therefore, the progeny were analysed by SDS-PAGE under non-reducing conditions (Fig. 4). Gliadins N1 to N5 and N13 from Neepawa, and C1 to C5 plus C11 from Costantino were recognised in progeny from their distinctive mobilities. The segregation of gliadins was also studied in seeds obtained from single F_3 plants.

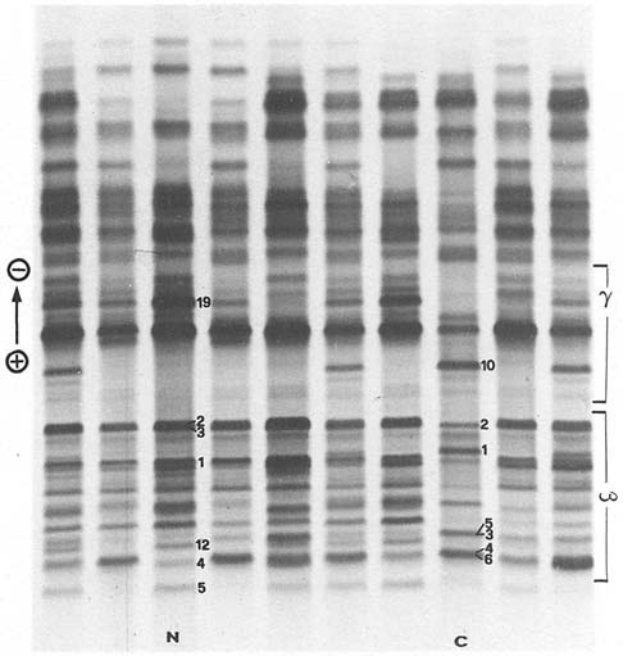

Fig. 3. A-PAGE fractionation of gliadins of the F_2 progeny from the cross Neepawa × Costantino. Gliadins analysed by recombination mapping are *numbered*. *N*, Neepawa; *C*, Costantino

The combined results from the progeny of this cross are shown in Table 1. Gliadins N1-N2-N3 always segregated as one unit, indicating their control by tightly linked genes. Similarly, bands N4-N5-N12 and N13-N19 from Neepawa, and C1-C2-C10 and C3 to C6 from Costantino behaved as single Mendelian units. Therefore, each of these gliadin blocks was considered as being controlled by one allele.

Genes coding for the gliadin blocks N4-N5-N12 and C3-C4-C5-C6 were found to be allelic. They were assigned to the *Gli-D1* locus which is known to control the synthesis of the slow-moving ω-gliadins in acid gels (Sozinov and Poperelya 1980). Similarly, the allelic blocks N1-N2-N3 and C1-C2-C10 were assigned to the *Gli-B1* locus because band C10, also designated as gliadin γ-40 according to the nomenclature of Bushuk and Zillman (1978), was found to be encoded by a gene on the satellite of chromosome 1B (Pogna et al. 1985). Finally, allelic gliadins N13-N19 and C11 were assigned to the *Gli-A1* locus on chromosome 1A.

After A-PAGE × SDS-PAGE fractionations of several progeny were scrutinised, it became clear that gliadins N8, N10 and N17 were always inherited with N1-N2-N3 (*Gli-B1*), and proteins N7-N9 and N18 were always inherited with N4-N5-N12 (*Gli-D1*). Moreover, no evidence of recombination was detected between N13-N19 (*Gli-A1*) and N11, between C3-C4-C5-C6 (*Gli-D1*) and C12, and between C1-C2-C10 (*Gli-B1*) and C7-C8. The assignment of gliadins of both parents to loci on the

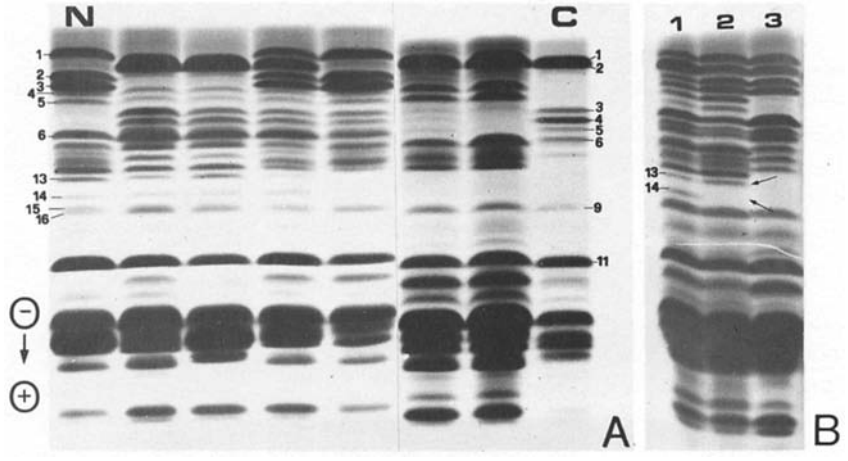

Fig. 4 A, B. SDS-PAGE fractionation of unreduced gliadins of the F_2 progeny from the cross Neepawa × Costantino. Gliadins analysed by recombination mapping are *numbered.* One recombinant pattern containing gliadin N13 without gliadin N14 (arrows) is shown in *lane 2* of **B**. In the remaining lanes gliadins N13 and N14 segregate as one unit. *N*, Neepawa; *C*, Costantino

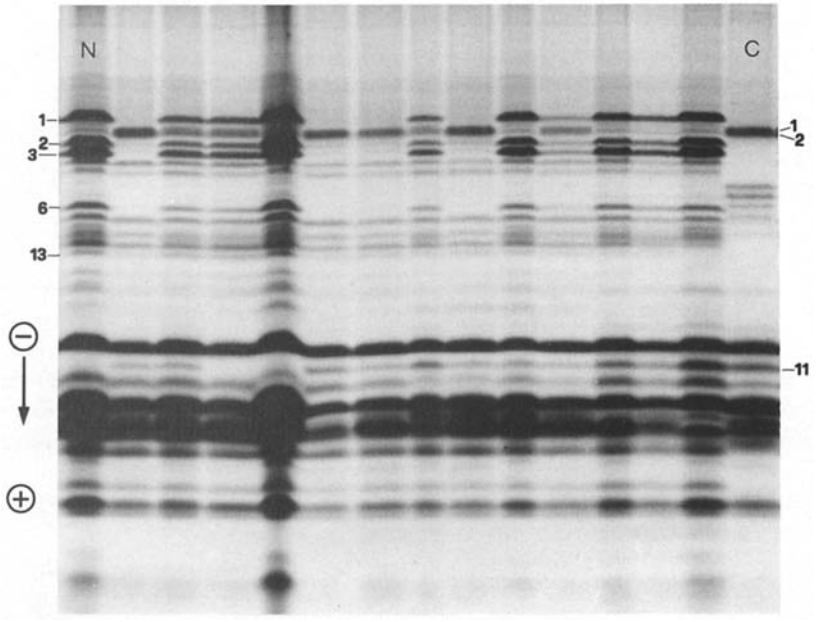

Fig. 5. SDS-PAGE fractionation of unreduced gliadins of single seeds from one F_3 plant homozygous for the *Gli-D1* allele of Neepawa. Gliadins analysed by recombination mapping are *numbered. N*, Neepawa; *C*, Costantino

Table 2. Assignment of gliadins of Neepawa and Costantino to loci on group 1 chromosomes

Gliadin		Chromosome	Locus
Neepawa	Costantino		
11, 13, 19	11	1A	*Gli-A1*
14, 15, 16	9		*Gli-A3?*
1, 2, 3, 8, 10, 17	1, 2, 7, 8, 10	1B	*Gli-B1*
6	–		*Gli-B3*
4, 5, 7, 9, 12, 18	3, 4, 5, 6, 12	1D	*Gli-D1*

homoeologous group 1 chromosomes is reported in Table 2.

Subsequent to this analysis, it was observed that gliadin N6 (Fig. 1 A) was segregating with respect to all the three *Gli-1* loci. Unfortunately, this gliadin overlapped with band C6 (*Gli-D1*) from Costantino during SDS-PAGE (Fig. 4). Therefore, segregation of gliadin N6 was determined by SDS-PAGE analysis of single seeds obtained from two selfed F_3 plants which were homozygous for the *Gli-D1* allele of Neepawa (Fig. 5). No significant linkage was found between N6 and the *Gli-A1* locus represented by gliadins N13 and C11 (Table 3). Yet, linkage betwewen N6 and the *Gli-B1*-encoded gliadins N1-N2-N3 and C1-C2 was significant. The recombination data for the two progenies were pooled and the combined estimate of recombination was found to be $20.2 \pm 3.0\%$. The χ^2 value of a homogeneity test of the two sets of data for this recombination value was not significant ($\chi^2 = 1.2$, $P > 0.25$) and, therefore, the map distance was calculated to be 22.1 ± 3.6 cM.

The segregation of the minor ω-gliadins N14, N15, N16 (Fig. 1 A) and C9 (Fig. 2 A) was difficult to follow

Table 3. Segregation data for gliadins in progeny of two selfed F_3 plants from the cross Neepawa (N) × Costantino (C)

Gliadin bands (and locus)			No. of seeds						χ^2 (6:3:3:2:1:1)	Conclusion	R (%) ±SD
a	b	c	abc	ab−	a−c	−bc	−b−	−−c			
1st progeny											
N6 (*Gli-B3*)	N13 (*Gli-A1*)	C11	58	33	29	13	11	10	2.8, ns	No linkage	
N6 (*Gli-B3*)	N1-N2-N3 (*Gli-B1*)	C1-C2	67	38	15	9	0	27	57.5***	Linkage	16.2±3.1
2nd progeny											
N6 (*Gli-B3*)	N1-N2-N3 (*Gli-B1*)	C1-C2	26	11	10	3	2	8	9.0, ns	Linkage	31.5±6.9

ns, not significant; *** significant at $P<0.001$

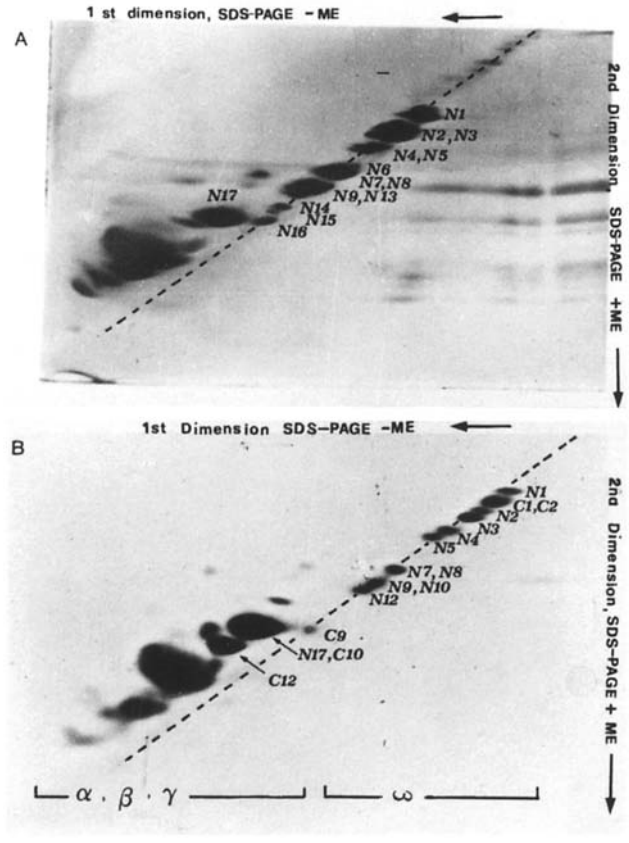

Fig. 6. Two-dimensional SDS-PAGE of gliadins from Neepawa (**A**) and one F_3 line from the cross Neepawa × Costantino (**B**). Gliadins are *numbered* as in Figs. 1 and 2

by one-dimensional SDS-PAGE (Fig. 4B). However, the proteins N14-N15-N16 were present in most of the A-PAGE × SDS-PAGE patterns of progeny homozygous for the *Gli-A*-encoded gliadins of Neepawa, and were absent in progeny homozygous for the *Gli-A1* allele represented by gliadin C11 of Costantino. Similarly, ω-gliadin C9 occurred in most F_3 lines containing band C11 and was absent when this latter band was missing. Our conclusion from these observations is that bands C9 and N14-N15-N16 are controlled by genes at a distinct locus gentically linked to *Gli-A1* on the short arm of chromosome 1A. These genes seem to be allelic because no F_3 line was found to lack both C9 and N14-N15-N16.

Electrophoretic analysis of gliadin N6

In order to characterize gliadin N6 with respect to the ω-gliadins coded at the *Gli-1* loci, ethanol extracts were fractionated by high-resolution two-dimensional electrophoresis. The two-dimensional A-PAGE × SDS-PAGE map of Neepawa was compared with that of one F_5 line which originated from the cross Neepawa × Costantino (Fig. 1B). This line contained all gliadins present in Neepawa except polypeptide N6, suggesting that N6 is the sole product of the additional gliadin locus of Neepawa. This was confirmed by two-dimensional A-PAGE × B-PAGE fractionation (see Fig. 8).

Gliadins were also analysed by two-dimension SDS-PAGE. Unreduced ethanol extracts were fractionated by SDS-PAGE in the first dimension and, after reduction with 2-mercaptoethanol, re-fractionated by SDS-PAGE in the second dimension (Fig. 6). The ω-gliadins differ from the α-, β- and γ-gliadins in that they lack cysteine residues (Shewry et al. 1986), accordingly they should have the same mobilities in both reduced and unreduced gels. The distribution of ω-gliadins, including band N6, N14-N15-N16 and C9, along a diagonal line in Fig. 6, is consistent with this expectation. In contrast, most α-, β- and γ-gliadins were located above the diagonal, indicating that they have slightly greater mobilities under non-reducing conditions than when reduced.

The two-dimensional IEF × SDS-PAGE fractionation of unreduced ethanol extracts of Neepawa demonstrated that N6 is more acidic than the other gliadins (Fig. 7). When analysed by two-dimensional A-PAGE × B-PAGE electrophoresis (Fig. 8), N6 was found to move faster towards the anode during B-PAGE than the other ω-gliadins.

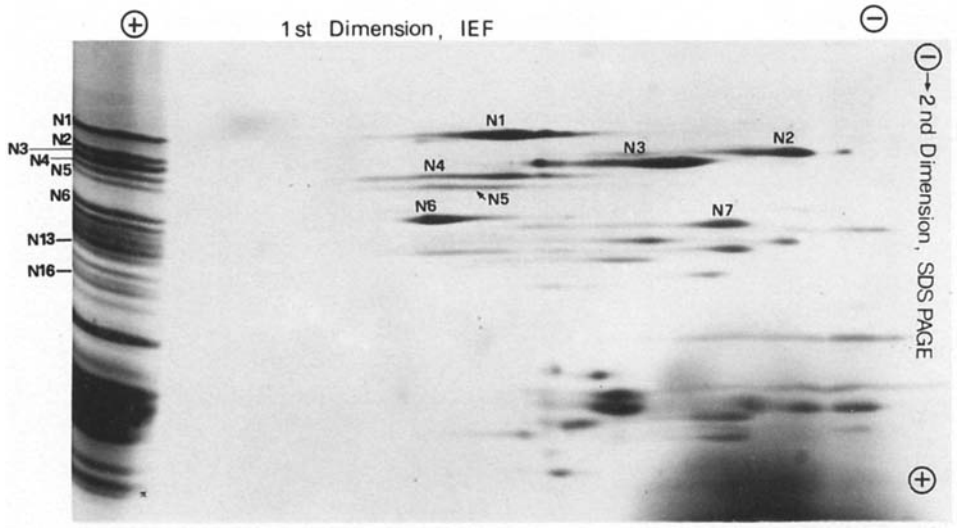

Fig. 7. Two-dimensional IEF × SDS-PAGE fractionation of unreduced gliadins from Neepawa. One-dimensional SDS-PAGE fractionation is shown on the left. Gliadins are *numbered* as in Fig. 1 A

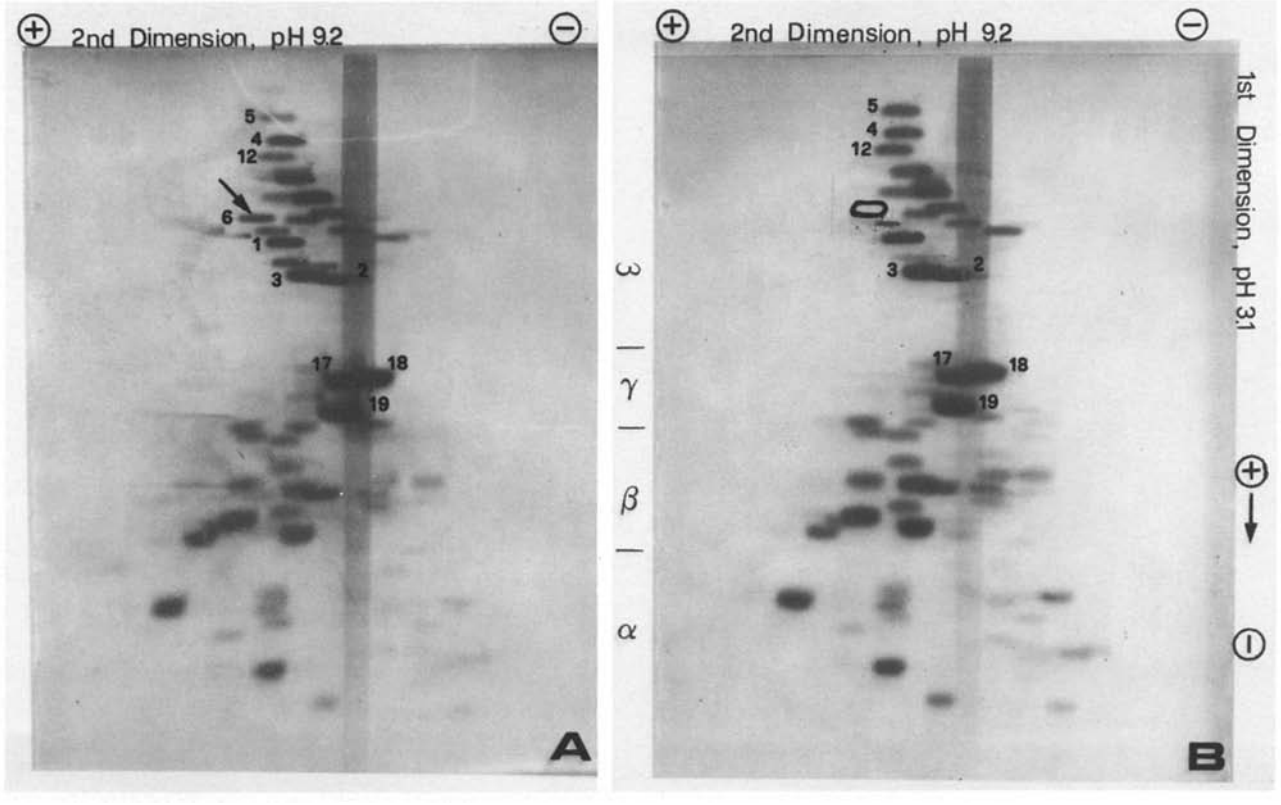

Fig. 8. Two-dimensional A-PAGE × B-PAGE fractionation of gliadins from Neepawa (**A**) and the F_5 line of Fig. 1 B (**B**). Gliadins are *numbered* as in Fig. 1 A. *Arrow* and *open circle* indicate gliadin N6

Discussion

The viscoelastic properties of gluten in both durum and common wheat are influenced by genetic variation in HMW- and LMW-glutenin subunits (Payne et al. 1981; Gupta and Shepherd 1988; Gupta et al. 1989). Significant associations have also been revealed between wheat quality and specific gliadin components (Damidaux et al. 1978; Pogna et al. 1982; Sozinov and Poperelya 1982).

Recent work (Payne et al. 1987; Pogna et al. 1990) has suggested that allelic variation at the *Glu-3* loci coding for LMW subunits of glutenin is primarily responsi-

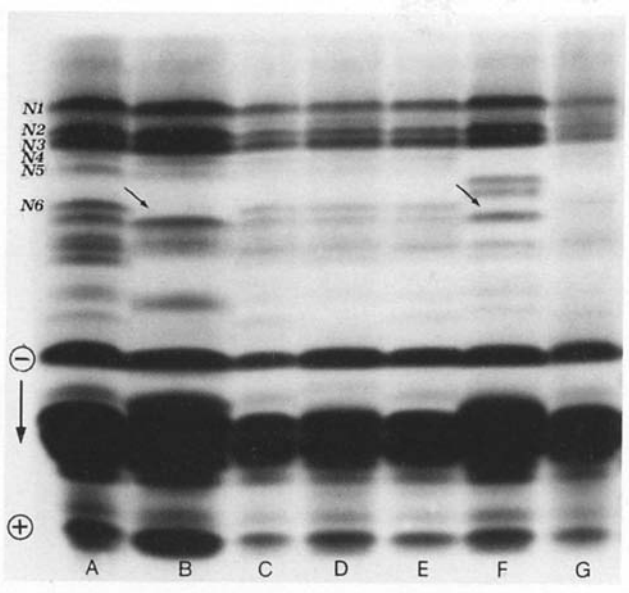

Fig. 9. SDS-PAGE fractionation of unreduced gliadins from Benito (**A**), Canuck (**B**), Katepwa (**C**), Leader (**D**), Neepawa (**E**), Sundance (**F**) and Thatcher (**G**). Gliadins are *numbered* as in Fig. 1 A. *Arrows* indicate the position of gliadin N6

ble for differences in gluten quality previously thought to be associated with the closely linked *Gli-1* loci coding for gliadins. However, alleles at *Gli-1* maintain their interest as genetic markers of quality because LMW glutenin subunits are difficult to obtain in pure form and are not fractionated adequately by one-dimensional systems such as A-PAGE or SDS-PAGE.

The cultivar Neepawa, along with several cultivars in the Canadian Western Red Spring (CWRS) class, including Benito, Canuck, Katepwa, Manitou, Napayo and Thatcher, possesses ω- and γ-gliadins which are sporadic or absent in the other classes of Canadian wheats as well as in European cultivars (Ng et al. 1989; Metakovsky et al. in press). The A-PAGE × SDS-PAGE and A-PAGE × B-PAGE analyses described in this study showed that Neepawa contain about 45 major gliadin components. Fifteen gliadins were found to be encoded by the complex *Gli-1* loci on the short arms of the group 1 chromosomes (Table 2). Judging by the A-PAGE patterns described in the catalogue of gliadin alleles (Metakovsky 1991), Neepawa has the *Gli-A1m* allele inherited from its grandparent cv Marquis, and the *Gli-D1j* allele from Thatcher. The recent work of Ng and Bushuk (1990) has shown that gliadin components designated as ω-37 and ω-38, which correspond to our *Gli-B1*-encoded gliadins N2 and N3, were inherited from a specific accession of cultivar Kanred. In the catalogue of gliadins only the *Gli-B1d* allele could be considered as present in Neepawa, judging by its component composition (Metakovsky et al. in press). However, we found at least six *Gli-B1*-controlled jointly inherited components in Neepawa, in contrast to only three bands in the *Gli-B1d* block. This discrepancy could be caused by difficulties in following the faint bands (N8 and N10) in one-dimensional separations, as well as the simplification of the component composition of some blocks for their easier identification (Metakovsky 1991). However, the difference may also indicate that Neepawa (and other Canadian wheats) has a unique allele at *Gli-B1* which is similar to *Gli-B1d,* but includes more gliadin polypeptides. The other possibility is that bands N8 and N10 are under the control of a separate *Gli* locus, tightly linked to *Gli-B1*. Recently Pogna et al. (submitted) described a gliadin gene distal to *Gli-B1* at a distance of about two recombination units from it.

In addition to *Gli-B1*, Neepawa contains a remote locus coding for the ω-gliadin N6 on chromosome 1B. During SDS-PAGE analysis of the gliadins of Canadian cultivars which share the *Gli-B1* allele of Neepawa, the N6 band was found to be present in cultivars Benito, Columbus, Conway, Kenyon, Katepwa, Lancer, Leader, Manitou, Napayo and Sinton, and absent in Canuck and Sundance (Fig. 9). On the other hand, gliadin N6 was also found in cv Chinook which does not contain the *Gli-B1* allele of Neepawa.

There are several reports of gliadin genes located at a distance from the *Gli-B1* cluster. Galili and Feldman (1984) analysed the inheritance of several gliadins in the substitution line CS (Thatcher-1B) and obtained a map distance of 28.1 cM between *Gli-B1* and the *Gld-B6* locus coding for protein B30. In this line the pair of 1B chromosomes of Chinese Spring was substituted from Thatcher, one of the Canadian wheats containing the *Gli-B1* allele of Neepawa. Recently, Payne et al. (1988) have found that *Gld-B6* and the *Glu-B2* locus, which codes for one LMW glutenin subunit of the D group (Jackson et al. 1985), are allelic, and renamed these loci as *Gli-B3*. The estimated distance (22.1 cM) obtained here between *Gli-B1* and the *Gli-B3* locus represented by gliadin N6 (= B30) is smaller than that calculated by Galili and Feldman (1984), but similar to the map distance of 22.4 cM obtained by Jackson et al. (1985).

According to Payne et al. (1988) the ω-type gliadins and the D subunits of glutenin coded by the *Gli-B3* locus are expressed in low amounts and allelic variation is rather limited. Moreover, D subunits are able to aggregate with glutenin by non-covalent bonds. However, band N6 is not a minor component of gliadins especially in the SDS-PAGE patterns (see Fig. 4). This could be due to the presence of the highly dissociating agent SDS in these gels, suggesting that protein N6 may aggregate with other proteins in the grain. The results given in this paper also indicate that band N6 is the only ω-gliadin coded by *Gli-B3*.

Two-dimensional SDS-PAGE showed that the mobility of band N6 is unaffected by the addition of a reducing agent (Fig. 6 A), suggesting the absence of intra-chain

disulphide bonds in its structure. IEF × SDS-PAGE also demonstrated that band N6 is the most acidic protein in the unreduced ethanol extract of Neepawa. The acidic nature of polypeptides (LMW glutenins of the D group and gliadins) controlled by the *Gli-B3* locus was shown earlier (Jackson et al. 1985; Payne et al. 1988).

The synthesis of ω-gliadins N14-N15-N16 in Neepawa was found to be controlled by genes at a locus recombining with the main *Gli-A1* cluster. This finding was supported by one-dimensional SDS-PAGE and two-dimensional A-PAGE × SDS-PAGE of progeny. Unfortunately the frequency of recombination between *Gli-A1* and the locus coding for N14-N15-N16 could not be determined with certainty because of the difficulties in identifying the presence of these minor components in some of the segregating F_3 lines. This remote locus and *Gli-B3* may form a homoeologous series, proteins N14-N14-N16 being allelic variants of the ω-gliadins coded by the *Gli-A3* locus on chromosome 1A at a comparable position to *Gli-B3* on chromosome 1B. Alternatively, this remote locus may be homoeologous to *Gli-A4* which is 10 cM from *Gli-A1* (Redaelli et al. 1992), or to the locus found by Metakovsky et al. (1986) at a recombination distance of about 5% from *Gli-A1*.

The identification of gliadin alleles in the parental cultivars Neepawa and Costantino will be useful for selection purposes in the breeding programme set up to determine the relationship between gluten viscoelastic properties and allelic variation at *Gli-1, Glu-3* and *Gli-3* in near-isogenic lines.

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Book review

Fedoroff, N.; Botstein, D.: The Dynamic Genome: Barbara McClintock's Ideas in the Century of Genetics. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. 1992. 422 pp., 36 figs., 32 photo., 8 tab. Hard bound $ 65.00.

This book is primarily a compilation of essays written by friends and colleagues of Barbara McClintock in honor of her 90th birthday. The perspectives of these contributions are particularly enlightening as they vary from former graduate students to laboratory coworkers to colleagues involved in related research endeavors. The structure of the book is more-or-less arranged according to a time-frame based on the career of Barbara McClintock. Dispersed among these contributions are reprints of a few manuscripts authored or coauthored by McClintock, including classical articles such as the relationship between cytological and genetic crossing-over and, of course, transposable elements. The book also contains a reprint of the Nobel lecture given in 1983 when she was honored with this distinguished award.

The essays written by former students give insight not only into the personal dedication and scientific approach of McClintock, but also the working relationship between major professor and student. Most of the other contributions excel in one of two ways. Some firmly establish McClintock's position in relation to other "giants" of genetics as this scientific field progressed during the century. Other authors specifically address the widespread impact her work had on the science of genetics, particularly in cytogenetics. For instance, several individuals relate the impact the insights elucidated by McClintock have had on their research careers with organisms other than maize. In many cases, importance is placed on the role played by personal relationships and discussions with McClintock on the careers of the authors. It is essential to note that a primary catalyst for these types of interaction was McClintock's interest in furthering the understanding of genetics. The reader does not perceive there to have been an attitude of superiority taken by this prominent scientist but one very willing to assist the development of other scientists as they pursued the answers to fundamental questions. McClintock's overall role in the science of genetics cannot be overstated as one examines the diversity of research thrusts she influenced throughout her career. This reviewer was also highly entertained by the contribution of G. Albrecht-Buehler titled "The Revenge of the Mayans", a humorously written critique of the scientific life of Barbara McClintock.

A reading of this book by current students would benefit their appreciation of the historical progress made in genetics during this century. It also serves as a pertinent example of the widespread impact an individual can have in the scientific community, and that significant breakthroughs are not limited in their scope of application to the immediate field of interest or specific organism of use. The scientific community suffered a tremendous loss with the recent death of this eminent scientist. The effect of Barbara McClintock's career will undoubtedly continue as she gave us insights into fundamental questions that we are now only beginning to address. The fact that many of her major discoveries were made with what would now be considered very crude technology only serves to establish the genius that was Barbara McClintock. In conclusion, I highly recommend this book to all scientists interested or involved in genetic research.

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