The D-Genome and the Control of Wheat Gluten Synthesis

It was recently reported by BOYD and LEE[1] that similar patterns were obtained by starch gel electrophoresis for gluten proteins of the hexaploid wheat variety 'Canthatch' (AABBDD) and for its tetraploid derivative 'Tetracanthatch' (AABB). 'Tetracanthatch' differs from 'Canthatch' in that it lacks the 7 pairs of chromosomes of the D-genome. Interpretation of these results was difficult since gluten extracted from a ditelocentric line of 'Chinese Spring', deficient in the short arm of chromosome 1D, showed an electrophoretic pattern deficient in 2 components of low mobility when compared with the pattern for its disomic parent, and because bands in the same region were missing in patterns for 2 of 3 reconstituted hexaploids derived from crosses between 'Tetracanthatch' and different accessions of *Aegilops squarrosa* L., the contributor of the D-genome.

Subsequently it was brought to our notice by Dr. K. W. SHEPHERD (Waite Agricultural Research Institute, Glen Osmond, South Australia) that the samples of 'Canthatch' and 'Tetracanthatch' examined by him showed distinct pattern differences. It is now established that the gluten of 'Tetracanthatch' wheat lacks at least 4 of the gluten proteins present in wheat of the variety 'Canthatch'. Due to a labelling error, 2 samples of 'Canthatch' were examined originally instead of the hexaploid and its tetraploid derivative. This paper presents protein analyses for authentic samples of 'Canthatch' and 'Tetracanthatch' and provides additional results which explain the patterns obtained for the reconstituted hexaploids.

Materials and methods. Wheat samples used are listed in the Table. Gluten proteins were extracted from a single grain by grinding with 0.4 ml 2*M* urea (LEE[2]). The clarified extract was applied directly to starch gel for electrophoresis in aluminium lactate buffer, pH 3.1, containing 2*M* urea (GRAHAM[3]).

Results. Starch gel electrophoresis of the gluten proteins of 'Canthatch' and 'Tetracanthatch' (Figure 1) shows that 'Tetracanthatch' lacks 3 slow-moving components and 1 of intermediate mobility present in the pattern for 'Canthatch'. No additional bands were detected for 'Tetracanthatch'. It therefore seems likely that the synthesis of these 4 components is controlled by genes on chromosomes of the D-genome.

Theoretically, the synthesis of these components should be restored in amphidiploids resulting from crosses between 'Tetracanthatch' and *A. squarrosa*. In order to examine this possibility, gluten proteins were analysed for 2 reconstituted hexaploids (Figure 2), in which different accessions of *A. squarrosa* contributed the D-genome. The pattern for each reconstituted hexaploid was similar to the pattern for a mixture of extracts from grain of the parents. Slow-moving components, when present in the pattern for the *A. squarrosa* parent (RL5271), were also present in the reconstituted hexaploid; when absent in the *A. squarrosa* parent (e.g. RL5003), they were also absent in the resulting hexaploid.

[1] W. J. R. BOYD and J. W. LEE, Experientia *23*, 332 (1967). There is an error in the legend of Figure 2 of this paper. Labelling of the second, third and fourth patterns from the left should read: 'Tetracanthatch' (AABB); 'Tetracanthatch' × *A. squarrosa* RL 5261 (AABBDD); 'Tetracanthatch' × *A. squarrosa* RL 5271 (AABBDD).

[2] J. W. LEE, J. Sci. Fd Agric. *19*, 153 (1968).

[3] J. S. D. GRAHAM, Aust. J. biol. Sci. *16*, 342 (1963).

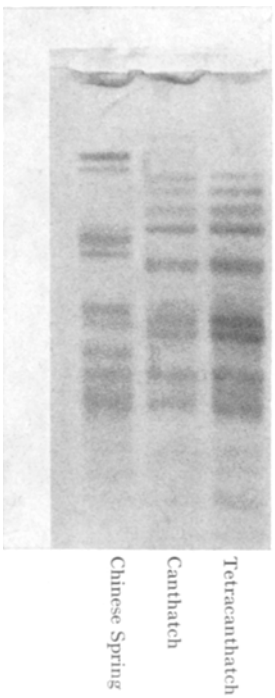

Fig. 1. Starch gel electrophoretic patterns of gluten proteins from Chinese Spring, Canthatch and the tetraploid from the latter. Origin at top.

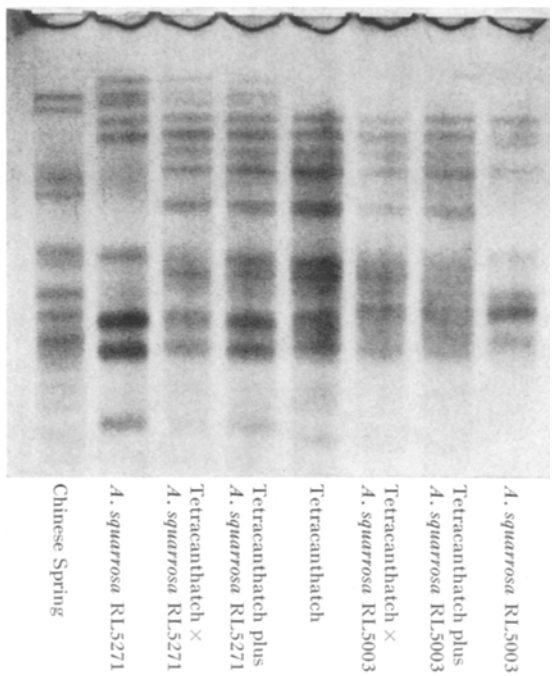

Fig. 2. Starch gel electrophoretic patterns for extracts of amphidiploids and of parent accessions and for mixed extracts of parent accessions.

The apparently conflicting results of the original paper can therefore be accounted for by a labelling error on one hand and by the variation in gluten protein pattern between accessions of *A. squarrosa* on the other hand.

Identification of wheat samples

Accession No. (U.W.A.[a])	Variety	Source and previous accession No.	
2921	Canthatch	R.L.[b]	
2922	Tetracanthatch	R.L.	
2879	Chinese Spring	U. of Mo.[c]	P62-61.4-1
2161	Chinese Spring DT 1D (long)	U. of Mo.	P56-17.1-3
2992	Tetracanthatch × *A. squarrosa* RL5271	R.L.	
2990	Tetracanthatch × *A. squarrosa* RL5003	R.L.	
2993	*A. squarrosa* var. *strangulata*	R.L.	RL5271
2995	*A. squarrosa*	R.L.	RL5003
2994	*A. squarrosa* var. *typica*	R.L.	RL5261
5463	*A. squarrosa*	P.B.I.[d]	C
5464	*A. squarrosa*	P.B.I.	E
5466	*A. squarrosa*	P.B.I.	A
5427	*A. squarrosa* var. *strangulata*	P.B.I.	
625	*T. monococcum* var. *nigro-flavescens*	U. of Man.[e]	2B28
5456	*T. monococcum*	P.B.I.	6
5454	*T. monococcum*	P.B.I.	13
5458	*T. monococcum*	P.B.I.	10
5453	*T. monococcum*	P.B.I.	13A
5460	*T. monococcum*	P.B.I.	19

[a] University of Western Australia. [b] Rust Laboratory, Canada, Department of Agriculture, Winnipeg. [c] University of Missouri. [d] Plant Breeding Institute, Cambridge. [e] Department of Plant science, University of Manitoba.

Variation of gluten protein composition between genotypes within a single species is well established for *Triticum aestivum* L. em. Thell. (ELTON and EWART[4]; GRAHAM[3]). This observation can now be extended to a number of other species of wheat. In the present study, considerable intraspecific variation of gluten protein composition was observed for most of 14 accessions of *T. monococcum* L., 6 accessions of *T. aegilopoides* L. and 11 accessions of *A. squarrosa*. Figures 3 and 4 show examples of patterns obtained for accessions of *A. squarrosa* and *T. monococcum*. Intraspecific pattern variations observed were in some cases as great as variations between representatives of different species. It was therefore hardly possible to establish any association between pattern und species.

Discussion. The association between slow-moving gluten components and the D-genome observed for a ditelocentric of 'Chinese Spring', for 'Canthatch' and for accession RL5271 of *A. squarrosa* is apparently not a general phenomenon since bands in this region are lacking in the patterns for some other accessions of *A. squarrosa* and for some bread wheats.

For the wheat-rye hybrid, *Triticale*, YONG and UNRAU[5] and BARBER et al.[6] have reported the appearance of 'new' protein bands which were present in neither parent. These reports for *Triticale* contrast with the present observation that the pattern for an amphidiploid is similar to the pattern obtained for a mixture of extracts from grain of the parents. Despite this simple relationship, it is not possible to infer from the patterns for 'Canthatch'

[4] G. A. H. ELTON and J. A. D. EWART, J. Sci. Fd Agric. *13*, 62 (1962).
[5] F. C. YONG and A. M. UNRAU, Can. J. Biochem. *42*, 1647 (1964).
[6] H. N. BARBER, C. J. DRISCOLL, P. M. LONG and R. S. VICKERY, Nature *218*, 450 (1968).

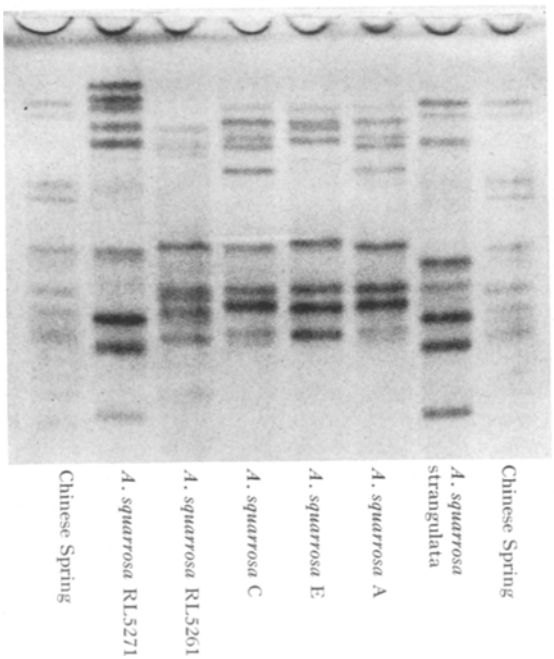

Fig. 3. Starch gel electrophoretic patterns of gluten proteins from accessions of *A. squarrosa*.

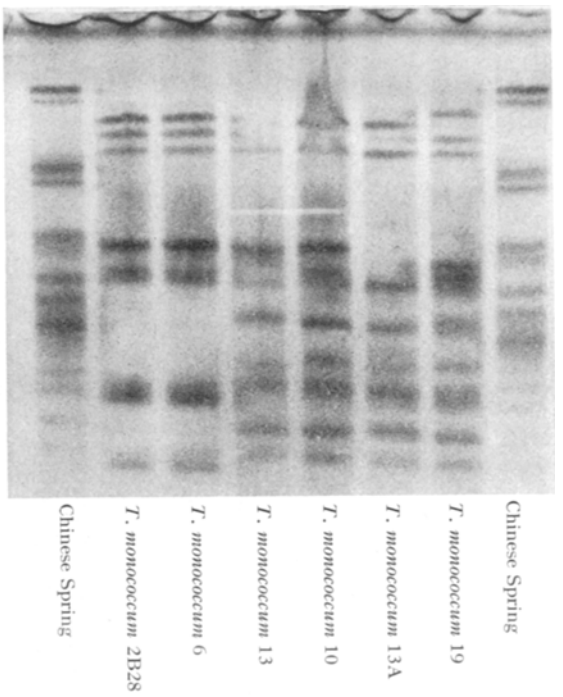

Fig. 4. Starch gel electrophoretic patterns of gluten proteins from accessions of *T. monococcum*.

and 'Tetracanthatch' the protein composition of the particular genotype of *A. squarrosa* involved in the evolution of 'Canthatch' since some bands due to the D-genome may be obscured by those contributed by the A- and B-genomes and since the 3 genomes may carry genes in common for protein synthesis[7].

Zusammenfassung. Die Entfernung des D-Genoms aus der Handelsweizensorte «Canthatch» führte bei der Stärkegelelektrophorese zum Verlust von 4 Komponenten des Klebermusters. Aus «Tetracanthatch» und verschiedenen Genotypen von *A. squarrosa* nachgezüchtete Hexaploide zeigten bei der Stärkegelelektrophorese eine ähnliche Zusammensetzung wie die Mischung der aus den entsprechenden Elterntypen extrahierten Proteine.

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[7] Helpful discussion with Dr. K. W. SHEPHERD and the technical assistance of Mrs. C. TRENOUTH are gratefully acknowledged.

The Influence of Oestradiol-17β on the Rat Uterine Na^+, K^+-Mg^{++} Activated Adenosine Triphosphatase Activity

The administration of oestrogens to ovariectomized rats can alter the electrolyte composition of the uterus[1,2]. The following experiments were undertaken to investigate the possibility that these changes are secondary to alterations in the Mg^{++}-activated ATPase activity brought about by oestrogens.

Materials and methods. The method used was that of W. W. KIELLY. Spayed adult rats (250 g $\pm$ 25) were injected s.c. with oestradiol-17β in arachis oil in doses ranging from 1–50 µg/rat. Animals were killed by cervical dislocation and their uteri were removed quickly and washed in ice-cold extracting solution (0.1 *M* KCl; 0.04 *M* $NaHCO_3$; 0.01 *M* Na_2CO_3) After weighing on a torsion balance, a portion of the tissue was removed for a dry weight estimation and the remainder was ground with sand in cold extracting solution. The final volume was adjusted to 20 ml/g tissue The suspension was centrifuged for 15 min at 900 *g* and an equal volume of de-ionized water was added to the supernatant After $^1/_2$ h this was centrifuged at 1000 *g* for 30 min to sediment the actomyosin precipitate. The supernatant was assayed for ATPase activity. This was carried out by incubating the following at 37 °C: 0.1 ml 0 05 *M* $MgCl_2$, 0.3 ml 0.2 *M* *Tris*-HCl buffer pH 6.9 (in 0.15 *M* KCl), 0.1 ml 0.02 *M* adenosine 3′,5′-triphosphate (disodium salt), 0.4 ml de-ionized water, 0.1 ml supernatant. The reaction was stopped after 5 min by adding 1 ml 5% perchloric acid. Phosphate assays were performed by a modification of the method of FISKE and SUBBAROW[3].

The concentration of protein of the supernatant was measured by a biuret method.

Results and discussion. There was no significant change in Mg^{++}-activated ATPase activity 30–40 min after administration of 5 µg oestradiol compared with ovariectomized controls. ATPase activity was decreased by 35, 44 and 39% 7, 19–25 and 45–50 h respectively following 50 µg oestradiol-17β administration. With 1 µg oestradiol-17β the change in ATPase activity was similar to that produced by 50 µg oestradiol (Table).

[1] D. F. COLE, J. Endocr. *7*, 12 (1950).
[2] N. B. TALBOT, E. C. LOWRIE and E. B. ASTWOOD, J. biol. Chem. *132*, 1 (1940).
[3] C. H. FISKE and Y. SUBBAROW, J. biol. Chem. *66*, 375 (1925).

Na^+, K^+, Mg^{++} activated adenosine triphosphatase activity of uterine tissue homogenate after removal of actomyosin expressed as µmoles phosphorus/g protein · 5 min

Treatment	Interval after administration	Mean	S.E. of mean	No. of animals	% difference from control
0.1 ml oil	7 h	1618.7	185.1	8	−39.6
1 µg oestradiol-17β	7 h	977.1	149.4	6	$0.01 < P < 0.001$
0.5 ml oil	7–50 h	1352.3	99.3	17	
50 µg oestradiol-17β	7 h	875.5	131.35	6	−35 $0.01 < P < 0.001$
50 µg oestradiol-17β	19–25 h	751.6	69.88	8	−44 $P > 0.001$
50 µg oestradiol-17β	45–50 h	824.2	49.4	7	−39 $0.01 < P < 0.001$
0.1 ml oil	30–40 min	1662.7	195.1	7	
5 µg oestradiol-17β	30–40 min	1867.5	66.76	6	+12.33 $0.2 < P < 0.1$