Acknowledgments.—The authors wish to thank Dr R. Yabo, for valuable suggestions on statistics, the Cardiology and Rheumatology Service of the P. Rossell Hospital for the cardiological examinations and Drs A. Hochman and H. P. de Reynés for clinical studies.

This investigation was supported by the Fund for Overseas Research Grants and Education, Inc.

6. References

BARTALOS, M., AND BARAMKI, T. A. 1967. *Medical Cytogenetics.* Williams and Wilkins Co., Baltimore.

CHICAGO CONFERENCE: Standardization in Human Cytogenetics. 1966. *Birth Defects: Original Article Series, II: 2.* The National Foundation, New York.

EDWARDS, J. H. 1962. Chromosome analysis from capillary blood. *Cytogenetics, 1,* 90-96.

LEJEUNE, J. 1965. In *Les chromosomes humains.* Gauthier-Villars, Paris.

MOORE, R. C., AND GREGORY, G. 1963. Biometrics of the karyotype of *Protemnodon bicolor,* with reference to the limitations in accuracy of identifying human chromosomes. *Nature* (Lond.), *200,* 234-237.

OHNO, S., WEILER, C., POOLE, J., CHRISTIAN, L., AND STENIUS, C. 1966. Autosomal polymorphism due to pericentric inversions in the deer mouse (*Peromyscus maniculatus*) and some evidence of somatic segregation. *Chromosoma* (Berl.), *18,* 177-187.

PHILIP, J., FRIDENBERG, O., AND SELE, V. 1965. Enlarged chromosome N° 1 in a patient with primary amenorrhoea. *Cytogenetics, 4,* 329-339.

SHARAT CHANDRA, H., AND HUNGERFORD, A. 1967. Identification of the human X chromosome: a reconciliation between results obtained from morphological and from radioautographic studies. *Ann. Génét., 10,* 13-17.

STEEL, R. G. D., AND TORRIE, J. H. 1960. *Principles and Procedures of Statistics.* McGraw-Hill, New York.

TURPIN, R., AND LEJEUNE, J. 1965. *Les chromosomes humains.* Gauthier-Villars, Paris.

A SPONTANEOUS CHLOROPHYLL MUTANT IN HEXAPLOID WHEAT

RONA PETTIGREW, C. J. DRISCOLL and K. G. RIENITS

Faculty of Biological Sciences, University of New South Wales, Kensington, N.S.W., Australia

Received 19.ii.69

1. Introduction

Only a limited number of chlorophyll mutants have been obtained in hexaploid wheat, *Triticum aestivum* L emend Thell. This is a consequence of the duplicated genetic material which buffers deletion of genes effecting chlorophyll production.

Those mutants that have been isolated and studied involve intragenic changes or deletion or two duplicate loci (Sears and Sears, 1963), Neatby's virescent, a spontaneous mutant, and *chlorina-1,* an E.M.S.-induced mutant, are examples of the former and Hermsen's virescent, a spontaneous mutant, an example of the latter (Sears and Sears, *loc. cit.*). This paper reports on a further chlorophyll mutant, of spontaneous origin, which involves an intragenic change.

Biochemical analysis of this type of mutant is of interest as it is clear that the mutant gene is an active gene rather than a deletion. It could not be a deletion as all 21 nullisomics of hexaploid wheat are normal green. Such analyses which are lacking in organisms with duplicated genetic material, may eventually elucidate the genetic control of chlorophyll production and the mechanisms of genetic diversification in polyploids.

2. Materials and methods

The spontaneous mutation was detected in a single plant in an F_2 population of the Cornell Wheat Selection 5075aB-2B-21 × Chinese Spring[6]. This population was uniform for the Chinese Spring phenotype except for the waxless character contributed by the non-recurrent parent. It has since been shown that the waxless character is inherited independently of the chlorophyll mutation.

Two dimensional thin layer chromatography on sucrose plates

All work involving pigment extracts was carried out as rapidly as possible in very dim light. A weighed sample of fresh leaf material (one or two leaves) was repeatedly ground in a Potter-Elvenhjem homogeniser with a total of 25 ml. of 100 per cent. acetone until all the pigment was removed.

Petroleum ether (10 ml.) was added to the extract clarified by centrifugation. The acetone was removed by washing three or four times with about 20 ml. of water, and the petroleum ether, containing the pigments, dried with a small amount of NaCl and evaporated under a stream of N_2 to 1-2 ml.

Thin layer chromatography was carried out by the method of Jeffrey (1968). The solvents were: (i) *n*-propanol (0·6 per cent. V/V) in 60-80° petroleum ether and (ii) chloroform (15 per cent. V/V) in 60-80° petroleum ether.

Column chromatography. This was carried out according to Strain (1958) using a column (1·8 cm. diameter × 15 cm.) of powdered icing sugar containing 5 per cent. corn flour.

The sample was applied as 1 ml. of petroleum ether extract prepared as for thin layer chromatography. Separation was achieved by addition of 15 per cent. chloroform in petroleum ether until solvent almost reached the bottom of column. The pigment-containing bands were removed from the column and pigments eluted with following solvents: chlorophyll *a* and *b* with acetone, carotene with petroleum ether and xanthophylls with 100 per cent. ethanol.

The concentrations of eluted pigments were calculated using coefficients given by Jeffrey (1968) at the observed absorption peaks.

Spectrophotometric analysis. Leaf material (0·2—0·3 gm.) was ground in 80 per cent. aqueous acetone with a Potter-Elvehjem homogeniser, centrifuged and the supernatant made up to 50 ml. with 80 per cent. acetone. The concentrations of chlorophyll *a* and *b* were determined using the equations of Vernon (1960) and also after quantitative transfer of pigments into diethyl ether, by the equations of Smith and Benitez (1955). For the determination of total carotenoids the chlorophylls were saponified and separated from the carotenoids as described by Boardman and Anderson (1967). The concentration of total carotenoids was calculated from the absorbance maxima (441 mμ for green and light green and 444 mμ for the yellow) using the factor of 0·24 given by Boardman and Anderson.

All spectrophotometric measurements were carried out using a Unicam S.P. 800 Recording Spectrophotometer. The spectrophotometric assay was considered to be more rapid and reproducible than the assays based on column chromatographic separation but did not enable the assay of separated carotenoids permitted by the latter procedure.

3. Results

Mutant plants. The original plant possessing the mutation was uniformly light green in colour. On selfing it and subsequently other light green individuals a ratio of 970 green or light green: 330 yellow individuals was obtained. The mutation is therefore due to a change in a single gene, which when homozygous produces a distinctly yellow phenotype. Although yellow

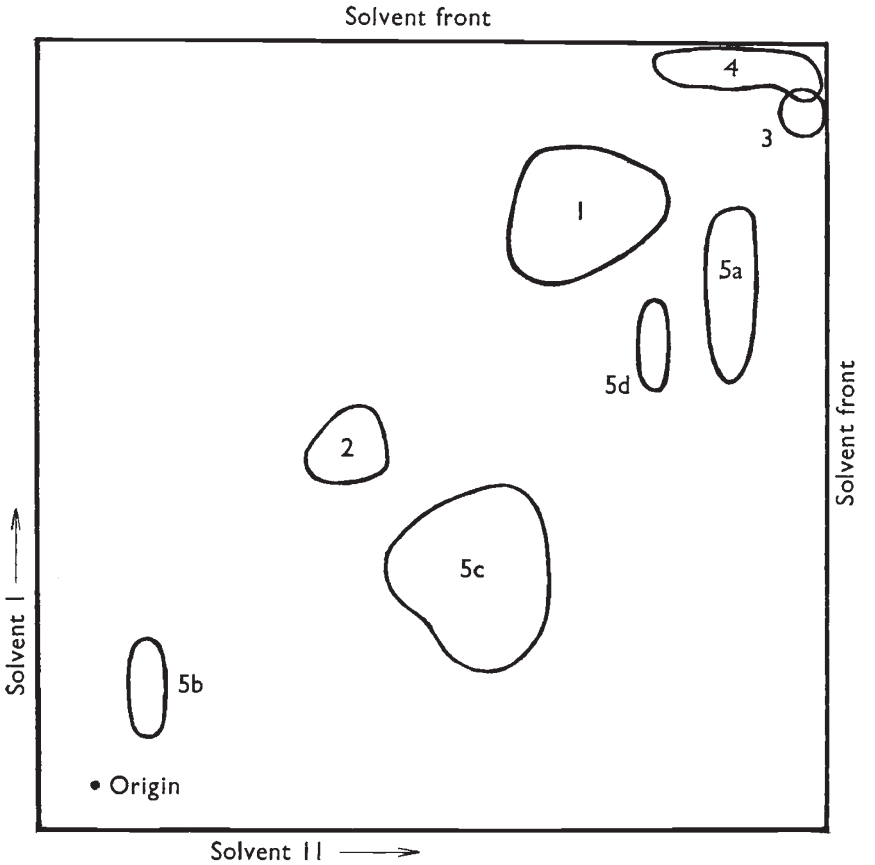

Fig. 1.—Two dimensional chromatogram of pigments found in leaves of green, light green and yellow plants.
Key. 1. Chlorophyll *a* (green); 2. Chlorophyll *b* (yellow green); 3. Pheophytin (grey trace); 4. Carotene (yellow); 5. Xanthophylls (yellow) *a.* Lutein, *b.* Neoxanthin, *c.* Violoxanthin plus unknown xanthophylls, *d.* Unknown xanthophyll (trace).

individuals mature four to six weeks later than heterozygotes, which are normal in this respect, they are nevertheless fully fertile. Also, gametes bearing this mutant gene compete equally with normal gametes.

Individuals heterozygous for this mutant allele can usually be distinguished from normal by virtue of being light green. The distinction between normal green and light green is most obvious at the one to two leaf stage under high light and low temperature conditions.

As the heterozygote is distinguishable from normal the mutation is looked upon as being incompletely dominant to normal. The original light green

plant was obviously a heterozygote and its lack of chimerism indicates that the mutation probably took place in one of the gametes involved in its production.

Qualitative pigment analyses. Fig. 1 illustrates the pigments separated by two dimensional thin layer chromatography. The green, light green and yellow genotypes were qualitatively identical. Chlorophyll *a* and *b* were always present with a trace of pheophytin *a* and no evidence of chlorophyllides (which would be clustered around the origin). The carotene spot was shown to be β-carotene from its chromatographic properties on a magnesia-celite column (Strain, 1958) and spectrophotometric properties using authentic β-carotene for comparison. The main xanthophylls were lutein and neoxanthin (spots 5*a* and 5*b*). The lutein spot was spectrophotometrically homogeneous despite its trailing nature. Spot 5*c* was large and diffuse and contained violaxanthin as well as some unknown xanthophylls. The material eluted from this area showed absorption peaks at 436 and 467 mμ in diethyl ether which are slightly lower than the 441 and 471 mμ given by Jeffrey (1961) for violaxanthin. Spot 5*d* present in trace amounts represented an unknown xanthophyll.

TABLE 1

Pigments of fresh leaf tissue of ninth leaves of yellow and green plants of a summer planting. Separations carried out by column chromatography.

Quantity of Pigment (μg/g fresh leaf) and Wavelength of Absorbance measurement (mμ).

Age of Leaf (days)	Chloro-phyll a $\lambda = 663$	Chloro-phyll b $\lambda = 646$	Carotene $\lambda = 447$	Lutein $\lambda = 444$	Unknown Xantho-phylls $\lambda = 437$	Neoxanthin $\lambda = 435$	Chl. *a*/ Chl. *b*
(a) Yellow plants							
3	335	23	40	51	21	10	14·7
10	490	66	62	65	9	12	7·5
15	568	85	57	55	8	9	6·7
20	672	104	75	62	16	19	6·4
Mean	—	—	58±23	58±9	14±10	13±7	—
(b) Green plants							
1	1520	504	88	123	24	36	3·0
9	1610	556	101	123	21	49	2·9
14	1508	456	112	143	24	41	3·3
17	1534	489	107	129	31	46	3·1
Mean	1543±73	501±66	102±17	130±16	25±7	43±9	3±0·3

Quantitative pigment analyses. Table 1 sets out the results of quantitative assays from summer-grown material for chlorophylls and carotenoids after column separation. Chlorophyll *a* and *b* are present in very much smaller amounts in yellow leaves than in green leaves. All the carotenoids are also reduced in yellow leaves but not to the same extent as are the chlorophylls. The chlorophyll content of yellow leaves showed definite trends with maturity as shown graphically in fig. 2.

Further observations on changes with maturity. Using the spectrophotometric assay technique the homozygous yellow, homozygous green and the heterozygote, grown in both summer and winter, were examined for changes in pigment content with maturity.

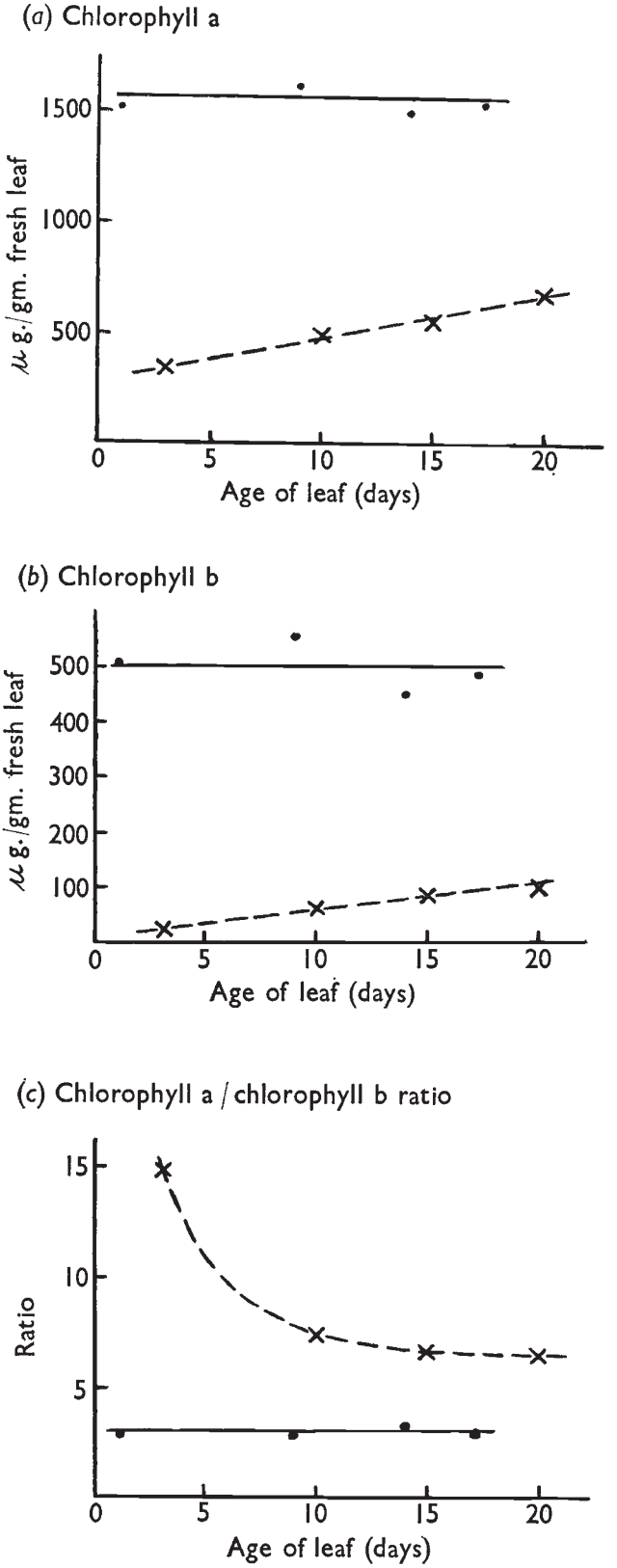

FIG. 2.—Changes in chlorophyll content with age of leaf as shown by column analysis of material grown in late summer.

● —● Green leaves × - - - - × Yellow leaves.

In all cases the amounts of chlorophyll *a*, chlorophyll *b* and total carotenoids were lowest in the homozygous yellow and highest in the homozygous green. The amounts of pigments in the heterozygote were much more similar to the homozygous green than to the homozygous yellow. For instance the chlorophyll a contents of the homozygous yellow, heterozygote and homozygous green in one set of determinations were 261, 1116 and 1372 μg/gm. of fresh leaf.

With the summer grown homozygous yellow material the chlorophyll *b* content increased from 41 to 72 μg/gm. fresh leaf between 1 day and 14 days after emergence of the fifth leaf. No definite trends were shown for chlorophyll *a* and the chlorophyll *a* : *b* ratio decreased from 6·3 to 3·4. Comparable observations on total carotenoids showed a decrease from 131 to 78 μg/gm. fresh leaf.

Similar observations on summer-grown heterozygous and homozygous green material showed no definite trends with maturity.

In contrast to the summer-grown material, the chlorophyll *b* content of the winter-grown homozygous yellow material remained fairly constant at a mean of $95 \pm 10\mu$g/gm. fresh leaf material between 1 day and 26 days after emergence of the fifth leaf. Similarly the chlorophyll *a* content showed no definite trends and the chlorophyll *a* : *b* ratio remained fairly constant at $4{\cdot}2 \pm 0{\cdot}5$. Comparable observations on total carotenoids, however, did show a decrease from 247 to 137 μg. The twelve sample points in this series of determinations were fitted to a regression line, the slope of which was significantly less than zero.

Similar observations on winter-grown heterozygous and homozygous green material showed an increase in all pigments with maturity of the leaf followed by a decrease with senescence.

4. Discussion

The trends in chlorophyll content noted in homozygous yellow material in late summer but not in mid-winter may have been caused by the change in one or more environmental factors, for example, day length, which would have been constantly decreasing in late summer and almost constant in mid-winter. Alternatively, these trends may be a consequence of a much more rapid development. The decrease in carotenoid content which was shown in both seasons with homozygous yellow material may be a constant feature of the ageing mutant leaf. This variability of the mutant shows the need, when describing it, for stating the environment under which it was grown and the stage of development of the sample material.

Although it is known that the mutant gene is an active one, its point of action is unknown. As there is no qualitative difference between the normal and the mutant we cannot point to any definite block in pigment synthesis. Possibly the mutation affects the chloroplast structure. D. von Wettstein (1961) has shown a relationship between different types of chloroplast structure and different chlorophyll mutants in barley but it has not been shown definitely whether the chloroplast structure abnormality is the cause or effect of pigment deficiency. Indeed this may vary from mutant to mutant.

Further light on the gene action involved may be obtained from the study of the effects of other alleles of the mutant and of varying dosages of corresponding genes on homologous chromosomes.

5. Summary

1. A spontaneous chlorophyll mutation isolated in hexaploid wheat involves intragenic change at one locus. The mutant allele is partially dominant to normal such that homozygotes are yellow and heterozygotes are light green.

2. Thin layer chromatography of leaf pigments did not reveal any qualitative differences between mutant and normal.

3. Quantitative analysis showed that chlorophylls, and to a less extent carotenoids, are reduced in the mutant.

4. As chlorophyll *b* was reduced more than chlorophyll *a* the ratio of chlorophyll *a* : *b* was increased above that of normal.

5. Quantities of chlorophylls and carotenoids vary with maturity of the leaf and with the environment.

Acknowledgments.—We are indebted to Dr Shirley W. Jeffrey, C.S.I.R.O., Division of Fisheries and Oceanography, for her advice and for details of the thin layer technique prior to publication.

One of us (R.P.) held a Reserve Bank of Australia Scholarship while carrying out this research.

6. References

BOARDMAN, N. K., AND ANDERSON, J. M. 1967. Fractionation of the Photochemical Systems of Photosynthesis. II. Cytochrome and Carotenoid Contents of Particles Isolated from Spinach Chloroplasts. *Biochim. Biophys. Acta.*, *143*, 187-203.

JEFFREY, S. W. 1961. Paper-Chromatographic Separation of Chlorophylls and Carotenoids from Marine Algae. *Biochem. J.*, *80*, 336-342.

JEFFREY, S. W. 1968. Quantitative Thin-Layer Chromatography of Chlorophylls and Carotenoids from Marine Algae. *Biochim. Biophys. Acta.*, *162*, 271-285.

SEARS, L. M. S., AND SEARS, E. R. 1968. The Mutants *Chlorina-1* and Hermsen's Virescent. *Proc. 3rd Int. Wheat Genetics Symposium* (in press).

SMITH, J. H. C. AND BENITZ, A. 1955. *Chlorophylls: Analysis in Plant Materials in Modern Methods of Plant Analysis, IV.* 142-196 (Eds. Paech, K. and Tracey), M.V-Springer-Verlag.

STRAIN. H. H. 1958. Chloroplast-Pigments and Chromatographic Analysis. *32nd Annual Priestly Lectures. Pennsylvania State University.*

VERNON, L. P. 1960. Spectrophotometric Determination of Chlorophylls and Pheophytins in Plant Extracts. *Anal. Chem.*, *32*, 1144-1150.

VON WETTSTEIN, D. 1961. Nuclear and Cytoplasmic Factors in Development of Chloroplast Structure and Function. *Canad. J. Bot.*, *39*, 1537-1545.

GENETICS OF SPECTACLE IN DIPLOID POTATOES

N. W. SIMMONDS

Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian

Received 20.ii.69

1. Introduction

The spectacle character in potatoes is a failure of pigmentation in sharply delimited patches around the eyes of tubers; the shape of the patches varies from oval to transversely streak-like. Dodds and Paxman (1962) showed