example, *Sl*), (C57BL/6-*Sl* × SEC)F_1 × C57BL/6; for recessive markers (for example, *ln*), (C57BL/6-*lnln* × SEC)F_1 × C57BL/6-*lnln*. In each case, the indicated marker gene and the Sol^1 allele of C57BL/6 were contributed by the same parent. Recombination percentages indicate absence of linkage with marker genes belonging to linkage groups III (Patch, *Ph*, 53 ± 8.0), XI (White, Mi^{w^h}, 68.2 ± 9.9), XIII (leaden, *ln*, 66.7 ± 10.3, and fuzzy, *fz*, 61.9 ± 10.6), and XVI (Varitint-waddler, *Va*, 52.4 ± 10.9). Results obtained for crosses involving *Sl*, in linkage group IV, suggest but do not prove loose linkage (36.6 ± 7.7) between the *Sol* locus and the *Sl* locus. Matings have been set up involving other genetic markers in linkage group IV, in order to test this possibility.

Particular peptide markers for both α-chains [from our work and that of Popp (*7*)] and β-chains (*11*) of certain mouse hemoglobins are now available to use for rapid screening of large numbers of mice, greatly facilitating definitive linkage experiments. Wherever it is applicable, the rapid one-dimensional chromatographic method for distinguishing hemoglobins with peptides α4 and α4H is preferable in linkage experiments to the currently used solubility test for two reasons. Several variables in technique, including concentration of hemoglobin in solution, temperature and time of incubation, *p*H (*13*), and molarity (*2*) of salt solution, alter the solubility of many mouse hemoglobins tested, so that within experiments which depend upon solubility for identification of hemoglobins, conditions must be carefully selected and controlled (*13*), and quantitative comparisons between systems may be misleading. Also, the chromatographic method may be applied reliably to hemoglobins from mice carrying the Hb^dallele, whose presence complicates identification of α-chain solubility effects.

The symbol now in use for the α-chain template locus, *Sol*, relates to a property not limited to effects of α-chain substitutions. The particular complex of non-α-chains found in mice carrying the Hb^d allele, whose presence the β^s-chain of human sickle-cell hemoglobin. If suitable mouse β-chain mutants become available for testing, it is probable that some of these also will influence hemoglobin solubility. In view of the accepted concept of a polypeptide chain as the direct product of a cistron, and in view of increased understanding of mouse hemoglobin genetics, we urge that serious consideration be given to replacement of the current *Sol* and *Hb* genetic designations by more appropriate symbols clearly associating each template locus with its respective polypeptide chain.

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12. Mice were killed; hemoglobin was prepared as usual except that dialysis was omitted (*1*), and 40 mg of undialyzed hemoglobin was digested in a final volume of 2 ml in the usual way for "fingerprinting" (*1*). Spots (100 μl) of each of 12 or 13 digests were placed on the long dimension (near the edge) of a sheet (46.3 × 57.2 cm) of Whatman No. 3 chromatography paper. The papers were developed in the short direction for 22 hours by descending chromatography with the upper phase of *n*-butanol, acetic acid, and water (4:1:5 by volume). After drying overnight, the spotted sheets were sprayed with Pauly's reagent and the presence or absence of the histidine-containing peptide α4H was noted.
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14. Solubility of hemoglobin was determined by a procedure simplified from those described previously (*2*, *13*); 40 μl of blood was drawn directly from the orbital sinus of a nonanesthetized mouse into heparinized capillary tubes, and mixed with 2 volumes of distilled water in a small beaker. After lysis of all cells, the hemoglobin plus stroma were added to 3 ml of 2.8*M* KH_2PO_4—K_2HPO_4 buffer solution at *p*H 6.8. This was immediately filtered through No. 2 Whatman paper, to remove precipitated stroma and serum proteins, and the filtrate was incubated for a period of 7 hours at room temperature. Solutions were again filtered through No. 2 Whatman paper and the density of hemoglobin remaining in solution (optical density) was determined in a spectrophotometer at wavelength 540 mμ.
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Wheat: Reconstitution of the Tetraploid Component (AABB) of Hexaploids

Abstract. *The tetraploid AABB genomic component of two varieties of common hexaploid wheat (AABBDD; 2n = 42) was reconstituted by a simple backcrossing technique in which known phylogenetic relationships between the hexaploid and tetraploid groups of* Triticum *were used. The reconstituted tetraploids do not closely resemble commonly described varieties of the present-day group of tetraploid species. The plants are dwarfed, lack vigor, and are partially or completely self-sterile, depending on the variety of the hexaploid source. Chromosome pairing is similar to that observed in a variety of durum wheat (AABB; 2n = 28). Synthetic hexaploids derived from hybrids between one of the reconstituted tetraploids and several strains of* Aegilops squarrosa *(D genome) are of normal growth and vigor and are highly fertile.*

The phylogenetic relationships among species comprising the allopolyploid wheat genus *Triticum*, and the related genus *Aegilops*, are perhaps more clearly understood than those of any other polyploid group of plants. The wheats fall naturally into three groups of species: the diploids, tetraploids and hexaploids with 14, 28, and 42 somatic chromosomes, respectively. The chromosome set of the diploid species has been designated as the A genome. The tetraploid group has the full complement of diploid wheat chromosomes and, in addition, a second set of seven chromosomes derived from another diploid species, supposedly *Aegilops speltoides* Tausch. (*1*). This second genome has been designated B so that the tetraploids have the genomic formula AABB. Hexaploid wheats have the two sets of genomes possessed by the tetraploids and a third set, the D genome of the diploid species *Aegilops squarrosa* L. The hexaploids have, therefore, the genomic constitution AABBDD. This last group has evolved by amphiploidization in two steps. First, the tetraploid component originated as an amphiploid through hybridization of diploid wheat (A genome) and *A. speltoides* (B genome) or a

Fig. 1. Adult plants of Canthatch hexaploid (left) and reconstituted tetraploid component (right).

closely related *Aegilops* species. Second, further amphiploidization occurred, in relatively recent times, between the tetraploid group and *A. squarrosa* (D genome) to give rise to the hexaploids. Conclusive evidence for this final step has been provided independently by Kihara (*2*) and McFadden and Sears (*3*).

The basic phylogenetic relationships among species of the *Triticum-Aegilops* group have been determined largely by means of genome analysis. This method is based upon the assumption that the relationship betwen species is correlated with the degree of meiotic chromosome pairing in their F_1 hybrids. The presence of a common genome or genomes is usually assumed by the close synapsis of the related sets of chromosomes. While this and other methods (*4*) have provided evidence for the establishment of a general outline of the origin and evolution of hexaploid wheat and of the phylogenetic relationships within the *Triticum-Aegilops* complex, there are still numerous details that are unknown or that require clarification. In this report a technique is described by which it is possible to reconstitute or extract the genomic components of hexaploid wheat by utilizing known phylogenetic relationships, thus providing a new approach to phylogenetic problems in *Triticum* and related species. In particular, this report is concerned with reconstitution of the tetraploid component (AABB) of common bread wheat.

The tetraploid component of *T. aestivum* L. emend. Thell. spp. *vulgare* MacKey cultivar Canthatch (AABBDD; $2n = 42$), a hard, red spring wheat, was reconstituted as follows: Initially a cross was made between Canthatch and *T. durum* L. cultivar Stewart (AABB; $2n = 28$). The pentaploid F_1 (AABBD; $2n = 35$), in which the genomes of *T. durum* pair with the corresponding genomes of the hexaploid to form most frequently 14 bivalents and 7 univalents at metaphase I of meiosis, was backcrossed to Canthatch (female). Pentaploids in which $14_{II} + 7_I$ were predominantly formed were selected from the backcross F_1 progeny and again crossed to Canthatch (female) to obtain pentaploids. A series of such successive backcrosses of the pentaploid type to Canthatch was made to eliminate the genetic material contributed by the *T. durum* parent in the initial cross. The pentaploids recovered at the conclusion of seven successive backcrosses were considered to be essentially homozygous for the Canthatch genotype. These F_1 pentaploids were self-pollinated, pollination being controlled by bagging. The tetraploid type subsequently recovered in the F_2 progeny presumably represents the AABB component of Canthatch. Supporting evidence to show that the backcrossing procedure was effective in reconstituting the Canthatch genotype was provided by the recovery of hexaploid siblings of the F_1 pentaploids and of the F_2 tetraploids which were indistinguishable from Canthatch in gross morphological characteristics.

The reconstituted tetraploid component of Canthatch does not resemble commonly described varieties of present-day tetraploid species. The plant is less vigorous than ordinary tetraploids or common wheat varieties; it is semi-dwarf, growing to about half the height of hexaploid Canthatch (Fig. 1). The leaves and stems are finer than those of Canthatch; the spike is very short and dense (Fig. 2), resembling somewhat the hexaploid spp. *compactum*. The rachis is not fragile and disarticulation is of the wedge type, as in Canthatch. The kernels are free-threshing, having about the same intensity of red color as those of Canthatch, but are considerably smaller with a roughly sculptured surface. Under relatively uniform conditions in a growth cabinet, self-fertility approaches 50 percent as compared with 95 percent or higher for Canthatch. At meiotic division in the microsporocytes, chromosome pairing is very similar to that observed in the *T. durum* cultivar Stewart; 14 bivalents, mostly of the closed type, are generally noted at metaphase I.

A similar attempt was made to reconstitute the AABB component of Selkirk, which is also a hard, red spring cultivar of *T. aestivum* spp. *vulgare*. All tetraploids recovered during the course of the backcrossing procedure were extremely dwarfed and of poor growth and vigor, even more so than the tetraploid of Canthatch. No seeds were produced on self-pollinated spikes despite the occurrence of normal bivalent pairing at metaphase I in the few plants examined.

From the results so far obtained it seems that the growth, vigor, and fertility of the extracted tetraploid component of hexaploid wheat varies from variety to variety. It is likely that this component of some strains cannot be maintained or perpetuated in the absence of one or more of the D-genome chromosomes. The pentaploid plants (AABBD) derived from all stages of the backcrossing cycle with both Canthatch and Selkirk were comparable to the hexaploids in growth, vigor, and general development, although highly sterile.

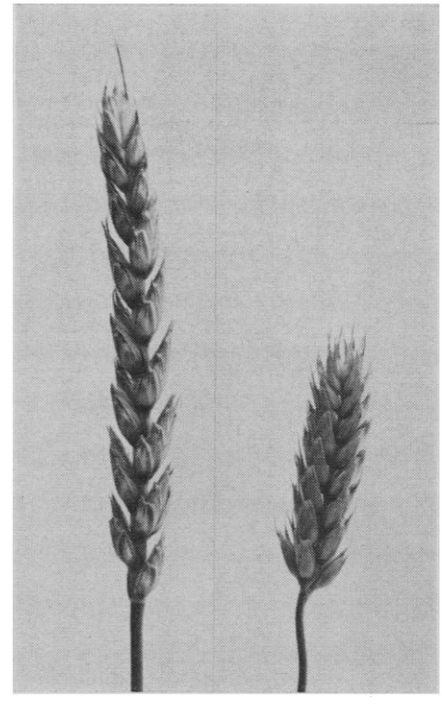

Fig. 2. Head of Canthatch hexaploid (left) and reconstituted tetraploid component (right).

The technique and results presented here are of practical and theoretical significance. The reconstituted genomic components of common hexaploid wheat may be of value in plant breeding. For example, the reconstituted AABB component of a commercial variety could be combined with varieties of *Aegilops squarrosa* to produce synthetic hexaploids. Differences noted between the synthetics and the commercial variety would be due to the newly added D genome of the particular *squarrosa* type used. Although preliminary evaluation of several such synthetic hexaploids indicates they are not of direct commercial value, as might be expected, desirable characteristics may be readily transferred to ordinary hexaploid varieties by routine breeding methods. Furthermore, if a synthetic were crossed with the common hexaploid strain from which the AABB had been extracted, segregation in the progeny would be for genes on the D chromosomes only. This would permit, therefore, a genetic analysis of the D genome.

By this technique it might be possible to separate or "dissect" hexaploid wheat into additional genomic components, either at the tetraploid (AADD and BBDD) or, less likely, at the diploid level (AA, BB, and DD). It might also prove useful in reconstituting the genomic components of other amphiploids in the *Triticum-Aegilops* complex and of similarly constructed polyploid types in other genera. The isolation of these components may provide a new approach to problems concerning the characteristics of the ancestral species of naturally occurring amphiploids, the genetic composition of the constituent genomes, and the genetic changes which presumably have taken place within them since incorporation into the polyploid form. It is obvious that genetic changes have occurred in the AABB component of common hexaploid wheat which adversely affect the growth and fertility of the reconstituted tetraploid. These deficiencies are not expressed at the hexaploid level because of the compensating effects of the D genome. This is substantiated by the normal growth, vigor, and high fertility of synthetic hexaploids produced from hybrids between the extracted AABB component of Canthatch and *A. squarrosa*.

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Joint Occurrence of a Lichen Depsidone and Its Probable Depside Precursor

Abstract. *A chemical race of the lichen* Cetraria ciliaris *Ach., known to produce the depside olivetoric acid, contains the corresponding depsidone, physodic acid, as well. Physodic acid may form by an intramolecular coupling of olivetoric acid.*

Lichens have been notoriously difficult subjects for physiological experimentation (*1*) and there is little information about the biosynthesis of their curious phenolic constituents. The routes of synthesis of some of these compounds have been proposed tentatively by analogy with known processes in other organisms and by the occurrence of chemically similar substances in certain closely related species or "strains." Conversions such as lecanoric acid to diploschistic acid by nuclear hydroxylation, alectoronic acid to α-collatolic acid by O-methylation, evernic acid to obtusatic acid by C-methylation, atranorin to chloratranorin by nuclear chlorination, and baeomycic acid to squamatic acid by alkyl side chain oxidation are suggested by the joint occurrence of these pairs of substances in the individual thalli of certain species of lichens.

Depsidones have a characteristic seven-membered ring joining two phenyl nuclei through ester and ether linkages, and are almost unique to lichens (*2*) (Fig 1). It has been proposed that depsidones are formed by dehydrogenative coupling of depsides, compounds containing the ester linkage preformed (*3*). This view is supported by the structural similarities between some depsides and depsidones. Also, a few examples of depside-producing lichens are known for which there is a morphologically indistinguishable segregate containing the corresponding depsidone (*4*). When a naturally occurring depsidone was synthesized in the laboratory, an oxidative coupling reaction was utilized, and the synthesis was cited as "good evidence in support of the suggestion that depsidones are formed *in vivo* from depsides" (*5*). The present report provides the first proof for the joint occurrence of a lichen depsidone and the corresponding depside.

During a microchemical survey of the lichen substances in the genus *Cetraria*, it became evident that specimens of *C. ciliaris*, producing olivetoric acid, contain another substance which interferes with analyses made by Asahina's microcrystallographic methods (*6*). Atranorin, a β-orcinol-type depside present in this lichen, was separated by its greater solubility in benzene and could not have interfered with the other tests. Chromatograms of extracts from fragments (approximately 25 mg) of 12 herbarium specimens were developed in n-butanol saturated with ammonium hydroxide, viewed with ultraviolet light (366 mμ), and sprayed with tetrazotized benzidine. Each sample showed spots for olivetoric acid and for another component, with a lower R_F value, tentatively identified as physodic acid by chromatographic comparisons with known lichen substances.

For confirmatory tests, a large sample of *Cetraria ciliaris* was collected from wooden fence rails at Laurel Springs, North Carolina. The lichen was air dried (179.2 g) and scanned with ultraviolet light (366 mμ). A few fluorescent plants (6.2 g, 3.5 percent of the sample) were segregated; they contained alectoronic acid and atranorin but were indistinguishable morphologically from the rest of the sample (*7*). A nonfluorescing, intact, fruiting plant (1.42 g) was extracted with boiling ethyl ether. The residue from evaporation of the extract was chromatographed on a column (50.0 × 4.0 cm) of silicic acid (Mallinckrodt, 100 mesh), and was eluted with benzene to which increasing concentrations of ethyl ether were added, up to 50 percent. The fractions (5 ml) of effluent were tested by paper chromatography (Whatman No. 1 paper developed with ammonium hydroxide-saturated n-butanol) and by Asahina's microcrystal tests (*8*).

Atranorin was identified in fractions