shown above, and contiguity is not excluded. Such linkage of two loci coding for similar polypeptides suggests that one may have arisen from the other or that they both stem from a common origin.

The fact that *B* and *C* polypeptides occur homologously in birds and in mammals suggests that the *B* and *C* loci had separate identities before the evolutionary separation of birds and mammals. If their separate identities are assumed to have originated by tandem duplication, these loci have thus remained closely linked for a long period of time, at least in birds.

Despite the close linkage, there is a wide difference in the biological behavior of the two loci. The activity of the *B* locus is manifest in both sexes and in all tissues throughout life, whereas the activity of the *C* locus is restricted to a particular sex, a particular tissue, and a particular stage of maturation. Thus, as is frequently the case in microorganisms, the control mechanisms involved may be specific to a short DNA segment constituting one or more loci. The linkage indicates that they are certainly not whole-chromosome mechanisms of the type occurring, for example, in normal "X-inactivation."

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Carbon Dioxide Compensation Points in Related Plant Species

Abstract. *Both high and low CO_2 compensation concentrations were found in the plant genera*—Panicum, Cyperus, *and* Euphorbia. *Within each genus, however, high and low compensations were found in different subgenera. Thus, they may not be genetically closely related. No significant differences in CO_2 compensation were found among 100 genetic lines of* Triticum aestivum *L. or among 20 lines of* Hordeum vulgare *L.*

The atmospheric concentration of CO_2 at which respiratory release of CO_2 within illuminated leaves is in balance with photosynthetic CO_2 fixation —the CO_2 compensation point—differs markedly among plant species (*1*). Species that have a CO_2 compensation point near zero part per million (ppm) have rates of photosynthesis in intense light and ambient air nearly twice as great as species with compensation points of 50 ppm or greater.

Species with low CO_2 compensation points apparently lack photorespiration (*2*), their net photosynthesis is not suppressed by O_2 (*3*), and they fix CO_2 by carboxylation of phosphoenolpyruvate (a 4-carbon pathway) (*4*). They are also characterized by having vascular bundle sheath cells containing specialized chloroplasts which appear to be particularly active in starch formation (*5*). Whether all these traits are necessarily related to high potential for photosynthesis has not been determined, nor has the genetic control of any of these processes been studied, because no significant genetic variability has been found for these traits within a species or within closely related species from which viable crosses could be obtained.

Compensation measurements were made in a closed system consisting of a test tube (2 by 15 cm) connected in series by glass tubing to an air pump, a desiccant column, and an infrared gas analyzer. A leaf, or a small branch in the case of small-leaved dicotyledonous species, was excised beneath air-free water. Air-free water (5 ml) was placed in the test tube, and the leaf or branch inserted into the tube with its base in the water. The test tube was connected to the system, immersed in a water bath which controlled leaf temperature at 23° ± 1°C, and illuminated through an additional 8-cm water filter by four 150-watt reflector spot lamps mounted 50 cm from the leaf. Photosynthesis in the leaves removed the CO_2 from the system until the CO_2 compensation concentration was reached. This required about 15 min-

Table 1. Occurrence of high and low CO_2 compensation points among species of *Euphorbia*, *Panicum*, and *Cyperus*.

Species with compensation point	
< 10 ppm	> 50 ppm
Euphorbia	
Subgenus Chamaesyce Raf.	Subgenus Agloma (Raf.) House
Euphorbia glyptosperma Engelm.	*Euphorbia corollata* L.
E. maculata L.	*E. hexagona* Nutt.
E. missurica Raf.	*E. marginata* Pursh.
E. serpyllifolia Pers.	Subgenus Poinsettia (Graham) House
E. supina Raf.	*E. dentata* Michx.
	E. heterophylla L.
Panicum	
Subgenus Eupanicum Godr.	Subgenus Dischanthelium Hitchc. & Chase
Panicum anceps Michx.	*P. maximum* L.	*Panicum oligosanthes* Schult.
P. antidotale Retz.	*P. miliaceum* L.	*P. praecocius* Hitchc. & Chase
P. capillare L.	*P. minus* Nash.	Subgenus unknown*
P. coloratum Walt.	*P. obtusum* H. B. K.	*P. bisculatum* Thumb.
P. dichotomiflorum Michx.	*P. plenum* Hitchc.	*P. milioides* Nees ex trin.
P. hallii Vasey	*P. virgatum* L.	
Subgenus unknown*		
P. laevifolium Hack		
P. prolutum F. Muell.		
P. stapfianum Fourc.		
P. turgidum Forsk.		
Cyperus		
Cyperus esculentus L.		*Cypserus papyrus* L.

* Not listed in Hitchcock (*9*).

utes. All measurements were replicated.

Within species, we sought variability for CO_2 compensation concentrations among 100 genetic lines of wheat *Triticum aestivum* L. and 20 genetic lines of barley *Hordeum vulgare* L. of diverse origin selected from the U.S. Department of Agriculture World Collections. Remarkably little variation in CO_2 compensation was found within these species, the compensation concentration being 52 ± 2 ppm (standard deviation) for wheat and 55 ± 2 ppm for barley.

Among the more than 300 monocotyledonous and 100 dicotyledonous species which we surveyed, CO_2 compensations were the same for all species within a genus except in three genera—*Euphorbia, Panicum,* and *Cyperus*—where we found both species with high and low compensation points in the same genus (Table 1). The *Panicum* species were of diverse origin and no single author has listed all of them in taxonomic groupings. In all cases, however, the phenotypes of species with high and low compensation points differed and, where subgenera classifications could be found, were grouped in separate subgenera.

In *Euphorbia,* only species of the subgenus Chamaesyce Raf. had low CO_2 compensation concentrations. In the *Cyperus* genus, *C. esculentus* L. of the subgenus Pycreus (Beav) C. B. Clarke had a low compensation point. *C. papyrus* L. had a high compensation point. The latter species is not listed by Fernald (*6*) but its form is greatly different from *C. esculentus.*

High and low CO_2 compensation points occur in different species of *Atriplex* (*7*). There, also, the traits were found in different subgenera. The recent report of Downton *et al.* (*8*) agrees with our observations within the *Panicum* genus. The fact that high and low CO_2 compensation points occur only in different subgenera indicates, perhaps, that the subgenera should be considered distinct genera. These contrasting compensation species do have many similar traits, however, and some of them may have sufficient genetic compatibility to permit viable crosses between them. Such crosses would provide unique material for study of the inheritance of basic physiological processes which are important in yielding ability of crop plants.

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Immunological Detection of Single Amino Acid Substitutions in Alkaline Phosphatase

Abstract. *Antiserums to wild-type alkaline phosphatase from* Escherichia coli *were prepared and tested for reactivity with phosphatases altered by point mutations. Eight out of the nine mutant enzymes were distinguished from the wild type with quantitative microcomplement fixation. The structural changes are among the smallest yet observed immunologically.*

Certain immunological techniques can distinguish between proteins differing in primary sequence by a very few amino acids (*1*), or even one (*2*). To obtain further information about the value of such techniques, we have determined the immunological effects of single amino acid exchanges in *Escherichia coli* alkaline phosphatase. This dimeric protein has approximately 400 amino acid residues per polypeptide chain (*3*). Our discrimination between mutant and wild-type alkaline phosphatase marks the smallest relative change in protein primary structure observed immunologically to date.

Wild-type *E. coli* alkaline phosphatase was purified from a constitutive strain (C90F1) according to the procedure of Malamy and Horecker (*4*). Chromatographically purified alkaline phosphatase was also obtained from Worthington Biochemical Corporation. Enzymatic activity was assayed by rate of hydrolysis of *p*-nitrophenyl phosphate (*5*). Both protein preparations were emulsified in Freund's complete adjuvant supplemented with 4 mg of BCG (bacillus Calmette-Guérin) per milliliter and injected intradermally at four sites on the backs of male New Zealand white rabbits. Each rabbit received 0.7 to 0.8 mg of protein. Bleedings were taken at weekly intervals, beginning 2 weeks after the initial injection. After 11 weeks, each rabbit was given a course of three intravenous injections (1 mg each) on alternate days. Serums were collected 4 and 7 days after the last intravenous injection and stored in the frozen state. Three rabbits gave serums which reacted with only a single component in crude extracts of *E. coli,* when examined by Ouchterlony double diffusion, immunoelectrophoresis, and microcomplement fixation. Diffusion methods indicated that the fourth rabbit produced a low concentration of antibodies to a second component in crude extracts. This did not interfere with the microcomplement fixation analysis presented below. Most antiserums differed in titer, but not in discriminating capacity, and results below are averages from experiments carried out with many antiserums.

Strains of *E. coli* which constitutively produced mutant alkaline phosphatases were isolated as phosphatase-positive revertants of nonsense mutations, mapping in the alkaline phosphatase structural gene (*6*). The mutant enzymes were altered in their electrophoretic mobility, but they had regained almost full enzymatic activity. The mutations occurred at five sites. The nature of the amino acid substitutions at four of the sites was deduced from the electrophoretic changes (*7*). Five revertants were from the nonsense mutation at a fifth site. The substitutions at this site were determined by amino acid analyses of tryptic peptides (*8*).

No differences were detected between the mutants and the wild type by the double-diffusion technique. A spur was seen in the comparison of the phosphatases of *E. coli* and *Serratia marcescens,* which indicates that the two enzymes share only a limited number of antigenic determinants. The *Serratia* en-