Spiroplasma and is the incitant of corn stunt disease. Whether the corn stunt spiroplasma deserves recognition as a new species depends on adequate characterization and comparison with strains of *S. citri*. We have deposited two isolates with the American Type Culture Collection. One of these (B isolate, clone 2) is nonpathogenic to plants (ATCC No. 27953). The second (E isolate) is the uncloned isolate that retained pathogenicity to plants through at least 19 broth passages (ATCC No. 27954).

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Genetic Linkage between the HL-A System and a Deficit of the Second Component (C2) of Complement

Abstract. *From a family of 14 individuals, evidence was obtained suggesting linkage between the HL-A haplotypes and the transmission of a 50 percent deficit in the functional activity of the C2 component of complement.*

Deficit of various complement (C) components is rare and is inherited in several species (*1–10*). In man, deficiencies of C1r (*1*), C2 (*2*), C3 (*3*), and C6 (*4*) are considered to be inherited as autosomal recessive traits (*5*). Deficiencies of C5 in mice (*6*), C6 in rabbits (*7*), and C4 in guinea pigs (*8*) are also reported to be inherited as autosomal recessive traits. Hinzová *et al.* (*9*) demonstrated that the H-2 gene complex in the mouse affects the level of serum complement and later showed a genetic association of the Ss-Slp mouse histocompatibility region with the production of the complement proteins (*10*). We now report data suggesting that the genes controlling C2 deficiency are linked to the genes controlling the HL-A system.

The propositus, a patient with total functional C2 deficiency, is a 17-year-old Caucasian female who has been intermittently ill since 1960 with an immune complex disease similar to systemic lupus erythematosus (SLE) (*11*). She initially was studied in greater detail because she consistently showed a total hemolytic complement (CH_{50}) too low to measure (*12*), no measurable renal disease, but consistently normal amounts of C4 and C3 (*13*). Her serum was totally deficient in hemolytically active C2 molecules (*14*). Addition of her serum to normal human serum

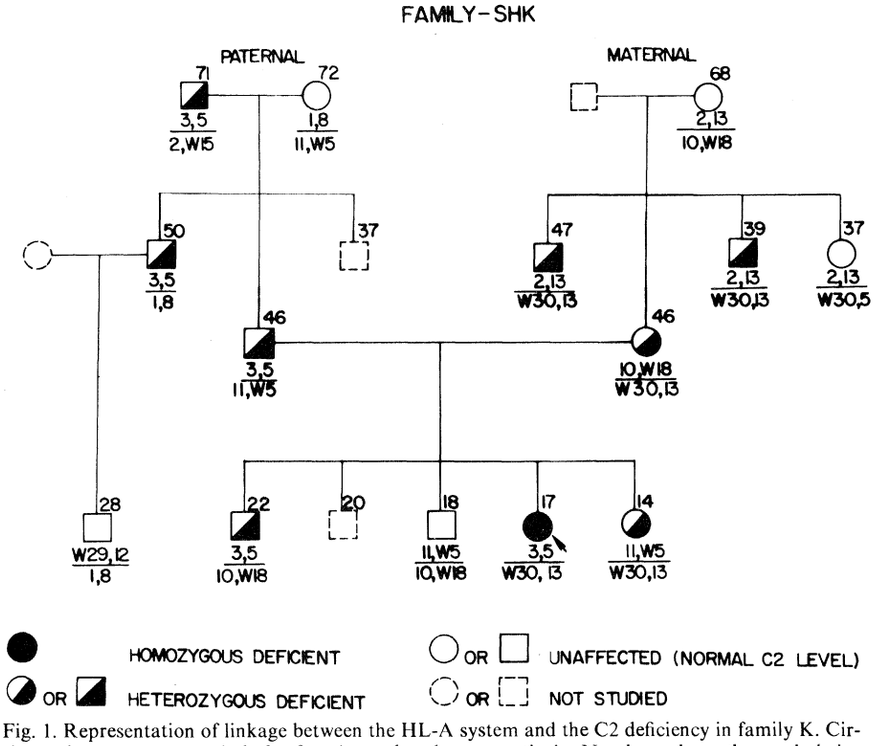

Fig. 1. Representation of linkage between the HL-A system and the C2 deficiency in family K. Circles and squares are symbols for females and males, respectively. Numbers above the symbols indicate age.

did not alter the complement value, indicating that her serum contained no inhibitor to C2. Addition of purified C2 (*15*) to her serum returned the number of CH_{50} units to control values, indicating she lacked only the functional C2 protein (*16*).

The patient, five members of her immediate family, four paternal relatives, and four maternal relatives were then examined for the C2 levels (*14*) and for the inheritance of the HL-A antigens (*17*) on two different occasions. Results of these determinations (double blind) were decoded after all data had been collected on each occasion. The C2 in serums of seven additional unrelated healthy individuals was determined on two separate occasions. We tested for HL-A antigens by the lymphocyte microcytotoxic test (*17*) with 112 well-characterized alloantiserums to HL-A representing the following specificities: HL-A1, HL-A2, HL-A3, HL-A9, HL-A10, HL-A11, W28, W29, W30, and W32 of the first segregant series and HL-A5, HL-A7, HL-A8, HL-A12, HL-A13, W5, W10, W14, W15, W16, W17, W18, W21, W22, and W27 of the second segregant series of the HL-A system (*18*). The HL-A genotypes and the respective C2 levels of all individuals tested are given in Table 1.

Table 1. HL-A genotypes and C2 levels in members of family K.

Individual*	Age (yr)	HL-A genotype†	C2 in serum‡ (10^8 molecule/ml)	Deficit of C2‡	C2-deficiency genotype§
		Immediate family			
Father	46	*3,5*/11,W5	1061	Half	*D*/*N*
Mother	46	10,W18/*W30,13*	1007	Half	*D*/*N*
Patient	17	*3,5*/*W30,13*	0	Total	*D*/*D*
Brother	22	*3,5*/10,W18	1085	Half	*D*/*N*
Brother	18	11,W5/10,W18	2325	None	*N*/*N*
Sister	14	11,W5/*W30,13*	930	Half	*D*/*N*
		Paternal relatives			
Grandfather	71	*3,5*/2,W15	1085	Half	*D*/*N*
Grandmother	72	1,8/11,W5	3489	None	*N*/*N*
Uncle	50	*3,5*/1,8	1415	Half	*D*/*N*
Cousin	28	W29,12/1,8	3179	None	*N*/*N*
		Maternal relatives			
Grandmother	68	2,13/10,W18	2946	None	*N*/*N*
Uncle	47	2,13/*W30,13*	1085	Half	*D*/*N*
Uncle	39	2,13/*W30,13*	1162	Half	*D*/*N*
Aunt	37	2,13/W30,5	2868	None	*N*/*N*

*Individuals identified by relationship to patient. †The HL-A haplotype carrying the C2-deficiency gene (*D*) is given in italics. ‡The number of C2 molecules ($\times 10^8$) per milliliter of serum in seven unrelated normal persons ranged from 2030 to 3601, and averaged to 2424. In this family, persons with values ranging from 2325 to 3489 have been considered "C2 normal," while those with values ranging from 930 to 1415 have been considered "nearly half C2-deficient." §The gene for normal C2 is named *N* and the gene for nearly half C2-deficiency is named *D*.

In this family, five individuals have normal C2 levels ranging from 2325×10^8 to 3489×10^8 effective or functional molecules per milliliter of serum (average 2961×10^8 molecules per milliliter), comparable to those observed in seven controls, whose values ranged from 2030×10^8 to 3601×10^8 effective molecules per milliliter of serum and averaged to 2424×10^8 molecules per milliliter. In eight other members, including both parents and two of the three siblings of the patient, the C2 ranged from 930×10^8 to 1415×10^8 molecules per milliliter, averaging to 1104×10^8. These values have been considered nearly half the normal C2 level (Table 1). The patient is the only individual in this group with a 100 percent functional deficit of C2 molecules.

If normal C2 level in man is a result of additive and codominant expression of two "normal" alleles at a single locus, each contributing equally to the total number of C2 molecules, then absence or suppression of one allele may lead to a 50 percent deficit in the total number of C2 molecules. If both of these genes were absent (substituted by "mutant") or suppressed, it could lead to a 100 percent deficit of the C2 molecules. If, instead, the C2 deficiency locus consists of a dominant normal gene (*N*) and a mutant (50 percent) recessive allele (*D*), heterozygotes would still exhibit full activity. Results in Table 1 support the former concept. The propositus is homozygote *D*/*D*, has inherited one mutant gene from each parent, and consequently has a 100 percent deficit. Two siblings, both parents, three uncles, and the paternal grandfather are heterozygous *D*/*N*, and have a 50 percent deficit. One brother, one cousin, one aunt, and the two grandmothers are homozygous *N*/*N* and have no C2 deficiency.

All four HL-A antigens were identified and both haplotypes were determined in all 14 individuals. A striking finding is the association between the presence of one or the other of the patient's two haplotypes, namely HL-A3-HL-A5 and W30-HL-A13, with the incidence of C2 deficiency among eight individuals in this family. All those individuals who shared an HL-A haplotype in common with the patient had nearly half the number of C2 molecules per milliliter of serum, while all those who had both HL-A haplotypes different from those of the patient had normal C2 levels (Fig. 1). This clear and definitive observation leads us to suggest that a genetic linkage exists between the C2 deficiency factors and the HL-A genes.

Since both haplotypes of the propositus carried different HL-A alleles and each haplotype carried a C2 deficiency allele, no population association between any given HL-A antigen and C2 deficiency can as yet be suspected from our results or from these together with those recently published (*19*). This does not, however, exclude the possibility of finding such an association when a large number of C2-deficient patients are tested for HL-A antigens. A genetic linkage between the HL-A system and the glycine-rich β-glycoprotein factor of the properdin system has been reported (*20*).

Chromosomal linkage of genes determining the HL-A antigens to those controlling C2 deficiency in man provides HL-A typing as a unique method for prognostic detection of C2 deficiency among relatives of patients with known C2 deficiency. Further studies of this linkage between the two systems may reveal significant, mutual functional or evolutionary relationships or both.

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Rates of Protein Evolution: A Function of Amino Acid Composition

Abstract. *Conservation of secondary and tertiary structure in proteins suggests that rates of sequence variation reflect differences in the total number of amino acid replacements that are compatible with preservation of structure. Consequently, rates of sequence variation depend on whether the constituent amino acids of individual proteins are, overall, more subject or less subject to evolutionary substitution than normal. Such rates correlate well with a mutability term based on amino acid composition.*

The rates of evolution of different proteins vary greatly. At one extreme is histone IV; only two changes in amino acid sequence have been accepted since the divergence of the calf and pea ancestral lines 1.5×10^9 years ago (*1*). Toward the other extreme is pancreatic ribonuclease; although the bovine and pig lines have diverged relatively recently, the sequences of their ribonucleases differ by 22 percent (*2*). Rates of such protein evolution can be expressed quantitatively as the number of accepted mutations per 100 residues per 100 million years. Such rates vary over three orders of magnitude (*2, 3*).

One plausible explanation for such variation is that constraints are placed on the overall structures of proteins and that these structural constraints are less stringent for the more rapidly evolving proteins. This argument works well for some proteins. In other cases, however, the argument may not be quite as applicable. Although at first there would seem to be only one major constraint for ribonuclease (that of maintaining activity), there is at least one other plausible constraint, namely, resistance to ready digestion by the various proteolytic enzymes found in the small intestine. Consequently, one would expect a somewhat slow rate of evolution for ribonuclease, whereas its rate is actually one of the fastest.

Other evidence also suggests that variation in structural constraint may not, in general, be sufficient to explain the variation in rates of protein evolution. X-ray crystallography has shown that there is a great conservation of secondary and tertiary structure in certain sets of proteins despite large variations in primary structure. Two examples will be cited here. One, the overall structures of myoglobin and of the individual chains of hemoglobin are similar despite changes in 80 percent of the amino acids (*2, 4, 5*). Two, the structures of trypsin, chymotrypsin, and elastase are quite similar despite their divergence in the remote past by gene duplication (*2, 6*). These two examples are sets of different, though similar, proteins; conservation of secondary and tertiary structure would be expected to be even greater for a single protein as long as its function is unchanged.

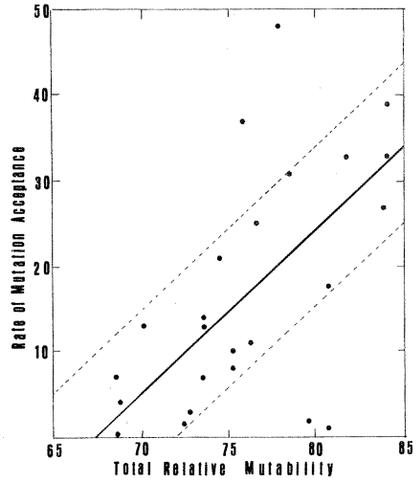

Fig. 1. Rate of mutation acceptance as a function of total relative mutability. Rates of mutation acceptance are in PAM's per 100 million years (one PAM = one accepted point mutation per 100 residues) (*2*). In the case of conflicting rates of evolution (trypsinogen) (*2, 10*), the data used are based on a comparison of mammalian sequences, which is the basis for most of the rates. The total relative mutabilities were calculated from average amino acid compositions of sequenced proteins; for simplicity, insertions and deletions were ignored. When available, the average amino acid compositions given by Dayhoff (*2*) were used; for the other proteins used, average amino acid compositions were calculated with the use of all the complete sequences listed in references (*2, 3, 10*). The immunoglobulin sequences used were those given in the alignments in Dayhoff (*2*). The proteins used were (in order of increasing rate of mutation acceptance): histone IV, glucagon, corticotropin, glyceraldehyde-3-phosphate dehydrogenase, cytochrome c, insulin, melanotropin beta chain, myelin membrane encephalitogenic protein, gastrin, lysozyme, pancreatic secretory trypsin inhibitor, luteinizing hormone beta chain, myoglobin, hemoglobin, trypsinogen, luteinizing hormone alpha chain, lactalbumin, lambda chain C region, gamma chain C regions, kappa chain V region, pancreatic ribonuclease, growth hormone, kappa chain C region, and amyloid protein A.

The above considerations lead to a second plausible explanation for the variation in rates of protein evolution: Stringent constraints on the secondary and tertiary structure of most proteins limit accepted mutations primarily to those in which an amino acid is replaced by another amino acid with similar properties. Consequently, proteins that evolve more rapidly should contain an increased percentage of the amino acids that have the greatest number of acceptable replacements.

In this report, I present a correlation between the rates of protein evolution and a