iting is similar to that recently carried out by Weare (*5*) and Rasmusson and Carpenter (*6*). Figure 3b also shows the corresponding average T_s. During January through April, Q_R stays quite near zero despite the fact that T_s is steadily increasing. Between April and May there is a dramatic increase in Q_R; this result suggests the dominance of the dynamical influences from May onward. One implication that may be drawn from Fig. 3b is that variations in Q_N may explain sea temperature changes in this region in the early phases of an El Niño, but dynamical influences dominate during the subsequent period. If, as suggested by the Wyrtki (*1*) hypothesis, h (in Eq. 1) is increased during El Niño, then Q_R would rise less steeply.

Further confirmation of these results is hampered by the general lack of data for the eastern Pacific, including ship reports, island surface observations, rawinsondes, and oceanic temperature and salinity data at depth. In addition, even if net surface heating is important or even dominant, the more difficult question remains as to the ultimate cause of the heating changes and their relation to the other known variations in the tropical Pacific, especially those related to the Wyrtki hypothesis. One might speculate that the heating and dynamical changes are both necessary for El Niño and that a full-fledged event occurs only when both processes are in proper phase. This might help to explain the apparent collapse of the early stages of an El Niño event in 1975 (*13*).

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Single Gene Mutations in Tomato Plants Regenerated from Tissue Culture

Abstract. *Plants were regenerated from cultured leaf explants of an inbred variety of* Lycopersicon esculentum. *Seeds were collected from the regenerated plants and sown in the greenhouse. The resultant plants were then evaluated in the field. Several monogenic mutations segregated in the progeny of regenerated plants. The recovery of single gene mutations is evidence that plant tissue culture can be mutagenic. Complementation tests revealed that one mutation was located on the long arm of chromosome 10.*

Tissue culture techniques, particularly shoot tip culture and somatic embryogenesis, have been advocated as methods for regenerating large numbers of genetically identical clones (*1*). However, extended culture periods can result in cell lines and regenerated plants with chromosomal abnormalities (*2*). Aneuploid and polyploid regenerated plants have been reported in many species, including tobacco, *Crepis*, and sugarcane (*3*). Larkin and Scowcroft (*4*) suggested that this variation among regenerated plants could be useful for the development of new cultivars. A component of the culture medium that is capable of inducing chromosomal variation could also cause nuclear gene mutation. If most of the nonchromosomal variation in regenerated plants has a genetic basis, plant regeneration in vitro could be used to introduce genetic variability rapidly into sexually propagated crop varieties. We have examined the variability in self-fertilized progeny of a large number of plants regenerated from leaf explants of a cultivar of a typical diploid seed-propagated species, the tomato *Lycopersicon esculentum* Mill., to ascertain to what extent the variation is sexually transmitted. We report on 13 morphological traits observed among regenerated plants, their sexual transmission, and their genetic characterization as controlled by single nuclear genes.

Seeds of a standard inbred tomato cultivar, UC82B (*5*), were used as a source of ten donor plants that were identified morphologically as typical of this cultivar. Young, fully expanded tomato leaves, after being sterilized at their surfaces, were placed on a culture medium (*6*). No known mutagens or extraneous chemicals were added to the culture medium. A callus mass developed shortly after inoculation and the first shoots were regenerated in 3 to 4 weeks. All regenerated shoots were transferred to a rooting medium (*7*) that was modified by adding 2 μM 1-naphthalene acetic acid. One to three plants were recovered from each explant that regenerated shoots.

Regenerated plants (R) were transferred to a greenhouse where observations were made on the morphological variation (*8*). The regenerated plants were self-fertilized, and seed was collected from each regenerated plant to evaluate the next (R_1) generation. Seed collected from 230 regenerated UC82B plants were sown in greenhouse flats to screen for seedling characters before transplanting the seedlings into the field. Progeny of each of the 230 plants (a total of at least 11,040 tissue culture–derived plants) were evaluated in replicated plots in the field in 1980 or 1981. Additional data were collected on self-fertilized R_1 and R_2 plants and on segregating populations of specific crosses (*9*).

As expected, chromosomal variation (particularly $4n = 48$ autotetraploidy) was frequently observed among the regenerated plants, but attention was directed instead to fertile variants whose R_1 progenies segregated for distinct morphological characters. Although some progenies appeared to segregate for new phenotypes, several were identified as being phenotypically similar to mutants reported previously. By examining the R_1 seedlings in the greenhouse and the R_1 plants in field plots, we identified 13 putative nuclear gene mutations arising from the 230 regenerated plants. No mutants were observed among more than 2000 plants from the seed of UC82B used as donor material. An examination of R_1 segregation data suggested that most of the variants originated by simple Mendelian mutation (Table 1). Each variant was regenerated from a separate leaf explant and, as far as we can determine, was a separate mutational event. A detailed analysis of single plant selections of generation R_2 was completed in the greenhouse for 9 of the 13 plants. The expression of two traits, *ms-tc1* and *G-tc1*, was highly variable in the greenhouse and could not be classified adequately, suggesting temperature sensitivity. An evaluation of R_2 seed of plants expressing *ms-tc1*, however, was completed in the field, where phenotypes were distinct. All of the remaining characters (Table 1) could be equally easily classified in field or greenhouse conditions. For all the

Fig. 1 (left). Segregation for the *la-tc1* trait among progeny of a UC82B tomato plant regenerated from tissue culture. Fig. 2 (right). Typical flower from the *ms-tc1/ms-tc1* mutant with abnormal anther cone development observed among R_1 progeny of a regenerated UC82B tomato plant.

recessive traits, the homozygote and heterozygote genotypes had to be deduced by progeny evaluation. Self-fertilized progeny of each of the heterozygotes examined segregated in a 3:1 ratio, demonstrating stability of these newly obtained mutations since they were transmitted through a second (R_2) generation. In addition, R_1 and R_2 seed were grown in Davis, California, where segregation of each mutant was also observed.

The orange fruit trait (*tv-tc1*) appeared among the R_1 progeny of a single red-fruited regenerated plant. All fruits on each variant plant were orange. The R_1 segregation data (Table 1) observed in the field suggested that orange fruit color was controlled by a recessive gene. Pooled progeny of three putative heterozygotes were observed to segregate in a 3:1 ratio (58 with red and 17 with orange fruit; $\chi^2 = 0.111$, 1 d.f.; $0.7 < P < 0.9$) in R_2. Thus, R_1 and R_2 segregation data of the heterozygotes were consistent with the hypothesis that a single stable recessive gene controls this trait. In addition to orange fruit, the mutated (*tv-tc1/tv-tc1*) plants also had modified flower color and delayed chlorophyll development in leaves (virescence). All of the 102 R_1 and R_2 plants with orange fruit also had orange rather than yellow flowers, permitting early classification of *tv-tc1* in either greenhouse or field. The cosegregation of flower, fruit, and leaf color in progeny of heterozygous red plants is evidence for a single pleiotropic mutation. Single pleiotropic mutations affecting both fruit color and chlorophyll development have been reported in tomato (*10*). No differences were observed when flowers and fruits of the tissue culture–derived variant were compared with those of the previously described tangerine (*t/t*) mutant of tomato (*10*). One R_2 *tv-tc1/tv-tc1* plant was crossed with an orange plant of LA158, a tester stock homozygous for the recessive tangerine allele. All of the 20 plants derived from this cross had orange flowers and fruits, demonstrating that the two mutations did not complement each other. Hence, the new orange mutation (*tv-tc1*) is allelic to the *t* locus on the long arm of chromosome 10 at map position 95. Progeny carrying the two mutant alleles were not virescent. Also, seed from these heterozygous *tv-tc1/t* plants segregated in a 3:1 ratio of normal to virescent seedlings. Therefore, functionally, the *t* locus contains at least two elements that can mutate independently.

Seedlings with the lethal albino mutation (*la-tc1*) have albino cotyledons and die before the development of true leaves (Fig. 1). Segregation in the R_1 generation (Table 1) was consistent with a 3:1 ratio. Fruits of surviving R_2 green plants were bulked during seed collection. This mixture, containing both +/+ and +/*la-tc1* seeds, fit the expected 5:1 ratio of normal to mutant among the R_2 seedlings (65 normal and 14 *la* mutants; $\chi^2 = 0.059$, 1 d.f.; $0.9 < P < 0.95$).

Two other chlorophyll-deficient mutations were observed among R_1 progeny: a virescent mutation (*v-tc1*), independent of the *tv-tc1* mutation, and a mottled mutation (*m-tc1*). The R_1 segregation data (Table 1) for *v-tc1* does not fit a 3:1 ratio. However, chlorophyll-deficient mutants that have reduced viability, resulting in distorted F_2 segregation ratios, have been isolated (*11*).

The regenerated parent of each of the four male sterile recessive mutants listed in Table 1 (see also Fig. 2) was fertile, and the corresponding R_1 progeny segregated into fertile versus sterile plants. No seed could be collected from these self-pollinated R_1 male sterile mutants. The R_1 data for normal versus male sterility fit a 3:1 ratio for each mutant.

Normal UC82B plants have jointed pedicels, a trait controlled by a single dominant gene (*12*). Among R_1 progeny in the field, *j-tc1* segregated as a single recessive mutation. Unlike *j-tc1*, the original regenerated plant of *j-tc2* was jointless. This plant bred true in both the R_1 generation (Table 1) and R_2 generation (96 jointless plants), suggesting that the original regenerated plant was a homozygous mutant. Earlier reported mutants for jointless pedicel (*j* and *j-2*) were both located on chromosome 11 (*13*). Homozygous *j-tc2/j-tc2* plants were crossed with a linkage tester line that was homozygous *j/j* (LA30), to test for complementation. Since all of the progeny were jointed, the new mutation *j-tc2* is recessive and is not allelic to *j*. Mitotic crossing over, visible as reciprocal homozygous somatic sectors on heterozygous leaf tissue, has been reported in a number of higher plants (*14*). It is pre-

Table 1. Segregation of single gene mutations in progenies of tomato plants regenerated in vitro. Symbols for mutants are based on the similarity of the phenotype to known tomato mutants. The *tc* notation designates that the mutations were derived from tissue culture. Values of χ^2 were calculated with Yates correction factor.

Mutant		Regenerated plant		R_1 segregation		
Symbol*	Phenotype	Phenotype	Putative genotype	Normal	Mutant	χ^2
ms-tc1	Male sterile	Normal	+/*ms*	29	10	0.008
ms-tc2	Male sterile	Normal	+/*ms*	38	10	0.250
ms-tc3	Male sterile	Normal	+/*ms*	36	6	2.032
ms-tc4	Male sterile	Normal	+/*ms*	8	2	0.000
j-tc1	Jointless pedicel	Normal	+/*j*	9	2	0.030
j-tc2	Jointless pedicel	Jointless	*j/j*	0	48	
tv-tc1	Tangerine fruit	Normal	+/*tv*	30	6	0.926
G-tc1	Green base	Green base	*G*/+	12	36	0.028
la-tc1	Lethal albino	Normal	+/*la*	64	19	0.197
v-tc1	Virescent	Normal	+/*v*	42	4	5.681*
I-tc1	Indeterminate	Indeterminate	*I*/+	8	40	1.361
I-tc2	Indeterminate	Indeterminate	*I*/+	11	25	0.333
m-tc1	Mottled	Normal	+/*m*	49	21	0.685

*Significant deviation from 3:1 ratio ($P < 0.05$).

sumed that the regenerated *j-tc2/j-tc2* tomato presumably arose by a mutation followed by mitotic recombination, both events occurring before or during shoot organization.

Two dominant mutations (*I-tc1* and *I-tc2*) with the same phenotype were isolated from independently regenerated plants. Tomatoes grown for canning, such as UC82B, are homozygous for the determinate (*sp/sp*) mutant. Mutation *I-tc1* segregated in a 3:1 ratio for indeterminate versus determinate growth in the R_1 progeny grown in the field. Single plant selections from the R_1 plants were analyzed in the R_2 generation. All determinate and some indeterminate plants bred true. Among heterozygous *I-tc1*/+ selections, a 3:1 ratio of indeterminate to determinate was observed (92 indeterminate and 33 determinate; $\chi^2 = 0.067$; 1 d.f.; $0.7 < P < 0.9$).

Thus, various types of morphological mutants were observed. In most cases, the mutations were similar to those that occur spontaneously or after mutagenic treatment of tomato seed. However, seed treatment often produces mosaics, since not all cells in the seed are equally affected by the mutagen. No mosaics were evident in our tissue culture–derived plants. Hence, the observed tissue culture mutations apparently occurred before shoot formation, and the 13 shoots were each derived from a single cell of a callus. As a consequence, 3:1 ratios were observed among R_1 plants in contrast to the distorted ratios often reported for progenies resulting when tomato seeds are treated with mutagens (*15*). Environmental factors and certain chemical and physical treatments can increase the frequency of mitotic recombination (*16*). The recovery of the *j-tc2/j-tc2* regenerated plant suggests that it may be possible to regenerate homozygous R plants after mitotic recombination in mutated heterozygous cells by adding chemicals that promote recombination to the tissue culture medium during regeneration.

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Multiclonal Origin of Polyps in Gardner Syndrome

Abstract. *Electrophoretic analysis of glucose-6-phosphate dehydrogenase was performed on polyp tissue from three black female patients with Gardner syndrome and who are heterozygous for the A and B forms of this enzyme. Polyp tissues from the three patients displayed the AB phenotype. This finding suggests a multiclonal origin of polyps in Gardner syndrome. Studies of tumors originating from such polyps may provide information about the sequence of cellular events leading to malignant transformation.*

During early embryonic development one of the X chromosomes of each mammalian female cell becomes functionally inactive. Whether the paternal or maternal X chromosome is expressed in any given cell is random (*1*). Females heterozygous for X-linked genes are thus mosaic with respect to the expression of the two alleles. Hence, X-linked genes can be used as developmental markers to trace the clonal origin of cells in heterozygous females (*2, 3*). The X-linked enzyme glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49) can be used in humans to determine the clonal composition of tumors. In black females who are heterozygous for the G6PD isozymes A and B (*4, 5*), tumors originating from a single cell express either the A or B allele, whereas tumors arising from more than one cell express both alleles. In most spontaneously arising tumors studied, including acute and chronic myelocytic leukemia (*6, 7*), Burkitt's lymphoma (*4*), carcinoma of the cervix (*8*), and leiomyomas (*9*), G6PD analyses have indicated that the tumor originated from a single cell or clone of cells. In contrast, similar studies of a carcinoma of the colon (*10*) revealed a possible polyclonal origin of this spontaneous tumor. However, the double G6PD-AB phenotype in this particular instance may have been due to stromal cell contamination in the samples (*4, 10*).

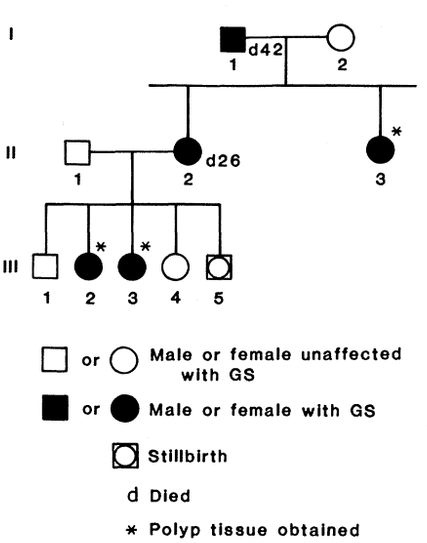

Fig. 1. A partial kindred pedigree identifying three patients with Gardner syndrome (*GS*).

To date, the electrophoretic forms of G6PD have been studied in black female heterozygotes for only three types of inherited tumors. Two of these disorders, inherited neurofibromas (*11*) and trichoepitheliomas (*12*), appeared to be multicellular in origin. Baylin *et al.* (*13*) reported that tumor tissues in black female heterozygotes with inherited medullary carcinoma of the thyroid and pheochromocytoma (Sipple syndrome) were monoclonal; the primary tumors contained either G6PD-A or G6PD-B, but not both. These data suggested that the inherited defect in the patients with familial Sipple syndrome resulted in the production of multiple clones of defective cells and that each tumor then arose as a final mutation in one clone of these cells (*14*).

Gardner syndrome is a precancerous bowel condition in which patients develop multiple polyps of the gastrointestinal mucosa. This syndrome, which has an incidence of 1 per 14,025 births (*15*), involves defects in the derivatives of all three primordial germ layers (*16*). The