Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Tomato Paste: A Concentrated Review of Genetic Highlights From the Beginnings to the Advent of Molecular Genetics

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THE following attributes account for the popularity of the tomato (*Lycopersicon esculentum*) for genetic research.

1. It is a basic diploid with minimal DNA duplication.

2. Its 12 chromosomes are highly differentiated and distinguishable.

3. The genome is replete with conventional and molecular markers and has well developed linkage maps.

4. The plant structure allows detection of a vast array of hereditary modification.

5. Related, intercrossable species afford a great wealth of readily accessible germplasm.

6. Excellent stock collections are maintained by the National Plant Germplasm System and the Tomato Genetics Resource Center.

7. The tomato is naturally self-pollinated, yet flowers are easily manipulated to yield large quantities of hybrid seed.

8. Tomato cells are readily cultured, hybridized, and whole plants regenerated therefrom.

9. The plants can be easily cultured in a wide range of environmental conditions; the tomato is amenable to sexual and asexual propagation.

10. The tomato offers the advantages of its edible crop status; much mutual benefit results from frequent exchanges between applied and basic research. You can study tomatoes and eat them too!

11. Recent developments reveal the tomato to be ideal for research in certain aspects of molecular genetics (RICK and YODER 1988). The maize *Ac* and *Ds* transposable elements have been incorporated into the tomato, where they actively transpose. Insertional mutagenesis may provide a means for cloning desired genes. Tomato has been transformed with DNA determining economic traits, including the delta endotoxin of *Bacillus thuringiensis* (which confers insect resistance), the capsid protein of tobacco mosaic virus (TMV) (which protects against infection by TMV), resistance to the herbicide glyphosate, and, via antisense RNA, reduced synthesis of polygalacturonase (which affects fruit firmness and disease susceptibility). Restriction fragment length polymorphism (RFLP) traits have been employed to greatly elucidate the genetics of several quantitative traits. These and other exciting developments were expedited by the pioneer studies of tomato genetics, the key events of which constitute the substance of this article.

The tomato was one of the many organisms investigated in the rash of genetic studies shortly after the "rediscovery" of MENDEL's work. The first publication was that of HALSTED, OWEN and SHAW (1905) on five distinctive morphological traits: dwarf plant habit, potato leaf, peach (fuzzy) fruits, yellow fruit flesh and colorless fruit epidermis. Although they ascertained dominance relations, it remained for PRICE and DRINKARD (1908) to demonstrate monogenic inheritance for these traits, in addition to lutescent foliage and pyriform fruit shape.

In the next three decades tomato investigations were sporadic and lagged far behind those in maize. Linkage studies trace back to D. F. JONES (1917), better known for his contributions to inbreeding and heterosis in maize, who reinterpreted data of HEDRICK and BOOTH (1907) on the cosegregation of dwarfness (*d*) and elongate fruit shape (*o*) as the consequence of linkage between them. E. W. LINDSTROM, whose major research was also in maize, followed with an intensive study of linkage on chromosome 2, utilizing JONES' markers in addition to *p* and *s*. He also investigated autopolyploidy and radium-induced mutation, and described the first tomato haploid.

WINKLER (1909) reported the first autotetraploid

tomato, a somaclonal variant from tomato callus tissue developed at the region of grafting between tomato and *Solanum nigrum*. J. W. LESLEY (1928) reported and intensively studied the first known autotriploid. In the progeny of his triploid, LESLEY obtained the first tomato primary trisomics and, via the standard trisomic-ratio method, assigned four markers to their respective chromosomes. In subsequent years LESLEY and his wife MARGARET MANN LESLEY made many other important contributions to tomato genetics and cytogenetics.

Other pioneers of this period were J. W. MACARTHUR and his student L. BUTLER. Their specialties were linkage and quantitative inheritance. Remarkable in both careers was their location in the Department of Zoology at the University of Toronto. Their research in tomato genetics was probably tolerated there because they concurrently investigated the genetics of mice (and muskrat ecology!).

In the 1940s and 1950s tomato genetics experienced a great expansion as a result of concurrent synergistic events. Until that time the tomato was regarded as poor material for chromosome cytology; only the tiny somatic and meiotic metaphase chromosomes seemed workable, but useful only for counts and extent of pairing. It is to BARBARA MCCLINTOCK that we owe an appreciation of the potential of the pachytene stage in tomato. Always active and scientifically curious, MCCLINTOCK applied her masterful techniques to demonstrate the potentialities of tomato cytogenetics. Her suggestions prompted S. W. BROWN (1949) to pursue the subject and reveal that the greatly extended chromosomes at this stage display good morphological differentiation into euchromatic and heterochromatic regions and that each arm terminates in a detectable telomere. Also significant was his observation that the contraction process after pachytene occurs primarily in euchromatin, the visible elements at metaphase being mostly heterochromatic. BROWN's student D. W. BARTON (1950) continued the research, providing the first descriptions and measurements for the identification of each of the 12 bivalents.

No account of this period would be complete without reference to the massive contributions of HANS STUBBE, for many years Director of the Institut für Kulturpflanzenforschung at Gatersleben, East Germany. Already renowned as successor to ERWIN BAUR as the world's authority on *Antirrhinum* genetics, STUBBE established an extensive program in the genetics and breeding of the cultivated tomato and the closely related wild species *Lycopersicon pimpinellifolium*. The large resources of this center and its professional staff were dedicated to various aspects of these subjects as well as the biochemistry and physiology of the tomato. As by-products of a search for agriculturally useful mutations induced by X-rays, about 300 monogenic mutants were induced in the former and 200 in the latter species. He documented the phenotypes and inheritance of all these mutants in a series of highly useful publications of the Institute. Eventually many of the mutants became well known as important linkage markers or genetic variants for a wide range of morphophysiological investigations. To mention examples, three of the *esculentum* mutants (*flc, not, sit*) tend to overwilt when drought-stressed. This phenomenon owes to aberrant stomate behavior caused by deficiency of abscisic acid. Also it is to the great credit of STUBBE that these mutants were freely exchanged internationally. He also conducted an elaborate investigation of the effects of grafts between 25 of these mutant strains and their isogenic normals, presumably to test claims by the LYSENKO group of graft-induced heritable changes. From 2,455 surviving grafts, some 30,000 first- and second-generation progeny were grown without detecting evidence of induced heritable changes (STUBBE 1954). Another experiment (STUBBE 1971) demonstrated that, by a program of induced mutation and selection, the fruit size of *L. pimpinellifolium* (~1 g) could be progressively increased within a few generations to approximate that of *L. esculentum* (150 g) and, similarly, fruit size of the latter could be diminished almost to the dimensions of the former.

Remarkable as these contributions were, it is all the more astonishing that they were accomplished behind the former "iron curtain." It is a credit to his personal courage that STUBBE could thus proceed in direct contradiction of the then prevailing LYSENKO dogma of graft hybridization and other aspects of the inheritance of acquired characters.

My role in this period was that of the lucky guy who happened to blunder onto the scene at the right time. Although I had experimented briefly with tomato genetics as a new graduate student under E. M. EAST, it was not until the late summer of 1942, after moving to my present position in Davis, that I delved into the subject in earnest. This renewed interest traces to a fertile suggestion by a fellow Department member, JOHN MACGILLIVRAY, that it might prove interesting to probe the causes of infertility of the so-called "bull" (unfruitful) plants commonly seen in tomato plantings. My first reaction was that this was a stupid, silly idea, but about a month later it dawned on me that such a survey might indeed be worthwhile. A few forays into nearby fields, then approaching harvest, revealed an unexpected wealth of genetic and cytological variation. By the end of that season we had acquired a series of male-sterile mutants in the three principal cultivars of that period, an array of meiotic and floral structural defects (all of which proved to be monogenic recessives), haploids, triploids (comprising two-thirds of the unfruitful plants), and tetraploids (RICK

1945). I was totally hooked and off to the races! In the next year 8 of the 12 primary trisomics, as well as other aneuploids, were identified morphologically in the progeny derived from the wealth of seeds in fruits naturally set on the triploids.

The timing of these events could not have been better. I teamed with BARTON for an attack on the primary trisomics; he identified the extra chromosomes in each trisomic type while I conducted genetic tests to identify the associated linkage groups (RICK and BARTON 1954). The acquisition of mutants from STUBBE and others provided the desired markers to populate the linkage maps; in fact, we had such a large array of mutants that we could afford to be selective, using only those with traits well expressed in early seedling stages. The program was also expedited by the establishment of the Tomato Genetics Cooperative (TGC) by BARTON and A. BURDICK in 1949 and administered at Davis for the following 32 years. The exchange of genetic stocks and information fostered by the TGC greatly facilitated and coordinated efforts to explore the genetic genome. Rules were adopted for systematization of tomato genetics and its nomenclature. We benefited in no small measure by the advice of M. M. GREEN and others with experience in genetically more advanced organisms.

The next great asset to tomato genetics was the arrival on the scene of GURDEV S. KHUSH. Having just completed his Ph.D. under G. L. STEBBINS (to my regret, not me, as so many assume), KHUSH joined our group at Davis, where his cytological skills provided a real shot in the arm for most of the 1960s. We embarked on a program of radiation-induced chromosomal changes that generated haploids, monosomics and trisomics of secondary, tertiary, telosomic and compensating types. Cytogenetic investigations of these aneuploids afforded localization of markers to all euchromatic arms and provided stocks for many other purposes and utilized in a wide variety of investigations.

In another phase of the project we induced deficiencies, irradiating normal (wild-type) pollen with fast neutrons to be applied to stigmas of various recessive marker stocks. Recessive progeny were selected for cytological study in the standard "pseudodominant" system used so effectively for cytological mapping in Drosophila. We chose to irradiate pollen rather than somatic tissue in order to avoid chimeral situations. The choice proved fortunate for another reason of which we were unaware at the time: recovery of deficiencies that would not survive gametogenesis. Because growth of angiosperm pollen tubes is presumably determined by the tube nucleus, defective sperm nuclei of irradiated mature pollen can be delivered at fertilization. The heterozygous deficiencies thereby generated were beautifully delineated at pachytene by KHUSH's expertise, infinite patience and diligence. In this fashion we localized many markers in the complement, thereby matching the cytological and genetic maps (KHUSH and RICK 1968). In contrast to maize, disappointingly, none of the many cytologically detectable euchromatin deficiencies were transmitted through either male or female gametogenesis, thereby precluding establishing stocks of any of them. The only locus (*ra*) for which deficiencies were transmitted proved to reside in the proximal heterochromatin of 9L (KHUSH, RICK and ROBINSON 1964). The possibility could not be discounted that this marker is situated in a tiny enclave of euchromatic within an otherwise heterochromatic zone. Monosomics were also generated, but only for chromosomes *5*, *11* and *12*, leading to the conclusion that the imbalance of monosomy can exceed the tolerance of sporophytes, again in contrast to maize, in which all monosomics are viable. This extreme sensitivity to deficiency served to reinforce the concept (RICK 1971) that the tomato is essentially a basic diploid with little duplication of DNA in its complement.

The events of this early period were reflected in rapid progress in mapping the genome. When BUTLER (1952) published an early summary, linkage had been detected for 35 markers. By 1956, 45 were situated to their loci among 56 allocated to their respective chromosomes. A summary in 1963 revealed 86 loci for 136 allocated markers. These categories reached 190 of 258 markers in 1975. About 70% of these determinations were made by our team at Davis. Thus, by the end of the 1960s, the framework of the tomato genome had been established.

The prime development of the 1970s was the application of electrophoretic characters to resolve problems in the genome. At the 1968 International Congress of Genetics in Montreal, DICK LEWONTIN encouraged me to approach these problems by means of isozyme markers, research then in its early days. The suggestion proved timely and eventually fruitful. In this effort we were assisted greatly by R. W. ALLARD and his student ALEX KAHLER, who were already proficient in electrophoretic techniques and applying them to the population genetics of barley and other plants.

Our preliminary effort was a survey of the nature of enzyme variation in representative cultivated *L. esculentum* and its wild var. *cerasiforme*. We were disappointed at the lack of variation, virtually nil in the former and only sporadic in the latter, and these mostly in the native Andean area. One bonus of this otherwise rather uninteresting situation was the discovery that older cultivated tomato stocks and var. *cerasiforme* from Mesoamerica have identical genotypes, thus supporting JENKINS' (1948) hypothesis that the former were products of the latter's domestica-

tion. Undoubtedly the many bottlenecks experienced by the predominantly self-pollinating var. *cerasiforme* during its migrations from the Andes to Mesoamerica (the generally accepted area of domestication) account for this extreme genetic uniformity.

These results stimulated us to turn our attention to the related wild species. Fortunately, the very closely related currant tomato (*L. pimpinellifolium*) proved to be rich in isozyme variation (RICK, FOBES and HOLLE 1977). Because *L. esculentum* and *L. pimpinellifolium* are conspecific according to genetic criteria (reciprocally crossable, homosequential chromosomes, highly fertile F_1 hybrids, and normal inheritance), inheritance patterns of electrophoretic banding pattern differences in crosses between these species resolved loci *vs.* alleles (hence allozymes). These markers were quickly mapped in tests against standard linkage markers, thereby enriching the array of useful markers and adding another handy mapping technique. We eventually adopted the LA716 accession of *Lycopersicon pennellii* (also homosequential with *L. esculentum*) as a key parent for linkage tests. This stock has the advantage of a self-pollinating pure line, in contrast to the strict allogamy and consequent extreme polymorphy of all other accessions of the species (RICK and TANKSLEY 1981). Additionally, alleles of LA716 and standard *L. esculentum* differ at 20 loci among 10 of the 12 chromosomes. A single cross between LA716 and any tomato line will therefore provide a linkage survey of about 70% of the genome. Such crosses have consequently become standard for linkage screening of new mutants.

In the meantime, allozyme surveys were made of various other *Lycopersicon* species. Extensive collections were made of these species in their native regions in a fashion appropriate for determining various population parameters. It was thereby possible to ascertain the comparative extent of genetic variation within and between populations. The data also permit estimates of outcrossing and analysis of mating systems. Among the tomato species, the situation varies from strict autogamy through intermediate, facultative types to obligate allogamy enforced by self-incompatibility. Major differences in these parameters exist even within several of the species, the autogamous groups always being geographically peripheral to the central, vastly more variable groups (RICK 1983). These findings have considerable bearing on evolution in the genus, on utilization of the wild species for tomato improvement, and on germplasm preservation.

Allozyme mapping has also been applied to investigations of quantitative traits. Analysis of cosegregation of these molecular markers with metric traits in interspecific hybrids has been particularly instructive. Thus, research on the potential insect antibiotic 2-tridecanone in hybrids of *L. esculentum* × *Lycopersicon hirsutum* f. *glabratum* revealed complex determination by genes at a minimum of five loci (ZAMIR *et al.* 1984), thereby providing valuable (if not discouraging) information to breeders. In another study, by TANKSLEY, MEDINA-FILHO and RICK (1982), the nature of inheritance of four quantitative traits was investigated in the *esculentum-pennellii* hybrid in cosegregation with 13 allozymic loci. A single backcross to *esculentum* progeny afforded a wealth of information: the minimum number of quantitative loci (QTL) could be estimated; the positive or negative effect of wild alleles could be detected; as anticipated, the trait with the best +/− balance exhibited the greatest degree of transgressive segregation; epistatic interactions could be detected between allozymic loci and QTLs; and pairwise tests of allozymic loci detected interactions between QTLs, sometimes revealing the existence of QTLs not ascertained by tests for epistatic interactions. The merits of molecular markers for simultaneous analysis of metric traits were thereby demonstrated, and foundations were laid for recent sophisticated mapping of QTLs with RFLP markers.

Tomato genetics has benefited greatly from interactions with applied research; in fact, I would be remiss not to cite mutual advantages of exchanges with workers in tomato breeding both in public agencies and private firms. Many valuable spontaneous mutants have been discovered and transmitted and important observations made by several members of my Department, Cooperative Extension, and many workers in private industry.

A by-product of tomato genetics research was the establishment at Davis of the Tomato Genetics Resource Center. As stocks of mutants and cytological deviants accumulated and more collections of wild species were made, it behooved us to take measures to preserve this material. With support from various public agencies and the tomato industry, it has been possible to perform the standard functions of this service of germplasm collections: acquisition, maintenance, evaluation, utilization, distribution and relevant research.

Tomato genetics has clearly become a very active field of research. The record proves that the tomato species offer unique advantages for certain investigations. The groundwork studies on the nature of the tomato genome paved the way for molecular genetic studies, some already completed and many others in progress. In conclusion, if I may be allowed a metaphor, if *Arabidopsis* is the *Drosophila* of plant genetics, then the tomato has become the mouse.

The editorial advice of R. T. CHETELAT and M. M. GREEN is gratefully acknowledged.

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