Antiquity of American Polyploid Cotton

Abstract. *Fragments of a boll of* Gossypium hirsutum *L. from archeological excavations near Tehuacán, Mexico, prove that this species existed in 5800* B.C. *No doubt remains that American tetraploid cotton species originated through natural hybridization.*

Much debate has ranged about the theory of origin of the American polyploid cottons. Since the basic genomes were recognized (*1*) as representative of both Old World and New World diploid cottons, there has been much speculation on the means by which such a hybrid could have arisen. The arguments have favored an ancient dispersal pattern for *Gossypium* and subsequent loss of one or both of the diploid parents or hybridization brought about relatively recently by man's transport of cultivated cotton across the Pacific (*2*, *3*). The searching analyses of chromosomal order in *Gossypium* by Gerstel (*4*) suggested to him a transatlantic route for the Old World parent in the hybridization, based on his conclusion that *G. herbaceum* L. was the species contributing the Old World genome to the allopolyploid.

Because there are three recognizable species forming the complex of American tetraploid cottons, two on the continental mass and one isolated in the Hawaiian Islands, the biological impossibility of differentiating and distributing several species within the relatively short time that man has been known to have inhabited the Pacific Islands is apparent. Similarly, the suggested transatlantic link for the origin of the New World allopolyploids raises fully as many problems as it solves. The alternative, favoring an ancient dispersal of the parental diploid types with their respective genomes, remains. Many archeologists and anthropologists have favored the transport theory because this enables them to solve easily additional problems in distribution of cultivated plants. Proof is now available that the human transport theory is untenable.

The archeological proof of antiquity of tetraploid cotton results from a search for the progenitors of cultivated maize (*5*). It was in this context that R. S. MacNeish settled upon the Tehuacán Valley of Mexico as the likely focal point of early cultivation of maize in a climate sufficiently dry to allow preservation of plant remains (*6*).

Initial analysis of plant remains recovered from five caves in the Tehuacán Valley area of southeastern Puebla (*7*) shows that cotton, maize, peppers, beans, squash, avocados, and other plants were cultivated nearby as long as 7000 years ago. Cotton appears both in manufactured material and in the form of crude fiber and boll fragments.

Gossypium remains in the form of cloth, string, assorted bits of fiber, and boll fragments are common among plant remains from upper levels of four of the caves. The caves, designated TC-50, Coxcatlán, TC-35, El Riego, TC-254, San Marcos, and TC-272, Purron by MacNeish, were apparently near areas under intense cultivation and constituted living quarters for persons working in the area. Included with the cotton fragments are remains of maize, beans, squash, peppers, avocados, sapotes, ciruelas, and other cultivated plants as well as abundant remains of plants which must have been gathered from the native vegetation in the area. The cultural complexes have been designated Venta Salada (dated from A.D. 700 to A.D. 1540) and Palo Blanco (200 B.C. to A.D. 700).

In earlier levels of the deposits, the fragments of cotton become less frequent, but they are found in association with obviously cultivated plant remains as early as the Abejas period, which MacNeish dates between 2300 B.C. and 3400 B.C. Preservation of plant remains is excellent; identifications of the material are unequivocal. No cotton remains have yet been recognized in the Coxcatlán phase (3400 to 5000 B.C.), but the samples of string excavated from this level have not been analyzed.

The most remarkable cotton find is the two segments of a cotton boll excavated in Coxcatlán Cave in zone XVI, an El Riego floor level dated between 7200 B.C. and 5000 B.C. Three carbon-14 dates for zone XVI are all around 5800 B.C. The segments are 4 cm long and 1.5 cm wide at their widest point (Fig. 1). The outer wall of the boll segment has curled completely back. When expanded by boiling, the segment became 5.1 cm long, 1.6 cm wide (the outer wall uncurled), and represented one-fifth of a boll about 3.0 cm in diameter. Because they are so similar in size and appearance, the segments apparently are fragments of the same boll. None of the fiber re-

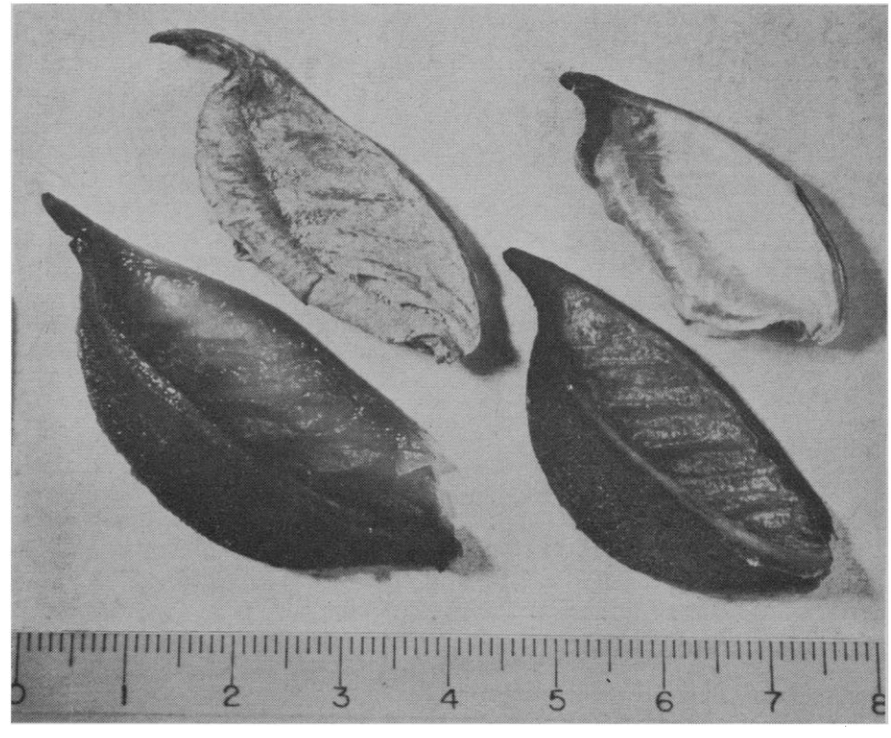

Fig. 1. Segments of bolls of *Gossypium hirsutum* L. On the left are two segments (one expanded by boiling) recovered from zone XVI, Coxcatlán Cave; on the right, two segments of a boll of cv. 'Deltapine' grown at Stoneville, Mississippi.

mains with the segments. The pieces have been examined by Kerr and Fryxell (*8*), who agree with our conclusion that these pieces represent tetraploid *Gossypium*. Comparison of the boll segments with modern bolls of *Gossypium hirsutum* L. cv. 'Deltapine' show no significant differences.

The earliest previous evidence of the use of cotton was the fragment of fabric and string reported from the excavation at Mohenjo-Daro, Pakistan (*9*). The cotton was the Old World *G. arboreum* which Hutchinson equated with *bengalense* cotton (*3*). Dated at about 3000 B.C., the fragments indicate a well-established knowledge of weaving.

In the New World, the cotton reported by Bird (*10*) from Huaca Prieta is *Gossypium barbadense,* one of the tetraploid species. Again, the workmanship indicates that the manufacture of textiles from cotton fiber was not crude but had advanced to a relatively sophisticated art. Cotton was probably well-established as a crop plant by this time.

The Coxcatlán Cave cotton boll fragments, clearly dated at about 5800 B.C., establish the knowledge of a tetraploid species of *Gossypium* among people who were developing a method of cultivation suited to the special climatic conditions under which they lived. However, it is only in the next archeological level (Coxcatlán phase) at about 5000 B.C. that the remains of a number of species clearly indicate the use of agricultural practices.

The fundamental significance of the Coxcatlán cotton boll fragments is that *Gossypium hirsutum* was present in North America at an archeologically early period and that it was well differentiated from the other tetraploid American species of *Gossypium*. Evidence for the time interval required for the establishment of a tetraploid species subsequent to initial hybridization is incomplete. It is not impossible that this time interval could be on the order of tens of years. If it must be assumed that all of the tetraploid American *Gossypium* species have been the products of hybridization of the same two parents (as the genetic background for these species seems to indicate), the time interval needed to establish the differences recognizable in distinguishing the species must be much longer than the time span during which cotton has been known to man. The original hybridization (or hybridizations) occurred long before man became aware of the use of cotton fibers. Since the tetraploid *Gossypium* species are restricted to America and the Hawaiian Islands, the parental stocks must have grown near one another in America, but have since become lost along with countless other species. Much additional knowledge assembled from future archeological excavations will undoubtedly unravel the story of the use of cotton fibers, but the parental stocks contributing to the original hybridization may never be found.

C. EARLE SMITH, JR.
U. S. Agricultural Research Service, Beltsville, Maryland

RICHARD S. MACNEISH
R. S. Peabody Foundation, Andover, Massachusetts

References and Notes

1. A. Skovsted, *J. Genetics* **28**, 407 (1934); **34**, 97 t. 1–3 (1937); J. M. Webber, *J. Agr. Res.* **51**, 1047 (1935); **58**, 237 (1939).
2. J. B. Hutchinson, R. A. Silow, S. G. Stephens, *The Evolution of Gossypium* (Oxford Univ. Press, New York, 1947).
3. J. B. Hutchinson, *Endeavour* **21**, 5 (1962).
4. D. U. Gerstel, *Evolution* **7**, 234 (1953).
5. P. C. Mangelsdorf, R. S. MacNeish, W. C. Galinat, *Science* **143**, 538 (1964).
6. MacNeish organized Project Tehuacán which has been supported for three field seasons by grants from the National Science Foundation and the Rockefeller Foundation.
7. R. S. MacNeish, *Science* **143**, 531 (1964).
8. We thank T. Kerr, Cotton and Cordage Fibers Research Branch, U.S. Department of Agriculture, Beltsville, Md., and P. A. Fryxell, Cotton Research Center, USDA, Tempe, Ariz., for critical evaluation of the specimens and for furnishing the modern bolls of *G. hirsutum* cv. 'Deltapine' for comparison.
9. A. N. Gulati and A. J. Turner, *J. Text. Inst. Trans.* **20**, 1 (1929).
10. J. B. Bird, *Nat. History* **57**, 296 (1948).

3 January 1964

Isotopic Molecules: Separation by Recycle Gas Chromatography

Abstract. *Gas chromatographic columns, greatly extended in length by the use of paired columns in a recycling apparatus, have been used to separate butane (n-C_4H_{10}) from deuterated butane (n-C_4D_{10}) and methane (CH_4) from deuterated methane (CD_4). The separation of monotritiated cyclobutane (C_4H_7T) from cyclobutane (C_4H_8) is nearly complete. This procedure is generally applicable to a wide variety of separations of isotopic molucules.*

The simple theory of gas chromatographic separation makes it quite apparent that increasing the column length under otherwise unaltered conditions results in better resolution of closely neighboring peaks (*1*). Indeed, A. J. P. Martin has often stated, "there is nothing excessive about a column a quarter of a mile long" (*2*). However, the technological problems of flow rates, overpressure, and so forth, have usually limited the lengths of packed columns to 15 or 30 m in most practical applications. Under these conditions, most isotopic molecules cannot be separated completely from one another, and isotopic separations by gas chromatography have been only infrequently reported (*3*). The only conspicuous exception has been the abundance of reports on the separation of H_2 and D_2, the *o*- and *p*- forms of H_2, and the various tritiated species (see *4*).

During the past 2 years, we have needed analytical techniques for separating a number of isotopic molecules, primarily hydrocarbons of low molecular weight such as CH_3T and CD_3T. We have worked out a recycling technique for extending the column length (*5*) almost indefinitely; this technique has proved applicable for separations of isotopic molecules, or for resolution of other molecular pairs that are difficult to separate on the usual columns.

The chromatographic system used in these experiments operates with two identical columns in series (C_1 and C_2 in Fig. 1) and a recorder in between (R_1). The columns are connected with four-way stopcocks (S_1 and S_2), for rapid reversal of the sequence of the columns in the series; and an additional recorder (R_2) is provided at the exit of the system. After injecting a sample containing a mixture of isotopic molecules, the isotopic mixture is allowed to proceed through C_1 and R_1 and into C_2. Almost all compounds with smaller retention volumes have by then passed

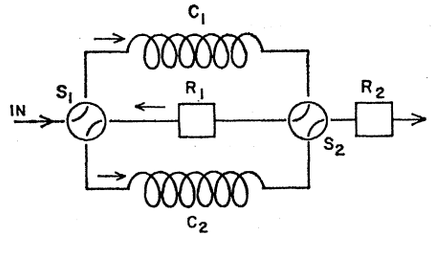

Fig. 1. Apparatus for recycle gas chromatography.