Cytologia **50**: 701–709, 1985

Enhanced Resolution of Somatic Chromosome Constrictions as an Aid to Identifying Intergeneric Hybrids among Some Triticeae

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Accepted May 4, 1984

Intergeneric hybridization research efforts among Triticeae have been the object of increased interest during the last decade. The genera utilized in crosses have been *Aegilops*, *Agropyron*, *Elymus*, *Haynaldia*, *Hordeum*, *Secale* and *Triticum*. Successful combinations set seeds i) that reach maturity on the female plant, or ii) from which the immature embryos require extraction and culturing; a procedure necessary for several intergeneric combinations as a consequence of endosperm collapse or of its complete absence. In either case, confirmation of hybridity is initially obtained by a root-tip cytological check conducted either before or after the transfer of young plantlets to peat pots or to soil in pots. A confirmed hybrid possesses half the chromosome complement of each parent, is self-sterile, and expresses explicit clarity at metaphase for primary and secondary chromosome constriction regions.

To facilitate the above cytological requirements, ideal chromosome contraction, metaphase spreads and quality staining are essential. In addition, sample storage and ease of preparation require that the cytological technique possesses adequate operational flexibility. This paper describes said technique, and includes photomicrographs of metaphase squashes of root-tip cells of intergeneric hybrids among some Triticeae so as to substantiate the technique's merits.

Material and methods

Germplasm

Some of the intergeneric hybrids among *Agropyron* species, *Elymus giganteus*, *Hordeum vulgare*, *Secale cereale*, *Triticum turgidum*, *T. aestivum*, some backcross progenies and a *Triticum aestivum* variety cytologically studied for chromosome constriction resolution are included in Table 1.

Production of intergeneric hybrids

Intergeneric hybrid production involves steps associated with emasculation, pollination, post-pollination hormone treatment, embryo rescue, embryo differentiation, plantlet growth and plantlet transfer to a potted soil medium. Specific details of each stage have been described by Mujeeb-Kazi and Rodríguez (1980, 1981a, 1984) and Mujeeb-Kazi *et al.* (1983).

Root-tip sampling

Root tips were collected at each of the following three stages:

i) At the time of removing the plantlets from the embryo culture vials, i.e. approximately a 3 weeks growth stage after embryo differentiation.

ii) From the plantlets growing in peat pots under growth chamber conditions, i.e. a 5 to 6 weeks growth stage. The growth regimes were 36,000 lux light intensity, temperatures of 15°C day/10°C night, 14 hours day/10 hours night, and approximately 65 to 75% relative humidity (RH).

iii) From plants growing in potted soil under greenhouse conditions of 14 hours day/10 hours night, 22°C day/15°C night, with 45 to 55% R.H. For sampling stages (ii) and (iii), repeated root-tip collections may also be made if chromosome banding techniques need to be applied after hybrid identification, or if chromosome elimination and/or aneuploidy are involved.

Cytological procedure

i) *Collection and pre-treatment*: Root tips were collected between 09: 30 AM and 11: 30 AM, then placed in a petri dish on a filter paper moistened with the pre-treatment solution mixture. This is comprised of colchicine, 8-hydroxyquinoline, and dimethylsulfoxide prepared according to the combined procedures of Bennett *et al.* (1977) and Sallee and Kimber (1981). Specific amounts for a 20 ml solution include 0.01 g colchicine + 0.005 g 8-hydroxyquinoline + 5 ordinary eyedropper drops of DMSO (dimethylsulfoxide). The samples were pre-treated from 2.5 to 3.5 hours depending upon material, but generally a 3 hour pre-treatment time (as used for samples in this study) gave satisfactory chromosome contraction and a high mitotic index.

ii) *Fixing and staining*: After pre-treatment, the root tips were transferred to vials containing 0.2% aceto-orcein and refrigerated (4°C) until used. Samples prepared as above have given excellent results, even after 25 days in refrigerated storage. Transfer to 2% aceto-rocein aids in intensifying the staining if done 2 days before squashing.

iii) *Squashing*: The sample vials for analyses were removed from refrigerated storage and allowed to reach room temperature, which took approximately one hour. A sample was then processed as follows:

a) The aceto-orcein stain was removed from the vial.

b) Enough 45% acetic acid was added to fill about a quarter of the vial.

c) The vial was heated over a spirit lamp flame so as to bring the contents to a slow boil.

d) The vial was then removed from the flame, shaken with a wrist action and re-heated to a slow boil.

e) After a second boiling, the vial contents (45% acetic acid + root tips) were emptied into an evaporating dish. A root tip was taken from this and placed on filter paper to remove excess 45% acetic acid.

f) The apical 2 to 2.5 mm tip was cut and placed on a dry microscope slide.

g) The root tip was then squashed with an arrow-head needle, and a small drop of 45% acetic acid was quickly added to the squashed tissue.

h) The tissue was again macerated. The slide was then slightly warmed and a cover glass placed gently over the macerated cellular area.

i) The cover glass sides were gently dabbed with coarse filter paper, the slide slightly warmed, placed in between folded filter paper on a flat surface, and thumb pressure applied directly to the cover glass.

iv) *Making permanent preparations*: The cover glass was removed by the dry ice procedure, and the slides were allowed to dry under laboratory temperature conditions of 20°C for one hour. They were next transferred to a coplin jar containing absolute ethanol for one hour, removed and again normally dried. The dried preparations were treated with xylene from 15 to 30 minutes, air dried, and made permanent with euparol or any other suitable mounting media.

A minor variation of the procedure for convenience scheduling involves transferring the slides after the cover glass has been removed to a coplin jar containing absolute ethanol and kept in the freezer (−12 to −15°C). The preparations were left in the freezer overnight. The follow-up procedures of drying, treating with xylene, drying again and mounting are similar to those earlier described.

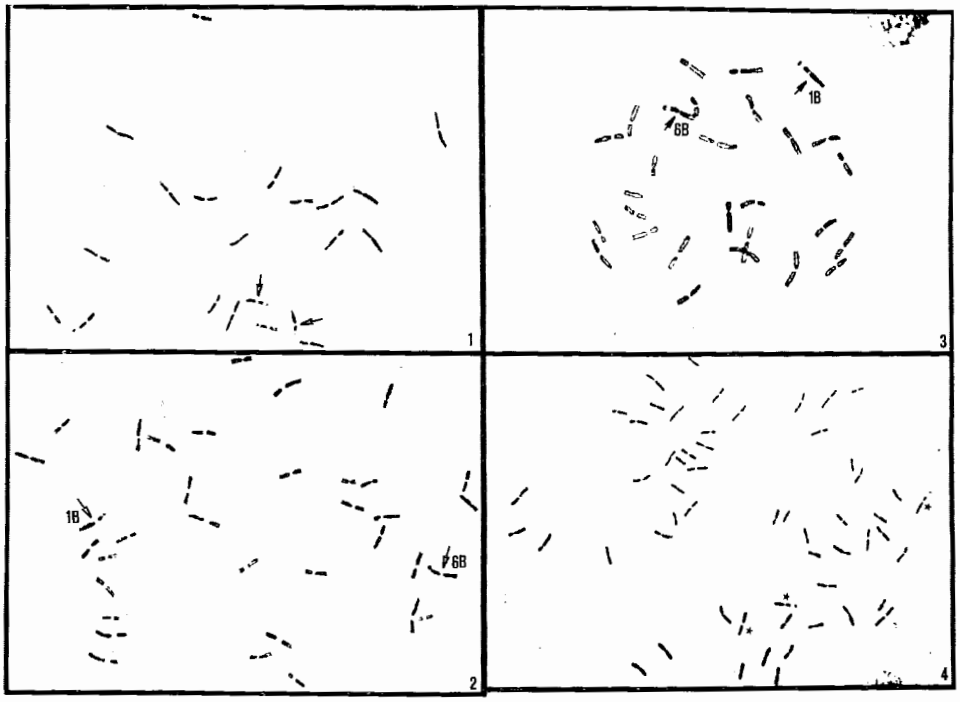
Results and discussion

The initial confirmation of an intergeneric hybrid is obtained from the somatic count made after sampling root tips of the "potential" hybrid. This can be done by collecting root tips over the three stages mentioned in the sampling methodology. In general, the time elapsed between sampling early in stage (i) and sampling at stage (ii) is about 8 to 10 weeks. For the over-anxious researcher, stage (i) sampling appears essential, but for the multifarious investigator a blend of sampling at stages (ii) and (iii) is desirable. For projects designed for agricultural practicality the essence resides in having a valid hybrid; the sampling stage thus becomes secondary. If, however, chromosome elimination and production of haploids or polyhaploids are an aspect of study, early sampling is advantageous for identifying the product. The mechanism of elimination will however remain elusive despite early sampling, since preferential chromosome loss occurs in the very early post-zygotic development as observed for *Triticum aestivum*/*Hordeum bulbosum* (Barclay 1975).

We have observed excellent primary and secondary constriction resolution in all intergeneric hybrids analyzed over all sampling stages, and now use these stages exclusively for analytical convenience in the overall program activities. Invariably, in all hybrid cells involving *Triticum* species the satellited 1B and 6B chromosomes can be identified in a single dosage, and in at least a few cells the 5D satellited wheat chromosome is detected.

For those intergeneric hybrids differing in polyploidy at the parental level, a simple chromosome number count is adequate for initial hybrid identification. Such combinations are exemplified by Figs. 1 to 4 for *H. vulgare*/*T. turgidum* (n=3x=21), 1B and 6B satellited (Fig. 1); *T. aestivum*//*Agropyron repens*/*A. desertorum* (n=5x=35), 1B and 6B satellited (Fig. 2); *T. aestivum*/*A. junceum* (n=4x=28), 1B and 6B satellited (Fig. 3); *T. aestivum*/*A. elongatum* (n=8x=56), 1B, 6B and an *Agropyron* satellited chromosome (Fig. 4).

Resolution of secondary constrictions gains significance when intergeneric hybrids have as parents genera of similar polyploidy. In this study, *T. aestivum* and hexaploid *Agropyron* species are examples of this. The somatic count in such intergeneric hybrids shall be 42. If amphiplasty is involved, the secondary constriction sites of the alien parent (*Agropyron* sp.) in the *T. aestivum/Agropyron* species hybrid do not express. The secondary constriction of the 5D wheat chromosome is observed infrequently, but can be resolved by decreasing the pre-treatment time to about 2 hours. Figs. 5 and 6 present somatic cell photographs of two hybrids with 42 chromosomes, in which variable numbers of satellites are expressed: a hybrid of *T. aestivum/A. varnense* with 1B, 6B satellited (Fig. 5) and *T. aestivum/A. intermedium* with 1B, 6B and possibly 5D satellited chromosomes (Fig. 6). The single dosage of the wheat satellited chromosomes seems a valid marker for hybrids involving *Triticum* with the similar polyploid alien species.

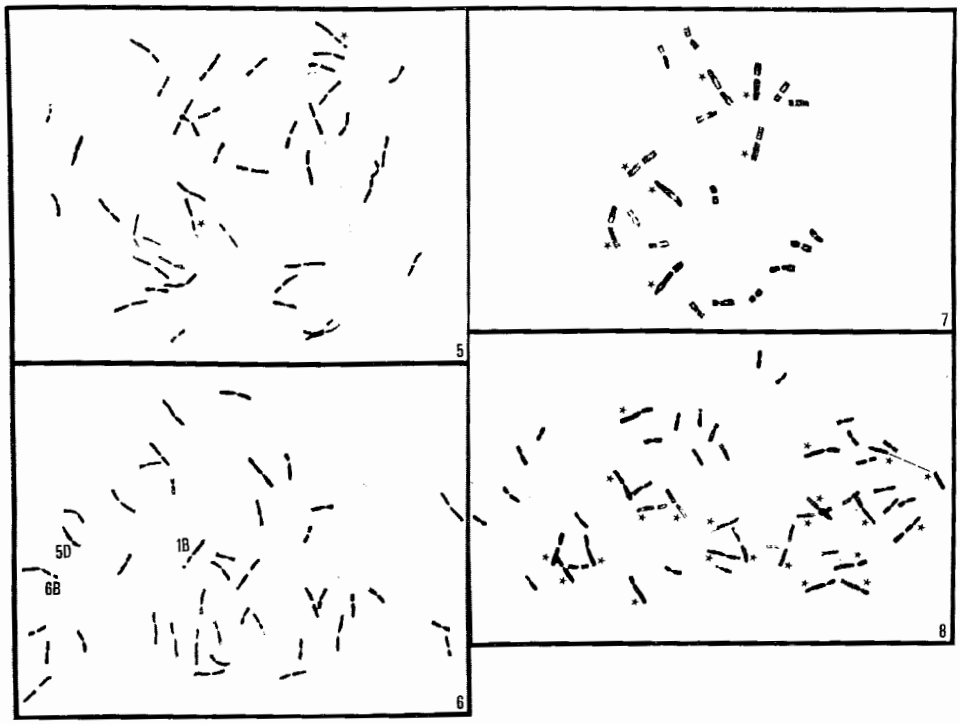


Figs. 1–4. Somatic chromosome number and satellited chromosome details in some intergeneric hybrids. 1, *Hordeum vulgare/Triticum turgidum* (n=3x=21) with 1B and 6B satellited chromosomes arrowed. 2, *T. aestivum//Agropyron repens/A. desertorum* (n=5x=35). 1B and 6B chromosomes arrowed. 3, *T. aestivum/A. junceum* (n=4x=28) with 1B and 6B satellited. 4, *T. aestivum/A. elongatum* (n=8x=56), with 1B, 6B and an *A. elongatum* satellited chromosome marked (*).

The technique also allows for parental chromosome identification based upon chromosomal size differences that the genera may possess. In intergeneric hybrids of *A. elongatum/Secale cereale* (n=3x=21), all somatic cells observed had 7 large chromosomes and 14 smaller *A. elongatum* chromosomes (Fig. 7). When C-banding was used, the large chromosomes were identified to be those of *S. cereale*

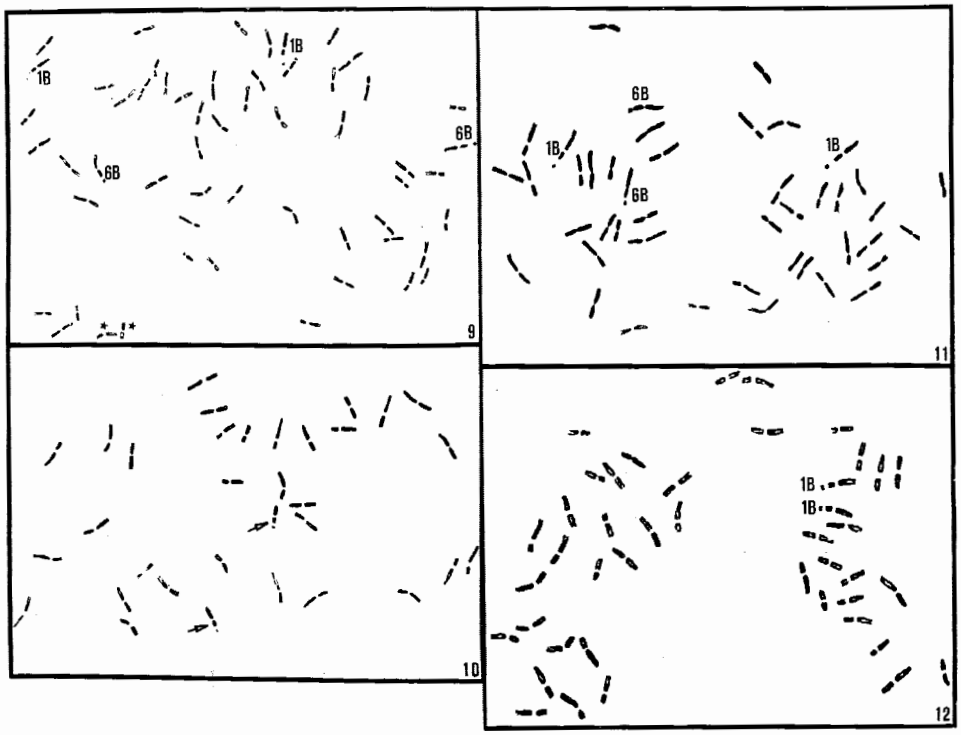
Table 1. Germplasm details for primary and secondary chromosome constriction resolution

Intergeneric hybrid (F_1), backcross progeny (BC) or parental variety details	Generation	Somatic chromosome number	Figure number in text
Hordeum vulgare/Triticum turgidum	F_1	n=3x=21	1
T. aestivum//Agropyron repens/A. desertorum	F_1	n=5x=35	2
T. aestivum/A. junceum	F_1	n=4x=28	3
T. aestivum/A. elongatum	F_1	n=8x=56	4
T. aestivum/A. varnense	F_1	n=6x=42	5
T. aestivum/A. intermedium	F_1	n=6x=42	6
A. elongatum/Secale cereale	F_1	n=3x=21	7
*A. elongatum//2*S. cereale*	BC_1	7x=49	8
T. aestivum/Elymus giganteus//T. aestivum	BC_1	2n=6x=42+14 (Jx)	9
H. vulgare/2 T. turgidum*	BC_1	2n=4x=28−2+7 (H)	10
*T. aestivum/E. giganteus//2*T. aestivum* ②	BC_2F_2	2n=6x=42+2 (J/X)	11
*T. aestivum/E. giganteus//3*T. aestivum* ①	BC_3F_1	2n=6x=42+1 (J/X)	12
T. aestivum var. Veery "S"/Chinese Spring	F_1	2n=6x=42 (1B/1R; 1B)	13
T. aertivum var. Veery/Chinese Spring	F_1	only 1B taken; 6B and 5D from Veery "S"	14



Figs. 5–8. 5–6: Somatic counts in intergeneric hybrids with 42 chromosomes. 5, *Triticum aestivum/Agropyron varnense* (n=6x=42) with 1B and 6B prominently satellited. 6, *T. aestivum/A. intermedium* (n=6x=42) with 1B, 6B and possibly 5D satellited. 7–8: Somatic counts in a *Agropyron elongatum/Secale cereale* hybrid (n=3x=21). 7, large *S. cereale* chromosomes are marked (*). 8, a backcross 1 plant from *A. elongatum//2*S. cereale* with 49 chromosomes. The 21 longer *S. cereale* chromosomes are marked.

(Mujeeb-Kazi and Rodríguez 1981). The size effects were also observed in the backcross 1 (BC1) hybrid, i.e. *A. elongatum*/2* *S. cereale* with 49 chromosomes (Fig. 8). The (BC1) 49 count involved the fertilization of a spontaneously doubled egg cell (21 to 42) by *S. cereale* pollen (n=7).



Figs. 9–12. 9–10: Somatic cytology of normal and aneuploid backcross progeny. 9, 56 chromosomes in a normal backcross 1 plant from *Triticum aestivum/Elymus giganteus//T. aestivum*. Satellited chromosomes 1B, 1B, 6B, 6B are marked. Two chromosomes were cropped in and are indicated by asterisks. 10, aneuploid BC1 from *Hordeum vulgare/2* T. turgidum* with 33 chromosomes. Note single dosage of 1B and 6B. 11–12: Satellites as critical chromosome markers in *Triticum aestivum/Elymus giganteus* backcross progenies. 11, a *T. aestivum/E. giganteus //2* T. aestivum* ② plant with 44 chromosomes. Note the double dosage of the 1B and 6B satellited wheat chromosomes. 12, a *T. aestivum/E. giganteus//3* T. aestivum* ① plant with 43 chromosomes. Only 1B, 1B present.

Production of BC1 progeny in intergeneric hybridization has been reported to be quite anomalous (Jewell and Mujeeb-Kazi 1982, Mujeeb-Kazi and Bernard 1982), or normal as for *T. aestivum/Elymus giganteus//T. aestivum* (Mujeeb-Kazi and Rodríguez 1981b). If the BC1 was normal and *T. aestivum* was involved in the cross and backcross four satellited chromosomes would be present, as appears in Fig. 9 for the BC1 of *T. aestivum/E. giganteus//T. aestivum* with 56 chromosomes. BC1 plants from *T. aestivum/E. giganteus//T. aestivum* were generally normal, with 56 chromosomes except for an occasional aneuploid with 55 chromosomes and an even rarer 55 chromosome BC1 plant with a dicentric chromosome.

The present technique provides excellent primary constriction resolution, hence identification of telocentric and acrocentric chromosomes is relatively easy. It is this superior constriction resolution that proves advantageous for detecting dicentric chromosomes. If the BC1 was aneuploid and mechanisms of chromosome loss resembled the randomness discussed by Jewell and Mujeeb-Kazi (1982), one could expect a BC1 plant with 33 chromosomes in which 1B and 6B were both missing. Fig. 10 illustrates this for the BC1 of *H. vulgare*/2* *T. turgidum*.

In developing advanced backcross progenies from the *T. aestivum*/*E. giganteus* combination we concluded that the production of all 14 *E. giganteus* chromosome addition lines may not be practically possible. Some disomic *E. giganteus* chromosome addition lines have, however, been produced, but have yet to be characterized. These plants possess 44 chromosomes and form 22 bivalents at metaphase I of meiosis. Fig. 11a shows a 44 chromosome somatic cell with a disomic *E. giganteus* chromosome addition and the double dosage of the satellited wheat chromosomes 1B and 6B. Developing addition lines were complicated by the preferential substitution ability of *E. giganteus* chromosomes for the satellited chromosomes of *T. aestivum* (Mujeeb-Kazi 1982), Where the preferential substituting ability is initiated, the cell has 42 chromosomes, of which three are satellited. The meiotic chromosome association in such plants was 20_{II} (bivalents) + 2_{I} (univalents), i.e. 6B and an *E. giganteus* univalent chromosome. The 43 chromosome cell in Fig. 12 shows where chromosome 6B has been completely substituted by an *E. giganteus* chromosome. Only 2 satellited wheat chromosomes (1B, 1B) are identified, and there is a potential monosomic *E. giganteus* chromosome addition since the plant expresses $21_{II}+1_{I}$ at meiosis. Hence, the satellited chromosomes are satisfactory markers for initially identifying preferential substitutions in intergeneric hybrids.

The practical potential of *T. aestivum* varieties possessing a 1B/1R translocation has been well demonstrated and recently reported (Rajaram *et al.* 1983). The translocation chromosome carries the short arm of the *S. cereale* satellited chromosome 1R and the long arm of the *T. aestivum* chromosome 1B. In somatic preparations of varieties with this 1B/1R translocation only 2 satellited chromosomes are observed, i.e. 6B, 6B. The 1RS satellite does not express. Translocation identification may then involve C-banding (Merker 1982), or N-banding (Mujeeb-Kazi and Miranda, unpublished), though we feel that with the present technique a rapid routine check can be accurately made without time-consuming banding procedures. To demonstrate this, F_1's between wheat varieties Veery 'S' (1B/1R, 1B/1R) and Chinese Spring (1B, 1B) were analyzed. Cells with 42 chromosomes possessed 3 to 5 satellited chromosomes, i.e. 1B, 6B, 6B and occasionally 5D, 5D. The satellite of 1RS on the 1B/1R chromosome did not express. The reciprocal cross expressed a similar number of satellite chromosomes. These three satellited chromosomes are enlarged for greater detail in Fig. 14.

The authors feel that the technique is adapted to several critical aspects of cytological investigations, whether these are associated with routine somatic number counts, or involve complex analyses dealing with aneuploid backcross progenies, chromosome size or morphology, or identification of translocations that involve

the satellited chromosomes. Its application has not been explored for the ability to resolve the tertiary constriction region, e.g. as in *S. cereale*. Despite the assets, we feel that positive intergeneric hybrid identification emerges from meiotic analy-

Fig. 13. Satellited chromosomes in a 1B/1R *Triticum aestivum* variety Veery "S"/Chinese Spring (CS). A somatic cell with 42 chromosomes in a 1B/1R *Triticum aestivum* variety Veery "S"/C.S. Satellited chromosomes 5D, 5D, 1B, 6B, 6B are marked ✪, 1B/1R does not express the 1RS satellite. One chromosome not in frame.

Fig. 14. Enlarged 1B, 6B, 5D chromosomes taken from *T. aestivum* variety Veery/Chinese Spring F_1.

ses. Such data not only reconfirm hybridity, but also possess the potential of directing intergeneric hybridization programs onto a path where chances of incorporating alien genes would appear more amenable to research manipulations.

Summary

A somatic cytological technique is described as an aid to superior resolution of primary and secondary chromosome constrictions. Initially, intergeneric hybrids among some Triticeae can be readily identified by routine chromosome number counts, chromosome size or morphology, and satellite number. This includes those hybrids that involve parental combinations of similar polyploidy. Superior secondary constriction resolution can accurately enable detection of 1B/1R translocation in wheat varieties, advanced lines, or hybrid progenies derived from varieties that have the 1B/1R translocation. The results are discussed and photographically documented.

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