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# *Teosinte crossing barrier1*, a locus governing hybridization of teosinte with maize

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**Abstract** A teosinte gene or gene cluster, *Teosinte crossing barrier1* (*Tcb1*), that restricts crossability with maize mapped 6 centiMorgans distal to *sugary-1* on chromosome 4. *Tcb1* is loosely linked with the *gametophyte-1* locus whose *Ga1-s* allele, present in many popcorns, confers nonreceptivity to the pollen of other maize varieties (*ga1*). Full-strength *Tcb1* (positive modifiers present) was nonreceptive to *Ga1-s* as well as to *ga1* pollen. Attenuated *Tcb1* (positive modifiers absent) was detectably more receptive to *Ga1-s* than to *ga1*, suggesting cross recognition between the two systems of incompatibility. Reciprocally, homozygous *Ga1-s* was unreceptive both to *Tcb1* and *tcb1* pollen, but heterozygous *Ga1-s*/*ga1* plants were somewhat more receptive to *Tcb1* than to *tcb1*. Discrimination by *Tcb1*/– females against *tcb1* pollen is prezygotic, accomplished without the loss of viable ovules. When introduced into maize, *Tcb1* incompatibility may be useful for isolating one category of commercial varieties from another.

**Keywords** Reproductive isolation · Pollen-pistil interaction · Cross incompatibility · Hybridization barrier · *Zea mays* · Teosinte

## Introduction

Hybrids between maize and annual teosinte are readily made by applying pollen from teosinte to maize silks. The resulting hybrids and $F_2$ progenies are vigorous and highly fertile. Nevertheless, the occurrence of hybrid, $F_2$ and backcross plants in Mexican maize fields where teosinte is endemic is relatively uncommon. Some wild and ruderal populations are isolated spatially or flower later than the local maize varieties, limiting the opportunity for cross-pollination. Curiously, hybrids are even less common among weedy populations, where flowering with maize is more nearly synchronous (Wilkes 1977). Physiological incompatibility (IC) between pollen and silks has been suggested in this circumstance (Wilkes 1967; Mangelsdorf 1974), based on analogy with the *Ga1-s:ga1* system which is polymorphic within maize.

In the *Ga1-s*:*ga1* system (reviewed in Nelson 1993), growth of *ga1* pollen tubes is retarded or arrested within *Ga1-s*/– silks (House and Nelson 1958). If *Ga1-s* is homozygous the cross fails; if heterozygous, success is variable (Nelson 1952). When *Ga1-s*/– silks are pollinated with a mixture of *Ga1-s* and *ga1* pollen, *Ga1-s* effects fertilization to the virtual exclusion of *ga1*. In contrast, full-sets and Mendelian expectations are realized among progeny of the reciprocal crosses, that is, when *ga1*/*ga1* silks are pollinated by *Ga1-s*/*Ga1-s* or *Ga1-s*/*ga1*. Such a one-way barrier is reminiscent of that sometimes encountered when self-incompatible species are pollinated with related, self-compatible species (e.g., Murfett et al. 1996; Bernacchi and Tanksley 1997).

Physiological IC between teosinte and maize was indicated from attempted hybridizations using teosinte as female and maize as male; that is, in the direction opposite to which the cross is regularly successful (Kermicle and Allen 1990). A good set of seed occurred only with certain introductions of teosinte, generally those found growing wild rather than as weeds. Barriers from two collections, "Chalco" and "Central Plateau", were transferred from teosinte to maize by sequential crossing, taking advantage of the fact that the barriers are unidirectional and simply inherited.

The two barriers proved to have different genetic bases. That derived from Chalco teosinte was not distinguished from the *Ga1:ga1* system of maize. In contrast, the Central Plateau – derived line transmitted the barrier to progeny as a linked, chromosome-4 cluster, designated the Teosinte Incompatibility Complex (TIC). One component of TIC is a pollen-specific allele of *ga1*. Pollen containing it fertilizes *Ga1-s*/*Ga1-s* plants as

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efficiently as *Ga1-s*. But plants containing this allele (*Ga1-m*) do not discriminate between *Ga1-s* and *ga1* pollen. Such an allele, possessing the male function of *Ga1-s* but lacking its female function, had been identified previously in inbred strain 4519 of White Rice popcorn (Jiménez and Nelson 1965; Ashman 1981). Another component of the TIC haplotype occurred among sublines in which *Ga1-m* had been replaced by *ga1* from the recurrent maize parent. This variant line (CP2) pollinated plants in a TIC tester strain successfully but, unlike TIC, was receptive to *Ga1-s* and *ga1* pollen. Thus, it bore the same relation to the TIC system as *Ga1-m* does to *Ga1-s*. It mapped 6 centimorgans (cM) distal to *sugary–1*. Not only were CP2 and *Ga1-m* stocks receptive individually to *ga1* pollen, so too was the combination (Kermicle and Allen 1990). The genes responsible for the crossing barrier in TIC pistils were not identified.

The present paper reports the mapping of the *teosinte crossing barrier1* (*tcb1*) locus within the TIC segment, tests for recognition between the *Ga1-s* and *Tcb1* systems of incompatibility, and examines whether *Tcb1* incompatibility acts before or after fertilization.

## Materials and methods

### Genetic stocks and nomenclature

Table 1 lists the IC stocks used and gives their compatibility relations. In order to standardize the genetic background, the genes of interest were incorporated through backcrossing into the Midwestern US dent inbred W22, which lacks known IC factors (*ga1 tcb1*). The *Ga1-s* counterpart line was developed by first crossing W22 with White Cloud hybrid popcorn, then backcrossing *Ga1-s*-containing progeny to W22 for five generations before self-pollinating to establish a homozygous lineage. IC genes from Central Plateau teosinte collection 48703 (Wilkes 1967) were transferred to maize first by crossing to various *ga1 tcb1* stocks, as available, for five generations and then successively to W22. Selection for strong IC resulted in the multifactoral haplotype designated TIC, established for routine use as a *Tcb1* tester after three generations of crossing to W22. *Ga1-m tcb1* and *ga1 Tcb1* were isolated from TIC, after additional generations of backcrossing (identified as classes B and C, respectively, in Table 3 of Kermicle and Allen 1990). Exceptionally, the experiment involving pollination first with *ga1 tcb1* and then a day later with the plant's own pollen was conducted using stocks derived before incorporating *TIC* into inbred W22 background.

### *Tcb1* mapping

Crossing a *Tassel seed5* (*Ts5*) strain of *Tcb1* to the chromosome-4 tester stock *virescent17* (*v17*) *brown midrib3* (*bm3*) *sugary1* (*su1*) (Maize Genetics Cooperation Stock Center) and the $F_1$ back to the recessive tester generated a 5-point testcross population for locating *Tcb1* relative to the four visual markers. Only the non-sugary kernel class was characterized due to reduced viability and difficulty in classifying the virescent seedling phenotype within the sugary class. A sample of non-sugary kernels was field-seeded, classified for *v17, bm3* and *ts5* phenotypes to identify crossover classes, then crossed reciprocally with TIC/*su1* to determine *Tcb1*:*tcb1* composition. Additional *v17-Su* crossover individuals were identified as virescent seedlings in a greenhouse planting, and then field transplants were classified for adult plant phenotypes and evaluated for *Tcb1:tcb1*. Five of the *v17-Su1* crossovers were established from a progeny in which virescent expression was incompletely penetrant, hence the effective population size could not be determined. In the remaining three progenies studied, 15 *v17-Su1* crossovers were present among 237 plants, or 6.3± 1.6%, which compares with the 7.6±1.3% reported previously (Stinard 1998).

### Simple sequence repeat analysis

Simple sequence repeat (SSR) markers were chosen that were known or suspected to map on the short arm of chromosome 4 based on data in MaizeDB. DNA was extracted from samples using the protocol of Dellaporta (1994). PCR reactions were performed on a PTC-200 Thermal Cycler (MJ Research). The amplification conditions were the same as the "touchdown" profile of Senior et al. (1998) except that the last cycle was repeated 30 instead of 20 times prior to terminating with a continuous 4°C cycle. The 15-µl reaction mix consisted of 3 pmol of each primer, 2.5 mM of $MgCl_2$, 100 µM of each dNTP, 10 mM of Tris pH 9, 50 mM of KCl, 0.1% Triton X-100, 1 mg/ml of purified BSA (New England Biolabs), 0.6 units of *Taq* DNA polymerase (Promega), and approximately 30 ng of template DNA. After amplification, 3 µl of loading dye (30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol) was added to each sample, and 6 µl of each mix was electrophoresed on 4% Metaphor (FMC Bioproducts) agarose gels in 1× TBE (Sambrook et al. 1989). After electrophoresis, gels were stained in 0.5 µg/ml of ethidium bromide and visualized on a UV transilluminator. Allelic constitution was first determined for the *v17 bm3 su1* multiple tester and the

**Table 1** Incompatibility stocks

| Stock | Source of IC genes | Distinguishing features |
|---|---|---|
| *ga1 tcb1* | Dent maize inbred W22 | Receptive to all pollen; unable to fertilize *Ga1-s*/*Ga1-s* or TIC/– |
| *Ga1-s tcb1* | White Cloud popcorn hybrid | Homozygotes unreceptive and heterozygotes variably receptive to *ga1* pollen; unable to fertilize TIC/– |
| *Ga1-m tcb1* | Central Plateau teosinte 48703 | Receptive to all pollen; able to fertilize *Ga1-s*/*Ga1-s* but not TIC/– |
| TIC haplotype | Central Plateau teosinte 48703 | TIC/– unreceptive to *tcb1*; fertilizes all pistil genotypes. TIC/TIC weakly self-incompatible |
| *ga1 Tcb1* | Central Plateau teosinte 48703 | *Tcb1*/– unreceptive or partly receptive to *tcb1* depending on modifier constitution; unable to fertilize *Ga1-s*/*Ga1-s* |

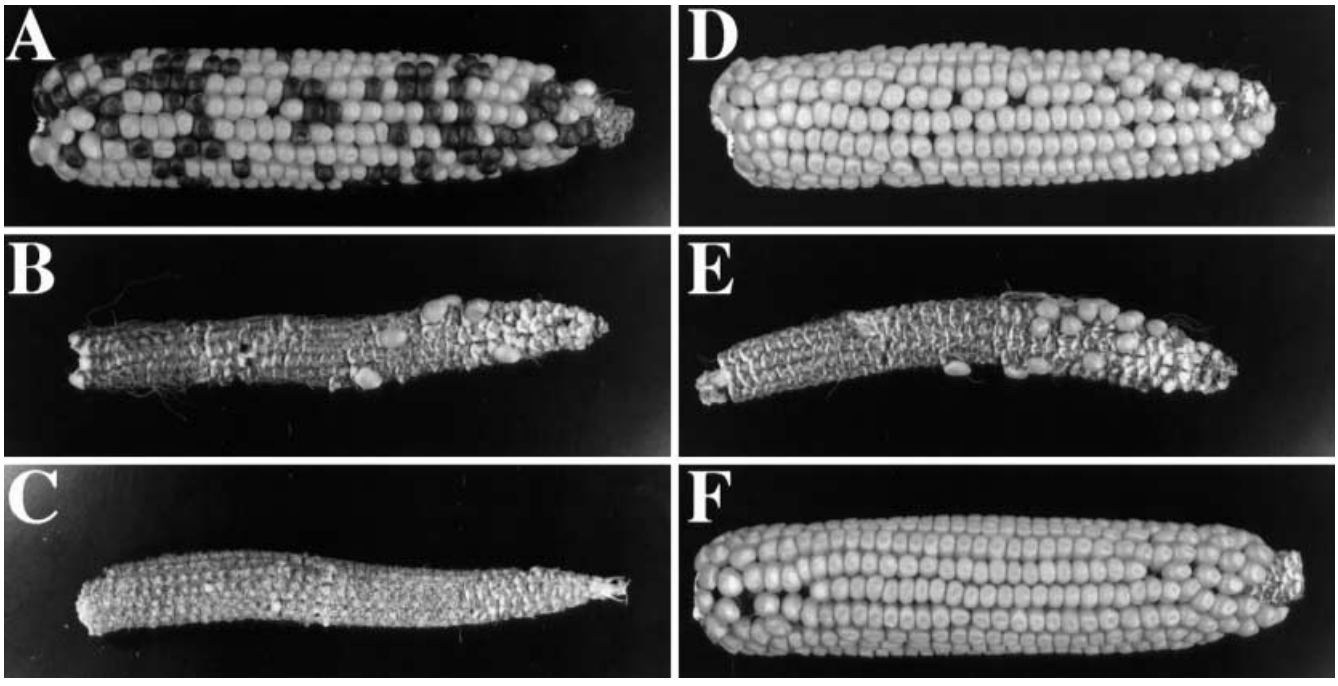


**Fig. 1A–F** Ears from crosses designed to distinguish between teosinte-derived incompatibility genes segregating in W22 backcross progeny. Pollen from a colorless kernel, true-breeding TIC strain was mixed with *ga1 tcb1* pollen that confers kernel color and then placed on silks of plants in the test strain (**A**, **D**). Pollen from each of the test plants was used in crosses to *Ga1-s* (**B**, **E**) and *Tcb1* (**C**, **F**) silks. Two classes of plants were present: (1) **A**–**C** those whose silks did not discriminate against the colored tracer pollen and whose pollen was unreceptive on both testers; and (2) **D**–**F** those whose silks were unreceptive to the tracer pollen and whose own pollen was unreceptive on the *Ga1-s* tester but receptive on *Tcb1*. Plants of category (1) lack both *Ga1* and *Tcb1* whereas those of category (2) lack *Ga1* but carry *Tcb1*

*Ts5 Tcb1* stock for all of the SSR markers. Those with detectable polymorphisms between the two parental types were then tested on the recombinants between *Ts5* and *su1*.

### Pollen mixtures

In tests for preferential usage, approximately equal quantities of *ga1 tcb* pollen and that of either *Ga1-s tcb1* or *ga1 Tcb1* were thoroughly mixed and distributed to silks of various genotypes. The *ga1 tcb* source used confers colored kernels (*R-sc* plus other complementary genes required for aleurone color) whereas the remaining strains confer colorless kernels (*r-g* plus complementary color genes). For each mix the proportion of viable pollen of the two classes was determined from the proportion of colored to colorless kernels obtained in crosses to colorless *ga1 tcb* (two crosses per mix). Values ranged from 28.8 to 54.2% colored. To standardize results across mixes, the proportion of colored kernels was divided by that determined for the two colorless *ga1 tcb* females, then averaged across the eight mixes made for each type. This transformation expresses results for the test females relative to *ga1 tcb*. Thus, if there is no difference in preference among pollen classes, the expected value is one, or 100%. Ears having fewer than 50 kernels were excluded from the calculation. Of the 144 crosses attempted in this experiment, 16 failed as anticipated because neither class of pollen was compatible with the pistil genotype, and four were unsuccessful for reasons extraneous to compatibility. The percentage seed set was estimated as described previously (Kermicle and Allen 1990).

## Results

### Joint segregation and mapping of *Tcb1*

Three features of teosinte-derived incompatibility by *Tcb1* were followed in a segregating testcross population. As female parent, plants containing *Tcb1* discriminate against *tcb1* pollen (Fig. 1). As male parent, *Tcb1* pollen not only functions preferentially on *Tcb1*-containing silks but also is discriminated against on *tcb1*/*tcb1* silks. Table 2 presents data testing the inheritance of these features relative to one another and in relation to four visual marker loci located in the proximal region of chromosome arm 4S. This region was chosen as likely to be of interest because only 3% *sugary-1* kernels had been recovered in $F_2$ progenies of TIC *Su1*/+*su1* heterozygotes (Kermicle and Allen 1990).

The backcross data are summarized according to single crossover classes, no multiple crossovers having been detected. A sample of six plants recombinant for *ts5* and *V17* (region I of Fig. 2A) retained all three *Tcb1* features. Conversely, 11 *bm3-Su1* crossovers (region III) retained none. The nine *v17-Bm3* crossovers (region II) fell into two classes: four retained all *Tcb1* features whereas five retained none. The regular cosegregation of the three features defines the *teosinte crossing barrier1* locus. Being approximately midway between *v17* and *bm3*, it maps to position 74 (Fig. 2B) on the 1995 maize genetic map of Neuffer et al. (1997).

Simple sequence repeat (SSR) markers polymorphic between the multiple tester and the *Ts5 Tcb1* parent were tested retrospectively on the recombinants in the four regions between *Ts5* and *su1* defined by the visual markers and *tcb1*. These data place *umc1117* and *phi074* distal to *tcb1* between *v17* and *ts5*, *bnlg490* proximal to *tcb1* halfway between *tcb1* and *bm3*, and *nc005* and *bnlg1937* be-

**Table 2** Location on maize chromosome arm 4S of *Teosinte crossing barrier1* (*Tcb1*) relative to four visual markers, based on a testcross population produced by crossing *Ts5 V17 Bm3 Su1* (*Tcb1*)/*ts5 v17 bm3 su1* (*tcb1*) heterozygotes to *ts5 v17 bm3 su1* females. Recombinant progeny grown from nonsugary kernels were evaluated for *Tcb1* through reciprocal crosses with TIC *Su1*/+ *su1* and by crossing to homozygous *su1*

| Recombinant class | Intercrosses with TIC *Su1*/+ *su1* | | | % Sugary kernels in crosses to *su1*/*su1* females (no. of plants) | *Tcb1*:*tcb1* constitution |
|---|---|---|---|---|---|
| | No. of plants | % Seed set with recombinant class as male | % Sugary kernels with recombinant class as female | | |
| I. *ts5-V17* | 6 | 70.8 | 3.8–21.9 | 54.5 (4) | *Tcb1* |
| II. *v17-Bm3* | | | | | |
| Group A | 4 | 66.3 | 4.2–19.2 | 57.8 (3) | *Tcb1* |
| Group B | 5 | 0.0[a] | 28.1–35.3 | 50.3 (4) | *tcb1* |
| III. *bm3-Su1* | 11 | 0.0[b] | 24.9–34.0 | 50.5 (7) | *tcb1* |

[a] Zero kernels; [b] A total of eight kernels

**Table 3** Average seed sets on plants differing in *Ga1*:*ga1* and *Tcb1*: *tcb1* constitution. De-tasseled plants were allowed to wind pollinate with *ga1 tcb1*

| Genotype of female parent | No. of plants | Average seed set (%) |
|---|---|---|
| *ga1 tcb1*/*ga1 tcb1* | 71 | 98.3 |
| *Ga1-s tcb1*/*Ga1-s tcb1* | 43 | 0.0[a] |
| *Ga1-s tcb1*/*ga1 tcb1* | 45 | 0.1 |
| *ga1 Tcb1*/*ga1 Tcb1* | 42 | 31.9 |
| *ga1 Tcb1*/*ga1 tcb1* | 45 | 42.9 |
| TIC/+ | 47 | 0.0[b] |

[a] A total of two kernels; [b] Zero kernels

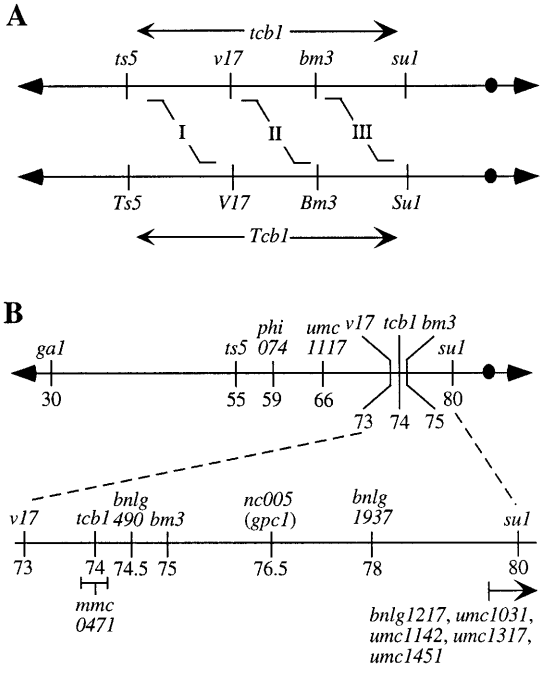


**Fig. 2A, B** Mapping of the *tcb1* locus on the short arm of chromosome 4. **A** An $F_1$ heterozygous genotype showing parental and recombinant classes I, II and III. **B** A chromosome-4 genetic map indicating the location of *tcb1* relative to visual and molecular markers in the proximal region of the short arm. The centromere is indicated by a *filled circle*

tween *bm3* and *su1*. In this population *mmc0471* was not separated from *tcb1*.

## Modification of *Tcb1* action

Exclusion of *tcb1* pollen by *Tcb1*/*tcb1* plants in the foregoing linkage study was variable. Nine backcross plants of the *Ts5 Tcb1* parental class produced from 1.5 to 15.3% sugary kernels rather than the Mendelian expectation of 25%. Similarly, the six *ts5-V17* crossover plants ranged from 3.8 to 21.9% sugary. This compares with a range of only 1.1 to 3.8% in the reciprocal cross, i.e., when pollen of the crossover plants was put onto TIC *Su1*/+*su1* females. This lower value is in the same range of *su1* kernels as when pollen of TIC *Su1*/+*su1* males is put onto TIC *Su1*/+*su1* females. The latter outcome, considering the 6% of recombination between *tcb1* and *su1* loci, is consistent with complete exclusion of *tcb1* pollen. Likely explanations for attenuation of *Tcb1* observed when the backcross plants were the female parent are that one or more modifier genes were lost during development of the *Ts5 Tcb1* stock from TIC or that *Ts5* itself dampens *Tcb1* action.

A second attenuated *Tcb1* strain was identified after repeated backcrossing of TIC to inbred W22. The derived *Tcb1* strain was compared with TIC/+ and *Ga1-s*/– genotypes for ability to prevent seed set when wind pollinated with *ga1 tcb1* (Table 3). Under condition of this test *Ga1-s*/*Ga1-s* plants were virtually barren and even *Ga1-s*/*ga1* heterozygotes produced less than 0.1% set. And not a single kernel set on the 47 TIC/+ plants. In contrast, a 32% set was obtained on attenuated *Tcb1* homozygotes and 43% on heterozygotes. This outcome indicates that inbred W22 carries one or more modifiers that decrease the effectiveness of *Tcb1* in rejecting *tcb1* pollen.

## Cross recognition between the *Ga1-s* and *Tcb1* systems of incompatibility

Separation of *Tcb1* from *Ga1-m*, present together in the TIC haplotype, provides material suitable for testing in-

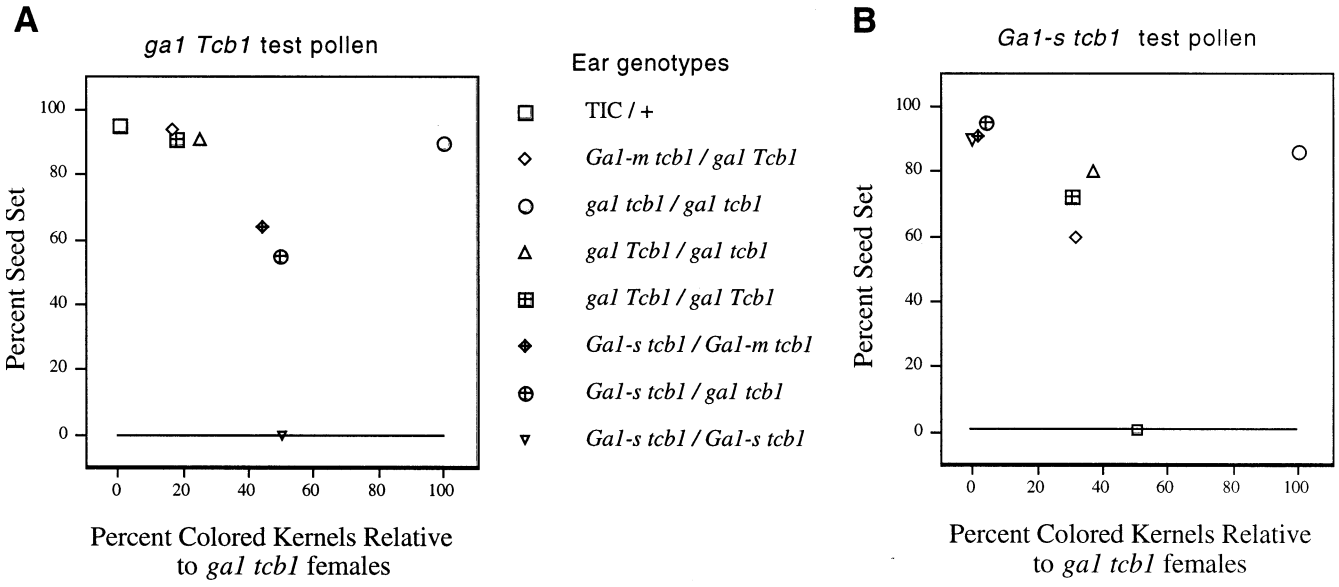


**Fig. 3A, B** *Tcb1* and *Ga1* pollen competition. **A** Success of *ga1 tcb1* pollen (colored kernel strain) in competition with *ga1 Tcb1* (colorless kernel strain). Pollen from the two sources was mixed then placed on silks of female strains, all of which confer colorless kernels. **B** Success of *ga1 tcb1* pollen (colored kernel strain) in competition with *Ga1-s tcb1* (colorless kernel strain). Pollen from the two sources was mixed then placed on silks of the same set of female strains, all of which confer colorless kernels

teraction between the *Ga1-s* and *Tcb1* IC systems. To this end *ga1 Tcb1* or *Ga1-s tcb1* pollen was mixed with *ga1 tcb1* and applied to silks of various IC genotypes. The *ga1 tcb1* strain used confers colored kernels, the other strains produced colorless kernels. Hence the *ga1 tcb1* pollen serves as a tracer to determine how efficiently the various female parents discriminate between *ga1 tcb1* and *Ga1-s*- or *Tcb1*-containing pollen.

Mixtures of *ga1 Tcb1* with *ga1 tcb1* pollen produced essentially a full set of kernels on *Tcb1*-containing ear parents (Fig. 3A), as expected based on mixtures of TIC with *ga1 tcb1* (Fig. 1D). TIC/+ females discriminated almost completely against *ga1 tcb1* pollen, whereas attenuated *Tcb1* homozygotes and heterozygotes (both homozygous *ga1*) averaged approximately 20% as many colored kernels as on compatible *ga1 tcb1*. (See Materials and methods for calibrating the proportion of viable pollen of the two classes in mixtures.) Interestingly, *Ga1-m tcb1*/*ga1 Tcb1* double heterozygotes plot with the attenuated *Tcb1* genotypes rather than with TIC/+. Thus the strong barrier of TIC is not due to the combination of *Tcb1* with *Ga1-m* as such, but due to enhancement by still other factors. Homozygous *Ga1-s tcb1* plants pollinated with this mixture were essentially barren. However, *Ga1-s* plants heterozygous with either *ga1* or *Ga1-m* (both homozygous *tcb1*) produced partial sets. There were only about half as many colored kernels as on fully compatible *ga1 tcb1*, showing a decided preference of *Ga1-s*/– pistils for *Tcb1* over *tcb1* pollen.

Mixtures of *Ga1-s tcb* with *ga1 tcb* pollen (Fig. 3B) produced good sets of seed on *Ga1-s* homozygotes and heterozygotes (all *tcb1*/*tcb1*) with almost complete discrimination against *ga1 tcb1* pollen, also as expected. TIC/+ females pollinated with the mix were almost barren, whereas attenuated *Tcb1*/*tcb1* heterozygotes produced fairly well-set ears. Neither homozygosity of the attenuated *Tcb1* stock nor addition of *Ga1-m* caused the level of incompatibility to approach that of TIC/+. That there was a smaller fraction of colored kernels on *Tcb1*/– females relative to *ga1 tcb1* again indicates partial cross recognition between the two IC systems.

### *Tcb1* as a prezygotic barrier

In principle, *Tcb1* could be expressed either before or after fertilization. If postzygotic, a reduced set of seed should accompany instances of distorted segregation, such as the deficit of sugary kernels among $F_2$ populations of TIC/*su1* heterozygotes. No reduction in set has been observed in this circumstance, providing evidence against a post-zygotic mechanism.

Conceivably, however, the barrenness observed following other types of crosses could reflect postzygotic lethality. To address this possibility ten plants in a backcross progeny segregating for heterozygous TIC and standard *tcb1*/*tcb1* plants were pollinated on successive days. Color-marked *ga1 tcb1* was applied on day 1 followed by the plant's own pollen on day 2. Six plants produced ears with a full set of mostly colored kernels, indicating compatibility with *ga1*. Four plants produced mostly or only colorless and weakly colored kernels characteristic of the TIC/+ parent, indicating maintenance of ovule viability despite prior pollination with incompatible *ga1 tcb1*.

## Discussion

### Multiple manifestations of *Teosinte crossing barrier1*

*Tcb1* – associated incompatibility is expressed in distinct ways depending on the parent genotypes and mode of

pollination. The most conspicuous effect is reduced or failure of seed set, such as that observed following pollination of full-strength *Tcb1*/*Tcb1* by *tcb1*/*tcb1*. A second manifestation is more subtle, reflecting selection among classes of pollen, with little or no reduction in seed set. Two circumstances of pollen selection should be distinguished. *Tcb1* and *tcb1* pollen may derive from different plants, as in artificial mixtures or natural wind pollination. In this circumstance the impact of preferential pollen function is global in that transmission of the entire genome is affected. This situation differs from when *Tcb1*-containing strains are pollinated with *Tcb1*/*tcb1* heterozygotes in controlled crosses. In this case, preferential functioning of *Tcb1* distorts the recovery of linked markers whereas the transmission of unlinked chromosome regions is unaffected. The differential functioning of pollen produced by *Tcb1*/*tcb1* plants clearly demonstrates that the pollen potential is controlled by the genotype of the pollen grain itself and not that of the parent sporophyte, analogous, that is to gametophytic self-incompatibility rather than sporophytic self-incompatibility (Thompson and Kirch 1992; McCubbin and Kao 1999).

Both manifestations of *Tcb1* involve recognition between pollen and pistil. Historically, cross IC has been interpreted in either of two ways. Failure can be viewed as a departure from the normal congruous relationship between pollen and pistil. If some function of one member is missing, fertilization fails due to incompleteness of the reaction, so-called “incongruity” (Hogenboom 1973). Alternatively, there may be genes that function to recognize foreignness and block an otherwise compatible reaction. Such gene functions are viewed as superimposed on the normal compatible reaction. In the present case, *Tcb1* silks would recognize *tcb1* pollen, and then respond to produce a barrier. A third possibility is to view *Tcb1* pistils as producing a barrier which is specifically overcome by *Tcb1*-containing pollen. Recognition in the third case involves pollen and pistil of the same constitution, and response is in the direction of compatibility. The present experiments do not distinguish between these possible causes. The term ”incompatibility” is used here in its general physiological sense, encompassing various interpretations.

## Organization of the TIC chromosomal region

During transfer from teosinte to inbred W22, the TIC haplotype behaved substantially as a single unit (Kermicle and Allen 1990). In present terms, most *Tcb1*-carrying plants in recurrent *tcb1*/*tcb1* backcross lineages are unreceptive to *tcb1* pollen, and *Tcb1*/*tcb1* heterozygotes resulting from crosses to standard *tcb1 su1* lines produce $F_2$ progenies segregating approximately 3% sugary kernels. However, after replacing the teosinte segment distal to *Tcb1*, the recovered plants in two separate lineages were partially receptive to *tcb1* pollen and produced variable numbers of sugary kernels in $F_2$ progenies, averaging about 10%. Reciprocal crosses showed only the pistil effect of *Tcb1* to be attenuated. Because *Ga1-m* from teosinte had been replaced by *ga1* from maize during the derivation of these lineages, *Ga1-m* was considered as a candidate modifier which stabilizes strong *Tcb1* activity. Nevertheless, adding *Ga1-m* to attenuated *Tcb1* as a *trans* heterozygote did not restore strong nonreceptivity to *ga1* and *Ga1-s* pollen. Presumably, removal of *Ga1-m* was coincident with loss of a modifier or modifier genes which stabilize the strong action of *Tcb1*.

Three properties of attenuated *Tcb1* were followed while mapping it relative to visual markers located on chromosome arm *4S*: discrimination in pistils between *Tcb1* and *tcb1* pollen, the ability as male to fertilize TIC/+ females, and reduced transmission of *Tcb1* pollen relative to *tcb1* in crosses to *tcb1*/*tcb1* females. These properties were either all present (ten chromosomes) or all absent (16 chromosomes) among the 26 crossovers characterized in the *ts5*-*su1* interval. Joint inheritance of the three properties could reflect pleiotropic action of one gene or the separate action of closely linked genes. Separate genes governing pollen and pistil functions would not be surprising, based on analogy with separate control in the case of self-incompatibility in *Brassica* (Shopfer et al. 1999; Takayana et al. 2000).

Chromosome rearrangement sometimes accompanies the linkage of separate genes of related function. Specifically, it has been proposed that *Ga1-s* acting in conjunction with a chromosome inversion might preserve the integrity of a chromosome-4S complex of teosinte traits (Galinat 1978). If an inversion were present in the teosinte segment bearing *Tcb1*, reduced recombination would be expected relative to heterozygotes involving only maize tester stocks. In the present study recombinants in the *v17*-*bm3* region, where *Tcb1* resides, constituted 2.5±1.0% of the total. This value agrees well with the 2.9±0.8% measured within a maize background (Stinard 1998).

Previously we reported a TIC lineage that had lost pistil function (Kermicle and Allen 1990). Pollen action, retained in this stock and referred to as Central Plateau factor 2 (CP2), mapped 6 cM distal to *su1*, i.e., approximately to the same position as *tcb*. CP2 may have originated by recombinational fractionation of *Tcb1*, by isolation of a separate gene whose pollen function duplicates that of *Tcb1*, or by mutation of *Tcb1*. Working out the origin of CP2 and other such variants should illuminate an understanding of *Tcb1* organization.

## *Tcb1* and the reproductive isolation of teosinte from maize

Simple, empirical tests suggest that physiological barriers prevent maize and teosinte from hybridizing freely. When pollinated with maize, plants of many teosinte populations, especially those of subspecies *mexicana*, set seed poorly or not at all (Ting 1963; Kermicle and Allen 1990). The reciprocal cross generally is successful, indicating that teosinte pollen is capable of fertilizing maize. However, teosinte pollen may compete poorly with

maize when both are present. When mixtures of teosinte and maize pollen were applied to maize silks, few or no hybrids were present among the progeny (Castro-Gil 1970). Fewer than half of such mixtures produced hybrid offspring and in only 5 of 57 test combinations were more than 10% hybrid offspring observed. The outcome was attributed to competition between maize and teosinte pollen, but the genetic basis for differential pollen function was not reported.

The *Ga1-s* system of IC might contribute to reproductive isolation (Wilkes 1967; Mangelsdorf 1974). If present only in teosinte or only in sympatric maize, it could prevent hybridization in one direction or the other. Indeed, *Ga1-s* was identified in a collection of Chalco teosinte (Kermicle and Allen 1990). The *ga1* composition of the maize populations sympatric to this teosinte is not known. Even if they were *ga1*, means of preventing them from being fertilized by teosinte is needed to maintain isolation over generations. Furthermore, in contrast with Chalco, the collection of Central Plateau teosinte reported here possessed the *Ga1-m* allele, allowing it to cross in both directions with *ga1* and *Ga1-s* maize. Thus, although *Ga1-s* might serve to prevent maize from fertilizing teosinte in local populations, this barrier is not universal.

Although preliminary, the present evidence suggests that *Tcb1* could play a significant role in isolating teosinte from maize reproductively. To do so generally, it should be widespread in teosinte, absent from sympatric maize, and act bidirectionally or be accompanied by other means of preventing maize from being pollinated with teosinte. *Tcb1*'s potential to act bidirectionally is intriguing. As presently observed in the genetic background of Midwestern US inbred W22, its transmission in *tcb1*/*tcb1* female×*Tcb1*/*tcb1* male crosses is reduced relative to *tcb1* only moderately. However, just as *Tcb1*'s action in pistils is attenuated in a W22 background, perhaps a difference in modifier genes between teosinte and Mexican maize may amplify *Tcb1*'s reduced function on *tcb1*/*tcb1* pistils. Clearly, more information is needed concerning the IC composition of sympatric teosinte and maize populations.

Successful transfer of *Tcb1* into maize recommends its consideration for avoiding contamination of one maize strain by another. Varieties to be protected might be pure breeding stocks, might possess special quality features or be free of transgenes. A barrier to crossing would suffice for certain purposes even if effective only in one direction. Such is the case whereby the *Ga1-s* system currently is employed to prevent the fertilization of popcorn varieties by dent hybrids. For the *Tcb1* system to work effectively, appropriate modifiers of the TIC haplotype need to be present and the maize to be avoided must be *tcb1*. If not present already, essential modifiers might be introduced jointly with *Tcb1* from the TIC haplotype, as evidently was done during development of the inbred W22 stock of TIC. It seems likely that dominant *Tcb1* is rare or absent from maize populations generally, since polymorphism for *Ga1-s* has accounted for repeated reports of IC within maize.

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## References

Ashman RB (1981) Failure to verify the *Ga9* locus on chromosome 4. Maize Genet Coop Newslett 55:50–51

Bernacchi D, Tanksley SD (1997) An interspecific backcross of *Lycopersicon esculentum*×*L. hirsutum*: linkage analysis and a QTL study of sexual compatibility factors and floral traits. Genetics 147:861–877

Castro-Gill MC (1970) Frequencies of maize by teosinte crosses in a simulation of natural association. Maize Genet Coop Newslett 44:21–24

Dellaporta S (1994) Plant DNA miniprep and microprep: versions 2.1–2.3. In: Freeling M, Walbot V (eds) The maize handbook. Springer, New York Berlin Heidelberg, pp 522–525

Galinat WC (1978) The inheritance of some traits essential to maize and teosinte. In: Walden DB (ed) Maize breeding and genetics. John Wiley and Sons, New York, pp 93–111

Hogenboom NG (1973) A model for incongruity in intimate partner relationships. Euphytica 22:219–233

House LR, Nelson OE (1958) Tracer study of pollen-tube growth in cross-sterile maize. J Hered 49:18–21

Jiménez JR, Nelson OE (1965) A fourth chromosome gametophyte locus in maize. J Hered 56:259–263

Kermicle JL, Allen JO (1990) Cross incompatibility between maize and teosinte. Maydica 35:399–408

Mangelsdorf PC (1974) Corn: its origin, evolution and improvement. Harvard University Press, Cambridge, Massachusetts

McCubbin AG, Kao TH (1999) The emerging complexity of self-incompatibility (S-) loci. Sex Plant Reprod 12:1–5

Murfett J, Strabala TJ, Zurek DM, Mou B, Beecher B, McClure BA (1996) S Rnase and interspecific pollen rejection in the genus *Nicotiana*: multiple pollen-rejection pathways contribute to unilateral incompatibility between self-incompatible and self-compatible species. Plant Cell 8:943–958

Nelson OE (1952) Non-reciprocal cross-sterility in maize. Genetics 36:101–124

Nelson OE (1993) The gametophyte factors of maize In: Freeling M, Walbot V (eds) The maize handbook. Springer-Verlag, New York Berlin Heidelberg, pp 496–503

Neuffer MG, Coe EH, Wessler SR (1997) Mutants of maize. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Schopfer CR, Nasrallah ME, Nasrallah JB (1999) The male determinant of self-incompatibility in *Brassica*. Science 286:1697–1700

Senior ML, Murphy JP, Goodman MM, Stuber CW (1998) Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci 38:1088–1098

Stinard P (1998) Three-point linkage data for *su1 bm3 gl7* on 4S. Maize Genet Coop Newslett 72:79

Takayama S, Shiba H, Iwano M, Shimosato H, Che F-S, Kai N, Watanabe M, Suzuki G, Hinata K, Isogai A (2000) The pollen determinant of self-incompatibility in *Brassica campestris*. Proc Natl Acad Sci USA 97:1920–1925

Thompson RD, Kirch H-H (1992) The *S*-locus of flowering plants: when self-rejection is self-interest. Trends Genet 8:381–387

Ting YC (1963) A preliminary report on the fourth chromosome gametophyte factor in teosintes. Maize Genet Coop Newslett 37:6–7

Wilkes HG (1967) Teosinte: the closest relative of maize. The Bussey Institute of Harvard University, Cambridge, Massachusetts

Wilkes HG (1977) Hybridization of maize and teosinte in Mexico and Guatemala and the improvement of maize. Econ Bot 31: 254–293