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The *Zea mays* Sexual Compatibility Gene *ga2*: Naturally Occurring Alleles, Their Distribution, and Role in Reproductive Isolation

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Abstract

Major genes govern the fertilization of teosinte ovules by maize pollen. A pollen–pistil compatibility system different from the previously described systems, *Ga1-s* and *Tcb1-s*, was identified among maize lines introgressed with chromosome segments from 2 teosinte populations. The pistil barrier is dominant, and pollen competence is determined by genotype of the individual pollen grain. A major gene governing this incompatibility behaves as a strong allele of *ga2*, a locus identified previously among maize genetic stocks on the basis of transmission ratio distortion. Additionally, pollen simultaneously carrying both *ga2* and *Ga2* was functional on *Ga2* silks, which have the pistil barrier, indicating that *Ga2* conditions acceptance of the pollen grain rather than *ga2* conditioning rejection of the pollen grain by *Ga2* silks. The strong allele (*Ga2-s*), a weaker one such as reported among maize genetic stocks (*Ga2-w*), and an allele having only pollen competence (*Ga2-m*), or some combination of these, was found in all 13 of the teosinte populations sampled. Sympatric and parapatric maize landraces carried *Ga2-m* or the presumed null allele *ga2*, but *Ga2-s* or *Ga2-w* was not found. The combination of exclusively *Ga2-s* teosinte with *ga2* maize, which could provide strong reproductive isolation, was not characteristic of the 5, paired populations tested.

Key words: *pollen–pistil compatibility, reproductive isolation, gametophyte factors,* Zea mays

Interaction between pollen and pistil governs reproductive compatibility in flowering plants. Accordingly, pollen–pistil incompatibility establishes the boundaries of hybridization by preventing both close inbreeding and wide dysgenic outcrossing. The genetic basis for the prevention of inbreeding through self-incompatibility is well known (reviewed in Franklin-Tong 2008). In contrast, what delimits the outer bound of hybridization is not well characterized. This is so despite plant hybridizers having established these bounds empirically in a number of genera.

One obstacle to characterizing cross-incompatibility is systemic. When incompatibility is reciprocal, no progeny are available for further analysis. However, when the cross is incompatible in only one direction, genetic analysis is feasible. The cross in one direction provides an assay for compatibility; the reciprocal cross provides progeny for propagation and genetic manipulation. A number of investigations scattered over different genera have taken advantage of this circumstance (for a review of tomato and its relatives, e.g., see Mutschler and Liedl 1994). However, even when distinct hybridization classes among offspring occur, it can be difficult to fit the data to a simple genetic model. One or both parents often are self-incompatible, which itself distorts progeny transmission ratios. This confounding is particularly acute when action of the major self-incompatible locus itself, or closely linked genes, confers cross-incompatibility (e.g., Murfett et al. 1996; Bernacchi and Tanksley 1997).

There are, of course, outcrossing species and relatives that are self-compatible but hybridize in only one direction. Some combinations of maize and teosinte strains fit this category. That is, they are unilaterally cross-incompatible (UCIC). In particular, maize pollen fertilizes plants of certain annual teosinte populations poorly if at all. Incompatibility is especially strong in ssp. *mexicana* teosinte populations that grow in intimate association with maize as a weed and that flower in synchrony with it (Baltazar et al. 2005; Ellstrand et al. 2007). Recently, there has been

renewed interest in relating teosinte × maize incompatibility to one class of "gametophyte factors." When the pistil carries a particular allele of such a gametophyte factor locus, pollen lacking that allele is disadvantaged or excluded from fertilization. If the male parent is heterozygous, the cross is semicompatible, resulting in preferential transmission of the allele. If no pollen carrying this allele is present, little or no seed is set (reviewed in Nelson 1994). In both cases, the reciprocal cross succeeds as artificial crossing typically is performed—applying an excess of pollen from a single source all at one time.

The first and best characterized of these *Zea* pollen–pistil compatibility genes is *Ga1-s*. When plants heterozygous for it and what behaves as a recessive null allele, *ga1*, are self-fertilized, *Ga1-s* pollen achieves fertilization to the virtual exclusion of *ga1* (Emerson 1934)—a striking early example of allelic conflict. Ratios of linked genes are distorted in proportion to their degree of linkage, a property by which the locus was mapped to the middle of the short arm of chromosome 4. Wind pollination of *Ga1-s*-carrying plants with a mixture of *ga1* and *Ga1-s* pollen has a more general effect on gene flow. Not only is the *ga1* allele excluded but also the entire genome with which it is associated. When only *ga1* pollen is present, fertilization of heterozygous *Ga1-s ga1* plants is variable depending on genetic background and environmental conditions (Schwartz 1950; Nelson 1952).

When present only in maize or only in teosinte, *Ga1-s* is expected to restrict hybridization between them. A survey of annual teosinte populations identified *Ga1-s* in 7 of the 14 populations tested, including all 5 spp. *mexicana* populations adapted to grow exclusively as weeds (Kermicle et al. 2006). For *Ga1-s* to isolate teosinte, the sympatric maize populations should be *ga1*. However, sympatric and parapatric maize populations carried a third allele, one described initially in popcorn inbred White Rice 4519 (Ashman 1981). It confers pollen compatibility on *Ga1-s* /— pistils but when present in pistils does not discriminate against *ga1* pollen. Presence of this allele (*Ga1-m*, for *male*) in maize neutralizes *Ga1-s* in teosinte as a barrier in reproductive isolation.

A gene analogous to *Ga1* has been identified within teosinte. Named *teosinte crossing barrier-1*, it was found in 7 of the 9 ssp. *mexicana* populations tested, and like *Ga1-s*, in all 5 weedy populations (Kermicle 2006). It was present in one of 4 collections of teosinte ssp. *parviglumis*, which although more closely related to maize than ssp. *mexicana* (Matsuoka et al. 2002), grows wild. *Tcb1-s* was absent in all 12 sympatric maize populations. When *Tcb1-s* was introgressed together with *Ga1-m* into a maize strain and then used as pollinator, compatibility with teosinte was improved significantly in 5 of the 8 populations carrying it, and completely restored in 3. As such, *Tcb1-s* is a candidate speciation gene for preventing teosinte from being fertilized by maize.

Although the mechanism of pollen–pistil recognition is not known for *Ga1-s* or *Tcb1-s*, results of a genetic test favor active acceptance over active rejection. Disomic pollen carrying both *Ga1-s* and *ga1* was accepted by *Ga1-s*/— pistils; similarly for disomic *Tcb1-s/tcb1* pollen on *Tcb1-s*/— pistils (Kermicle and Evans 2005). That is, the presence in pollen of *Ga1-s* or *Tcb1-s*, rather than *ga1* or *tcb1*, was determinative. In the terminology of Hogenboom (1975), the relation of *ga1* pollen on *Ga1-s*/— pistils, and likewise *tcb-1* on *Tcb1-s*/— pistils, is incongruous.

In the course of introducing *Ga1-s* and *Tcb1-s* from teosinte into maize by backcrossing, cross-incompatibility was encountered that was attributable to neither gene. The present report concerns inheritance of this incompatibility system, the functional relation of this system with *Ga2*, interactions with *Ga1-s* and *Tcb1-s*, and the ability of pollen carrying both *ga2* and *Ga2* alleles to function on *Ga2*-containing silks. The prevalence of different *ga2* alleles within teosinte and maize was evaluated, and the implications of this distribution on a role for the *Ga2* system in the reproductive isolation of *Zea* subspecies are discussed.

Materials and Methods

Introgression of UCIC from Teosinte into Maize

Plants of 13 annual Mexican teosinte populations (Supplementary Table S1) were crossed, and their F_1 progeny backcrossed recurrently to maize inbred W22 as female to transfer possible incompatibility factors into a genetic background suitable for genetic analysis. As a US Midwest inbred, W22 is free of known incompatibility factors. The initial 2 generations of crossing were performed without selection under the short days of a winter nursery located near Homestead, FL. Thereafter, lineages that segregated cross-incompatible plants were propagated by crossing plants that received W22 pollen poorly to ear parents of this inbred line, either at Homestead or in a summer nursery at Madison, WI. Of interest here are lineages descended from teosinte collections 101 and 104 that segregated UCIC plants that were unable to fertilize the previously defined UCIC stocks, *Ga1-s* and *Tcb1-s*. After 5 generations of backcrossing, true-breeding strains (UCIC-101 and UCIC-104) were established by self-fertilization. Table 1 gives the distinguishing features of these near-isogenic lines and of related UCIC stocks utilized in the present investigations.

UCIC Phenotyping of Pollen

Separate tests determine whether a plant expresses a UCIC barrier in pistils from whether its pollen is competent to fertilize pistils having that barrier. To assess pollen competence, pollen of a test plant is placed on silks of a UCIC plant of the strains described above. A given population of teosinte may be true breeding or polymorphic, and individual plants may be homozygous or heterozygous. Accordingly, 3 plants representing each of the 13 teosinte collections were first crossed to inbred W22 to haploidize the contribution from teosinte. Then, several plants in each F_1 progeny were evaluated by crossing as male to an established UCIC strain as female. Pollen parents producing testcross ears having less than 20% seed set were classified as lacking pollen competence; those having more than 40% set were classified as possessing it, with 30% being an ambiguous outcome.

Table 1 *ga2* pollen-pistil cross-compatible stocks

Stock[a]	Source	Compatibility		
		Fertilizes UCIC	Discriminates between UCIC and non-UCIC pollen	Rejects non-UCIC pollen
UCIC = *Ga2-s* (strong)	Teosinte collections 101[b] and 104[c]	+	+	+
Ga2-w (weak)	Maize genetic stocks	+	+	±
Ga2-m (male)	Maize and teosinte	+	−	−
non-UCIC = *ga2*	Maize inbred W22	−	−	−

[a] All are *ga1/ga1*; *tcb1/tcb1*.

[b] *Zea mays* ssp. *mexicana*, Cocotitlán, Chalco, Edo de Mexico.

[c] *Zea mays* ssp. *parviglumis*, Alcholoo, Teloloapan, Guerrero, Mexico.

UCIC Phenotyping of the Pistil

The first of 2 methods used to detect a UCIC pistil barrier relies on reduced seed set. Seed set ranged from full to barren (Figure 1, Panel A) depending on strength of the barrier and conditions at pollination. When large numbers of plants were to be evaluated, such as in tests of UCIC inheritance, gene dosage, and allelism, wind pollination was employed. For this, detasseled plants in an isolation block were wind pollinated by interplanted rows of W22 males. A similar condition was simulated for greenhouse grown teosinte. In that case, the apical tassel and staminate parts of lateral inflorescences were removed at least every other day. Maize pollinators introgressed with UCIC in addition to *Ga1-m* and *Tcb1-s* were furnished throughout the teosinte silking period. In a separate greenhouse, teosinte plants grown from the same collections of seeds were allowed to interpollinate, providing a baseline of potential seed production. For teosinte females, counts of the number of filled fruitcases were made, whereas for maize ears seed set was estimated to the nearest 10%, then averaged, as described previously (Kermicle and Allen 1990).

The second method to evaluate strength of the pistil barrier is based on competition between UCIC and non-UCIC pollen in artificial mixtures (Figure 1B). This test covers a broader range of UCIC pistil actions than seed set itself. For example, weak UCIC pistil action that does not prevent fertilization when non-UCIC is the sole source of pollen may discriminate against it in mixtures with UCIC. For the present experiments, non-UCIC pollen typically carried the genes required for aleurone kernel color, whereas UCIC pollen, together with the female parents under test, lacked one or more functional alleles of these genes. Crosses of a given mix to non-UCIC W22 females established the ratio of viable pollen from the 2 sources. UCIC strength of test plants pollinated with the mix is expressed relative to the proportion of non-UCIC pollen that functioned in the control mating.

UCIC Evaluation of Mexican Maize

Twelve collections (Supplementary Table S1) of landrace (open pollinated) maize, sympatric or parapatric to the teosinte populations under study, were analyzed as described above, with one difference relative to teosinte. Phenotyping for pistil barrier by artificial pollen mixtures used first-generation W22-backcross plants. This differs from teosinte where it was necessary to make one or more additional backcrosses in order to obtain plants having sufficiently maize-like ears suitable for analysis.

Test for Acceptance of *Ga2* Pollen versus Active Rejection of *ga2* Pollen Using Pollen Carrying Both *Ga2* and *ga2*

Plants carrying a translocated chromosome as an addition to the standard diploid set produce a fraction of functional

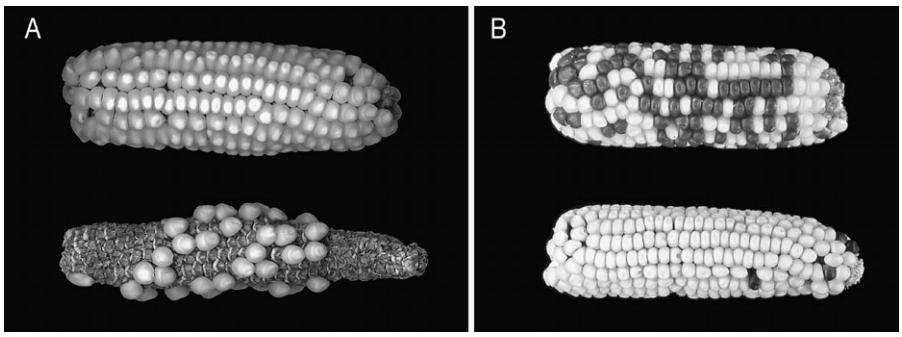

Figure 1. Criteria of pistil UCIC barrier strength. (**A**) Differential seed set. Ears resulting from pollinating a non-UCIC plant (above) and a UCIC plant (below) with non-UCIC. (B) Preference in pollen mixtures. Ears resulting from pollinating non-UCIC (above) and UCIC (below) plants with a mixture of UCIC and color-marked non-UCIC pollen.

pollen having the *ga2* region duplicated. The B^5 chromosome of translocation B-5Ld, used here, comprises most of the long arm of chromosome 5 attached to the centromeric portion of maize's supernumerary B chromosome (Beckett 1994). *ga2* lies one map unit distil to the interchange and *pr1* lies another 11 units distal. (*pr1/pr1* confers red kernels in the colored aleurone stocks used, whereas *Pr1/—* confers purple.) Due to close linkage, *pr1* serves as a surrogate marker for *ga2* and B^5. All 3 chromosome-5 long arms of the $5/5/B^5$ stock carried *ga2*; the 2 nontranslocated arms carried *pr1*, whereas B^5 carried *Pr1*. Reciprocal crosses of this stock with a diploid *pr1* tester served to propagate the partial trisomic plants verified by reduced transmission of the B^5 chromosome—marked with *Pr1*—through the male and female with a greater effect on the male.

The second parent used to construct the test heterozygotes combined *Ga2*, either *Ga2-w* or *Ga2-s*, with *pr1*. Stocks of *Ga2-w pr1* were obtained from the Maize Genetics Cooperation Stock Center. A stock of *Ga2-s pr1* was generated by coupling *Ga2-s* in the UCIC-104 stock with *pr1* in a *pr1 brittle1* (*bt1*) tester through recombination.

Pollinating the $5/5/B^5$ plants with *Ga2-s pr1/ga2 pr1* males or *Ga2-w pr1/Ga2-w pr1* males produced progenies having a subset of the desired test genotype, *Ga2 pr1/ga2 pr1*/$B^{5(ga2\ Pr1)}$. Pollen from single plants derived from these crosses was used to fertilize *ga2 pr1* and *Ga2 pr1* plants, constituting compatible and semi-compatible crosses, for testing the frequency of B^5 transmission. The frequency of function of pollen of the genotype *Ga2 pr1*/$B^{5(ga2\ Pr1)}$ in crosses of *Ga2 pr1/ga2 pr1*/$B^{5(ga2\ Pr1)}$ onto *ga2 pr1* and *Ga2 pr1* females was estimated from the frequency of transmission of *Pr1* through the male. A subset of the *Pr1*-marked kernels from the cross onto *Ga2 pr1* females was progeny tested to verify that they were indeed trisomic and thus had inherited the $B^{5(ga2\ Pr1)}$ chromosome.

The presence of *Ga2* in the $5/5/B^5$ males above was verified by mixing pollen of these plants, which are homozygous for *R1*, with pollen of *ga2* plants homozygous for *r1*, and using this mix to pollinate ears of *Ga2 r1* and *ga2 r1* females. The presence of *Ga2* in these plants was then indicated by the ability of the pollen mix to successfully fertilize *Ga2* females and also have a much higher ratio of purple (*Ga2 R1*) kernels to yellow (*ga2 r1*) kernels in the cross to the *Ga2 r1* females than in the cross to the *ga2 r1* females. Heteroallelic pollen function tests were performed in both the summer nursery of 2008 in Stanford, CA, and the following winter nursery in Molokai, HI.

Ability of *Ga2-s* to Function on *ga2* Silks

To test for the ability of *Ga2-s* pollen to compete with *ga2* pollen on *ga2* silks, plants with the *Ga2-s pr1* recombinant chromosome described above were crossed onto a *ga2 Pr1* W22 stock. The resulting F_1 plants were crossed as males onto both *Ga2 pr1* and *ga2 pr1* females to test pollen competition from a heterozygote producing a 1:1 mix of *Ga2* and *ga2* pollen. These crosses were performed in the winter nursery in Molokai, HI.

Cross-Recognition between *Ga2* and the *Ga1* and *Tcb1* Systems

A mix of pollen from *Ga2-s r1* plants and *ga2 R1* plants was applied to silks of *ga1 ga2 tcb1 r1* females, *Ga1-s ga2 tcb1 r1* females, and *ga1 ga2 Tcb1-s r1* females to determine if *Ga2-s* would provide a measurable increase in compatibility with *Ga1-s* or *Tcb1-s* silks. Likewise, mixes of *ga1 ga2 tcb1 R1* pollen with either *Ga1-s ga2 tcb1 r1* pollen or *ga1 ga2 Tcb1-s r1* pollen was applied to silks of *ga1 ga2 tcb1 r1* females and *ga1 Ga2-s tcb1 r1* females to test for the ability of *Ga1-s* or *Tcb1-s* in the pollen to overcome the barrier produced by *Ga2-s*. Cross-recognition experiments were performed in the summer nursery of 2007 in Stanford, CA.

Results

Inheritance, Pollen Action, and Chromosome Location of Unilateral Cross-Incompatibility

UCIC near-isogenic stocks derived from teosinte collections 101 and 104 (Supplementary Table S1) fertilize plants of maize inbred W22 (non-UCIC) readily, but receive its pollen poorly, likewise with F_1 hybrids between UCIC and W22. Successive generations of backcrossing to W22, with selection for incompatibility, produced segregating progenies useful for determining UCIC inheritance. Detasseled plants tested through wind pollination by W22 males gave a wide range of seed set scores (Figure 2A). The data for both collections suggest a bimodal distribution, with a valley at 30% and 40% set. Approximately, equal numbers in the 2 classes implicates a major dominant gene in the determination of UCIC.

Figure 2B addresses UCIC dominance. Tested for seed set, again when wind pollinated by W22 (non-UCIC) pollen, true-breeding strains of the 2 UCIC collections showed somewhat lower set than after outcrossing onto non-UCIC W22, indicating either incomplete dominance or, possibly, dilution of modifiers introduced with the UCIC parent.

Control of pollen behavior in some systems of incompatibility is governed by genotype of the individual pollen grain, in others by genotype of the parent plant (sporophyte). If control is exerted at the level of pollen (haploid male gametophyte), and given that pistil control is dominant, F_2 progenies are not expected to contain plants compatible with non-UCIC pollen. Whereas, if control is imposed by the paternal sporophyte, all pollen classes from F_1 plants would function, producing one-fourth compatible F_2 progeny, assuming major gene control. Many fewer than one-fourth compatible offspring occurred (Figure 2C, graphs 8 and 9). The distributions are intermediate between the respective true-breeding UCIC (Figure 2B, graphs 3 and 5) and the F_1 controls (Figure 2C, graphs 10 and 11). This outcome is consistent with gametophytic control of pollen action.

The UCIC stocks 101 and 104 used in the preceding experiments were isolated in parallel but independently. Incompatibility could be due to different genes or to

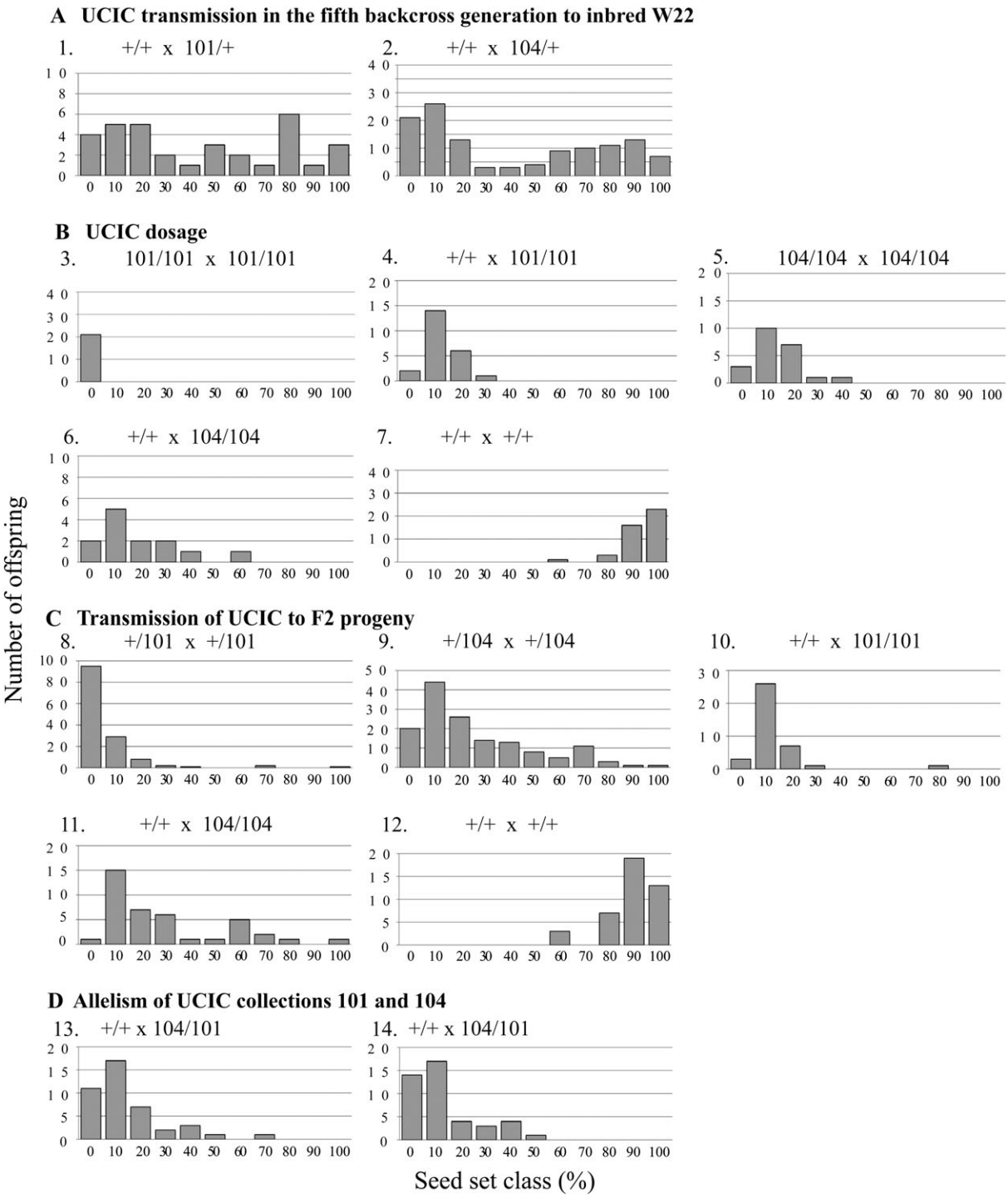

Figure 2. Inheritance of UCIC isolated from annual teosinte collections 101 and 104. The parental cross is given. The offspring were wind pollinated with non-UCIC then evaluated for seed set. UCIC parentage is indicated by source, strain 101 or 104; non-UCIC is indicated by "+."

different alleles of the same gene. To test allelism, the 2 true-breeding stocks were intercrossed, next testcrossed on W22 (non-UCIC) and the resulting progeny then evaluated for UCIC as above. Incompatibility of all 85 offspring was within the range expected for UCIC heterozygotes (Figure 2D). The absence of fully compatible offspring is consistent with allelism.

To locate UCIC to chromosome arm, advantage was taken of the distortion following semicompatible crosses in the transmission of genes linked with UCIC. A set of maize

reciprocal translocations couples the short arm of chromosome 9 with each arm of the other 9 chromosomes. The 9*S* arm in each case carries the recessive allele of *waxy1* (*wx1*) conferring high amylopectin starch to the kernel's endosperm, giving it a "waxy" appearance. If the major UCIC locus is linked with *wx1* through the translocation (T), crosses of *wx1* T(non-UCIC)/*Wx1*(UCIC) onto UCIC *wx1* females are expected to produce a deficit from the expected 50% of waxy kernels. Three translocations involving chromosome 5 showed a marked excess of nonwaxy kernels, the allele entering the cross in *cis* with UCIC (Table 2). Translocation T5–9 (4817), having a breakpoint near the centromere, shows the largest excess at 96.2% nonwaxy. (The 3.8 % of waxy kernels could result from recombination between UCIC and *Wx1*, or *wx1* (non-UCIC) pollen may have escaped the incompatibility screen. As such, 3.8% represents a maximal estimate of escapes.) Similar translocations involving chromosomes 1, 3, and 4 produced from 53.2% to 57.8% nonwaxy. A modest but statistically significant excess of the nonwaxy class also has been observed in *Wx1/wx1* testcrosses involving standard chromosomes (e.g., Sprague 1933).

Compatibility and Allelic Relations of UCIC 101/104 with Maize *ga2*

A long-known gene, *Gametophyte factor-2*, that causes ratio distortion when present in pistils and heterozygous in the pollen parent also maps to the proximal region of chromosome *5L* (Burnham 1936; Brieger 1937; Longley 1961; Neuffer et al. 1997). As an initial test of functional relationship between UClC and *Ga2*, the UClC-104 stock was pollinated with 2 *Ga2* stocks: a colorless kernel (*anthocyaninless-2*) one and a colored-kernel (*A2*) one obtained from the Maize Genetics Cooperation Stock Center. Use of both pollinators resulted in full seed sets, indicating compatibility. Comparing the fertilizing ability of *a2 Ga2* and UClC-101 pollen in mixtures provided a more sensitive test of relative pollen competence. Complementary kernel-color factors marked the 2 sources of pollen to distinguish kernels sired by each (*a2 R1* for *Ga2* vs. *A2 r1* for UCIC). Pollination of an *A2 A2* ; *r1 r1* ; *ga2 ga2* strain determined the percent of viable *Ga2* pollen (colored kernels) in 3 mixes at from 51.8% to 75.0%. Similar proportions obtained in crosses of the mixes onto plants in the 2 pollen-donor strains (colorless kernels on *Ga2 Ga2* ; colored on UCIC) is consistent with equal competence of *Ga2* and UCIC pollen on these 2 females (Figure 3; $P = 0.10$).

In the first of 2 tests conducted to compare strengths of the UCIC pistil barrier with that of the maize tester stocks *a2 Ga2* and *A2 Ga2*, the 3 lines together with control *ga2* were crossed reciprocally in all possible combinations. As expected, poor sets were obtained from the UCIC female × *ga2* male crosses, averaging 26% (Table 3). In comparison, the *a2* and *A2* sources of *Ga2* received *ga2* pollen with averages of 74% and 46%. Although the values for *Ga2* stocks clearly differ from UCIC-104, they fall below values obtained when the UClC-104 and *Ga2/Ga2* strains were crossed among themselves, ranging from 92% to 98%. The comparatively small reduction in seed set in *Ga2/Ga2* × *ga2/ga2* crosses possibly accounts for why this effect was not noted in initial reports concerning *Ga2* (Burnham 1936; Brieger 1937).

In contrast, the early reports showed *Ga2/Ga2* pistils to discriminate strongly against *ga2* pollen when both *ga2* and *Ga2* are available. To compare levels of discrimination, 3 mixtures of approximately equal quantities of fresh *ga2* and *Ga2* pollen were tested on *ga2/ga2*, *Ga2/Ga2*, and UCIC-104 females. As before, complementary, recessive kernel-color genes marked the 2 sources of pollen to distinguish kernels sired by each (*A2 r1* for *ga2* vs. *a2 R1* for *Ga2*). In fully compatible crosses on *ga2 ga2* females, *ga2* sired from 43.5% to 59.5% of the offspring. In all 3 mixes, the level of discrimination against *ga2* pollen was stronger by UCIC than by *Ga2* (Figure 4), although for unknown reasons, discrimination between the 2 pollen classes varied from mix to mix.

To test whether the major compatibility gene of UCIC is transmitted as an allele of *Ga2*, F_1 hybrids of the 2 were crossed to W22 (non-UCIC, *ga2*). If UCIC and *Ga2* are alleles, each offspring should receive one or the other gene, conferring compatibility in a subsequent cross to a UCIC tester female. Crosses of all 43 UCIC-104/*a2 Ga2* testcross progeny fertilized UCIC plants well; likewise with 42 of 43 UCIC-104/ *A2 Ga2* progeny. The remaining ear produced a scattered set of kernels, which is not uncommon following hand pollination even between compatible parents. Although of low resolution, the outcome of these tests is consistent with very close linkage or allelism of UCIC-104 with *Ga2*.

Based on compatibility relations and similar chromosome location, it seems reasonable to conclude that a major component of the teosinte UCIC phenotype is allelic to *Ga2*. In parallel to allelic series of the *ga1* and *tcb1* loci, UCIC is given the allelic designation *Ga2-s*, denoting its strong pistil barrier. Similarly, *Ga2-w* denotes the weak pistil action characteristic of the 2 maize *Ga2* genetic stocks.

Table 2 Linkage of UCIC with translocation breakpoints in chromosome 5, observed in crosses of UCIC; *wx1* females × non-UCIC; *wx1* translocation/UCIC; *Wx1* males

Translocation			No. of kernels		
Stock	Breakpoints	Entry	Non-waxy	waxy	% waxy
T5-9 (022-11)	9L.27;5S.30	P6039	1150	76	6.2
T5-9 (4817)	9S.05; 5L.06	P6041	1111	44	3.8
T5-9 (a)	9S.17;5L.69	P6043	685	322	32.0
T1-9 (4995)	9S.20;1L.19	P6046	333	280	45.7
T3-9 (8562)	9L.22;3L.65	P6047	579	509	46.8
T4-9 (e)	9L.26;4S.53	P6048	755	551	42.2

Distribution of *ga2* Alleles among Mexican Annual Teosinte and Their Counterpart Maize Populations

For *Ga2-m* and *Ga2-s* to be factors in reproductive isolation, they should be present either in teosinte or maize but not

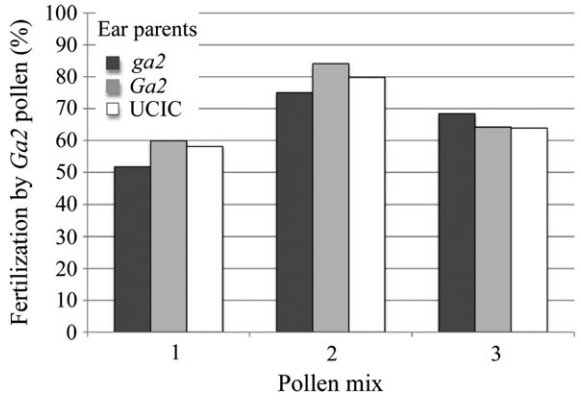

Figure 3. Competition between *Ga2* and UCIC pollen in mixtures on *ga2/ga2*, *Ga2/Ga2*, and UCIC females.

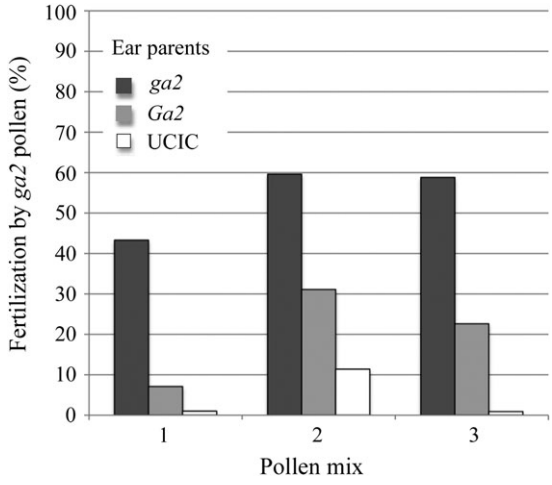

Figure 4. Competition between *ga2* and *Ga2* pollen in mixtures on *ga2/ga2*, *Ga2/Ga2*, and UCIC females.

both. Thirteen sympatric populations were characterized for pollen competence and 5 for pistil barrier strength. To evaluate pollen competence, plants of teosinte and of their associated maize population were first hybridized with inbred W22 maize (*ga2/ga2*) and F_1 offspring then crossed as pollen parent to *Ga2-s/Ga2-s* females. If the original Mexican *Zea* parent were homozygous *Ga2* (generic designation for pollen competence, i.e., *Ga2-s*, *Ga2-w*, or conceivably, an allele having only pollen action), all its offspring are expected to be compatible with the tester; if heterozygous with *ga2*, one-half would be; if *Ga2* were absent, all would be incompatible.

All 37 teosinte plants tested in this manner carried *Ga2*, and it was homozygous in all plants but possibly 2, for which the test was inconclusive. The 12 associated maize populations were mixed: 7 had only *Ga2* and 3 only *ga2*, whereas 2 contained both allele classes (Table 4). The maize populations included multiple collections of the landraces Cónico and Cónico Norteño. Whereas plants of the 3 collections of Cónico were all *Ga2*, one of Cónico Norteño was *ga2*, one was *Ga2*, and one had both. This finding suggests the possibility of local coadaptation of crossing behavior between teosinte and given populations of maize landraces. Among the maize landraces associated with the 5 teosinte populations growing in close association with maize as weeds, only collection 107 carried *ga2*, offering the possibility of isolation from *Ga2-s* teosinte.

For comparison with the Mexican *Zea*s, 10 Midwest US inbreds in addition to W22, shown earlier to be *ga2 ga2*, were evaluated for *Ga2* vs. *ga2* composition by crossing directly to *Ga2-s/Ga2-s*. Each of the 4–5 plants tested in inbreds A188, A619, A632, B73, CM105, Pa405, SDp312, W153R, W540Ht, and W23 failed to fertilize plants of the *Ga2-s* test strain. The absence of *Ga2* among these lines is parallel to the *Ga1* system in which pollen-competent alleles are common in Mexican maize landraces but uncommon or absent in US dent inbreds.

Five *mexicana* teosinte populations (collections 101, 102, 107, 205, and 207), together with their counterpart maize varieties, were chosen for determination of pistil barrier strength. For this, a total of 193 plants in introgressed W22 backcross lines were crossed to *Ga2-s/Ga2-s*, thereby genotyping for *Ga2*, and were also pollinated with a mixture of *Ga2-s* and color-marked *ga2* to test for discrimination against *ga2* pollen. One-hundred and six teosinte-derived and Mexican maize-derived backcross plants were classified as *ga2/ga2* based on 20% or lower seed set on a *Ga2-s/Ga2-s* test plant. As female parents these plants gave pollen discriminate indices (i.e., *ga2* vs. *Ga2* ratios relative to control *ga2/ga2* females, see Materials and Methods) clustered around 1.0, indicating compatibility with *ga2* pollen (Figure 5A). Ratios similar to these were observed for the 38 *Ga2*-carrying plants (those producing >40% seed sets on *Ga2-s/Ga2-s*) in the backcross lineages of sympatric/parapatric maize landraces (Figure 5B). By analogy with the *Ga1* and *Tcb1* systems of cross-incompatibility, this class is designated *Ga2-m*, denoting male-only action. Neither *Ga2-s* nor *Ga2-w* was found in the 5 maize populations.

In contrast, the 49 *Ga2*-carrying plants in teosinte-derived introgression lines range from almost complete discrimination against *ga2* pollen to none, with modal classes toward

Table 3 Compatibility between 4 stocks differing in UCIC, *Ga2*, and *ga2* constitution (% seed set)[a]

		Pollen parent			
		UCIC	Ga2		
Ear parent		Col. 104-3	*a2*	*A2*[b]	*ga2*
UCIC	Col. 104-3	88	96	92	26
Ga2/Ga2	*a2*	96	92	96	74
	A2[b]	92	98	96	46
ga2/ga2	*a2*	96	92	96	96

[a] % of full seed set averaged over 5 crosses.

[b] Stock with heterogeneous genetic background, whereas the other 3 are sublines of inbred W22.

Table 4 *Ga2* pollen competence of maize landraces associated with 13 annual teosinte populations collected in Mexico

Collection		Teosinte		Maize			
Number	State	ssp[a]	Habitat	Landrace	*ga2* constitution[b]		
101	Mex.	mex.	weedy	Chalqueno	GG	GG	Nt
102	Mex.	mex.	weedy	Cónico	GG	GG	GG
104	Gro.	par.	wild	Pepitilla	GG	GG	G/-
105	Mich.	par.	wild	Cónejo	gg	gg	gg
106	Mich.	mex.	weedy	Cónico	GG	GG	GG
107	Mich.	mex.	weedy	Cónico Norteño and Tabloncillo	g/-	gg	g/-
109	Gto.	mex.	r/y[c]	Cónico Norteño	gg	Gg	g/-
110	Mex.	mex.	weedy	Cónico	GG	G/-	GG
201	Chih.	mex.	r/y	Christalino de Chihuahua	GG	GG	GG
202	Gro.	par.	wild	Blanco Tardío	gg	g/-	gg
203	Jal.	par.	wild	Criollo Blanco de Ocho	g/-	Gg	GG
205	Dgo.	mex.	r/y	Cónico Norteño	GG	GG	GG
207	Jal.	mex.	r/y	Criollo Blanco de Ocho	see collection 203		

[a] Subspecies: mex., mexicana; par., parviglumis.

[b] Genotype based on ability of F_1 hybrids with *ga2* to fertilize *Ga2-s/Ga2-s*. G designates *Ga2-s*, *Ga2-w*, or *Ga2-m*; g designates *ga2*. The 3 plants indicated for each collection are listed in the same order as those reported for *ga1* and *tcb1* compositions (Kermicle et al. 2006; Kermicle 2006). Nt = not tested.

[c] r/y designates ruderal and weedy.

the ends of the distribution (Figure 5C). Nine of the 14 plants having compatibility values in an intermediate range of between 0.2 and 0.8 descend from teosinte collection 102. This group may constitute a distinct allele class, perhaps equivalent to the weak *Ga2* pistil action characteristic of the 2 maize genetic stocks reported in Table 3 and Figure 4.

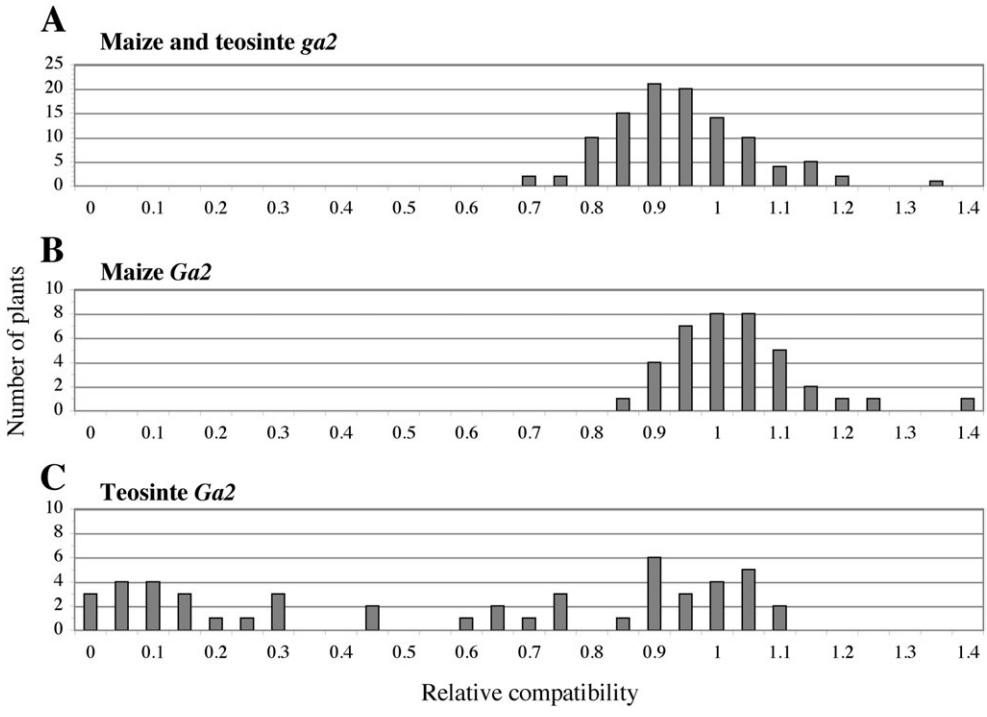

Figure 5. Discrimination by *Ga2/ga2* and *ga2/ga2* females between *Ga2-s* and *ga2* pollen in mixtures. The *Ga2/ga2* and *ga2/ga2* plants occur as sib plants segregating in W22 (*ga2/ga2*) backcross progenies descended from teosinte and sympatric Mexican maize *Ga2* sources. Results are expressed relative to fertilization by *ga2* pollen in the mixture on compatible, *ga2/ga2* females. Pistil discrimination values range from strong (near zero, *Ga2-s*) through weak to absent (near 1.0, *Ga2-m* and *ga2*). The figure excludes 12 teosinte-derived and 2 maize-derived plants that were scored as 30% seed set in crosses to *Ga2-s/Ga2-s*, and therefore were of uncertain *Ga2:ga2* genotype. (**A**) One-hundred and six *ga2/ga2* plants from the teosinte and maize sources combined. (**B**) Thirty-eight *Ga2* plants derived from the sympatric maize sources. (**C**) Forty-nine *Ga2* plants derived from teosinte sources.

Included in the analysis is teosinte collection 107, for which the corresponding maize proved *ga2*. The *Ga2* descendants of one plant tested as *Ga2-m*. Descendants of the second gave relative compatibility scores of from 0.46 to 0.93. Variable presence of a weak pistil barrier indicates that *Ga2* is not a regular contributor to reproductive isolation between maize and teosinte at this site.

Multiple Alleles or Multiple Loci?

The previous section attributes variation in pistil barrier strength among diverse Mexican *Zea* sources to allelic differences in *ga2*. Alternatively, the variation might be due to genes at other loci that confer compatibility with the *Ga2-s* tester. To locate pollen competence to chromosome region in these accessions, the *Ga2-s* compatible offspring in backcross lineages descended from 10 teosinte and 5 sympatric maize plants were evaluated by the same means by which UCIC initially was associated with the centric region of chromosome 5. That is, each was first crossed to a waxy form of reciprocal translocation T5-9 (4817) (breakpoints 5L.06 and 9S.07), then testcrossed onto *wx1 Ga2-s*. All the lineages, including 4 *Ga2-s*, 4 *Ga2-w*, 2 *Ga2-m* from teosinte, as well as 5 *Ga2-m* from maize, contained *Ga2*-s compatible plants that produced a preponderance of nonwaxy kernels, reflecting the *Wx1* allele carried in *cis* with the putative *Ga2* allele, thereby confirming the source of variation to reside in the centric region of chromosome 5.

Restoration by *Ga2* of Teosinte × Maize Compatibility

Plants of the same 5 teosinte populations evaluated for strength of the *Ga2* pistil barrier in a preceding experiment were used to determine whether the addition of *Ga2* to a maize line would restore compatibility with teosinte. In an earlier test, populations 101, 102, and 207 produced, respectively, 56%, 37%, and 69% as many seeds when pollinated with *Ga1-m Tcb1-s ga2* maize as when the teosinte populations were intermated (Kermicle 2006). This compares with 110% and 89% for populations 107 and 205, indicative of full or nearly full restoration. *Ga2* was incorporated into the *Ga1-m Tcb1-s* maize pollinator for present use. Now, populations 107 and 205 produced an average of 129% of their teosinte intermated counterparts (Supplementary Table S2). (The excess over 100% in this experiment may be due to supplying maize pollinators throughout teosinte's silking period, after pollen in the teosinte intercross group had begun to wane.) Populations 101, 102, and 207 produced 35%, 44%, and 72% as many seeds as the 2 comparison populations, 107 and 205. None of the 3 percentages is appreciably higher than that obtained in the earlier test, that is, before *Ga2* had been added to the pollinator strain. That adding *Ga2* to the pollinator did not improve compatibility with teosinte was unexpected, especially in population 101 from which one of the original UCIC strains was isolated. A possible explanation is that action of *Ga2-s* in these teosinte populations is muted by modifiers relative to its action in the genetic background of the near-isogenic inbred W22 lines. The modifiers could include yet unidentified cross incompatibility genes that overshadow compatibility for *Ga2*.

Ga2 and *ga2* Pollen have Equal Competence on *ga2* Silks

To test whether the *Ga2* system is indeed unidirectional, pollen of males heterozygous for *Ga2-s pr1/ga2 Pr1* were crossed onto both *ga2 pr1* and *Ga2 pr1* females. The *Ga2-s pr1* chromosome was isolated from recombination between the teosinte *Ga2-s* allele from stock 104-1 and a maize *pr1* allele. Crosses onto both *Ga2-s pr1* and *Ga2-w pr1* showed segregation distortion of *pr1* averaging only 24.9 ± 2.8 (standard error of the mean [SEM])% and 24.7 ± 3.4 (SEM)% inheriting *Pr1*, respectively, rather than a Mendelian 50%. In contrast, crosses onto *ga2 pr1* silks did not reveal segregation distortion of *pr1*, with 48.6 ± 1.0 (SEM)% inheriting *Pr1*, demonstrating equal competence of *Ga2-s* and *ga2* pollen on *ga2* silks.

Receptivity of *Ga2* Pistils to Heteroallelic *Ga2/ga2* Pollen

To test the fertilizing ability of pollen having both a receptive and unreceptive allele of the *Ga2-s* or *Ga2-w* barrier, collections of pollen containing the heteroallelic class were used in paired compatible crosses to *ga2 pr1* and semicompatible crosses to *Ga2 pr1* (Figure 6). The incidence of heteroallelic pollen function was scored by the frequency of seeds carrying the partial trisomic 5 *ga2 pr1*/5 *Ga2 pr1*/B$^{5\ ga2\ Pr1}$ among the progeny as indicated by the purple aleurone conditioned by *Pr1* versus the red aleurone conditioned by *pr1*. The critical

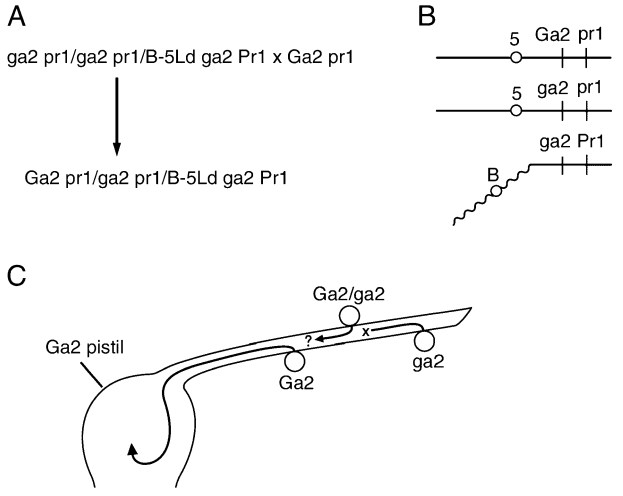

Figure 6. Genetic maps and genotypes for incompatibility versus incongruity test. (**A**) Cross used to derive progenies segregating for partial trisomic 5/5/B-5 plants carrying *Ga2*. (**B**) Chromosome constitution and crossing barrier genotype of test plants used to generate *Ga2/ga2* pollen. (**C**) Cartoon depicting the behavior on a *Ga2* pistil of *Ga2* pollen (successful), *ga2* pollen (unsuccessful), and the uncertain fate of heteroallelic *Ga2/ga2* pollen, being tested.

comparisons involve transmission of the heteroallelic 5 *Ga2 pr1*/$B^{5\ ga2\ Pr1}$ class via pollen in fully compatible versus semicompatible crosses. In the paired crosses, most show a slight reduction in partial trisomic offspring resulting from the semicompatible cross relative to the fully compatible cross, although many heteroallelic 5 *Ga2 pr1*/$B^{5\ ga2\ Pr1}$ pollen grains are functional on *Ga2* silks. In fact, in some crosses, the heteroallelic 5 *Ga2 pr1*/$B^{5\ ga2\ Pr1}$ pollen has higher function on the *Ga2* silks than the *ga2* silks. The average transmission of the heteroallelic pollen across all the paired crosses was not significantly different between crosses onto *Ga2 pr1* silks and onto *ga2 pr1* silks (Figure 7). Taken together, heteroallelic pollen can clearly function on *Ga2* silks indicating that the presence in pollen of *Ga2-s*, not *ga2*, was determinative, although the presence of *ga2* may compromise this function slightly. In the terminology of Hogenboom (1975), the relation of *ga2* pollen on *Ga2*/— silks is incongruous, like *ga1* on *Ga1-s*/— pistils and *tcb1* on *Tcb1-s*/— pistils, reported previously (Kermicle and Evans 2005).

Cross-Recognition of *Ga2* with *Tcb1* or *Ga1*

If the *Ga2*, *Ga1*, and *Tcb1* systems produce cross-incompatibility by the same biochemical mechanism, one would predict cross-recognition between the systems and the ability of *Ga2* pollen to function on *Ga1-s* and *Tcb1-s* silks and vice versa. To test this model, *ga1 ga2 Tcb1-s* or *Ga1-s ga2 tcb1* or *ga1 Ga2-s tcb1* pollen was mixed with *ga1 ga2 tcb1* and applied to silks of various cross-incompatibility genotypes.

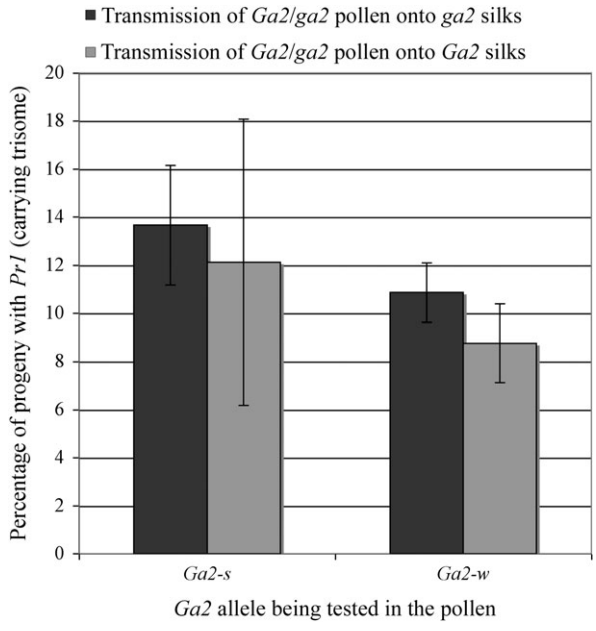

Figure 7. Ability of heteroallelic *Ga2-s/ga2* pollen or *Ga2-w/ga2* pollen to function on *Ga2* silks. The frequency of transmission of *Pr1* marking the $B^{5(ga2\ Pr1)}$ chromosome was compared between crosses onto *ga2 pr1* silks (black bars) and onto *Ga2 pr1* silks (gray bars). Error bars equal plus or minus 2 SEMs.

The *ga1 ga2 tcb1* strain used confers-colored kernels, the other strains produced colorless kernels. Hence the *ga1 ga2 tcb1* pollen serves as a tracer to determine how efficiently the various female parents discriminate between *ga1 ga2 tcb1* and pollen containing one of the crossing barrier genes. In parallel, the ratio of colored kernels to colorless kernels in the cross to a neutral *ga1 ga2 tcb1* silk parent measures the ratio of viable pollen in the mix. Mixtures of *ga1 ga2 tcb1* pollen with *Ga1-s*, *Ga2-s*, or *Tcb1-s* pollen when applied to the cognate silk genotype (e.g., *Ga1-s* pollen onto *Ga1-s* silks) demonstrated strong selection against *ga1 ga2 tcb1* pollen (Table 5). Most crosses between different cross-incompatibility genes failed to produce any seed indicating that *ga1 Ga2-s tcb1* pollen was no more effective than *ga1 ga2 tcb1* on *Ga1-s* or *Tcb1-s* silks when the barrier is strong. Similarly, *Ga1-s ga2 tcb1* and *ga1 ga2 Tcb1-s* were unable to overcome a strong *ga1 Ga2-s tcb1* silk barrier. However, in the set of crosses involving Mix 5, in which the *ga1 Ga2-s tcb1* silk barrier was not as strong (as indicated by the ability of some *ga1 ga2 tcb1* pollen to function), there was a slight advantage to the *Ga1-s ga2 tcb1* pollen over the *ga1 ga2 tcb1* pollen.

Discussion

A Family of Pollen–Pistil Cross-Compatibility Genes

The genetic behavior of *Ga2* reported here parallels that of the pollen–pistil cross-compatibility (PPCC) genes *Ga1* and *Tcb1*. The pistil barrier is dominant, and pollen competence is determined by genotype of the individual pollen grain. One allele at each locus confers a strong pistil barrier and pollen competence (designated-*s*), another only pollen competence (*-m*) and a third neither one. And, to the extent tested, interactions between pollen and pistil functions are locus specific. That is, a pollen-competent allele of one locus does not substitute substantially for that of another (Burnham and Clark 1954; Kermicle and Allen 1990; present study). Furthermore, the control of compatibility shows a consistent pattern. Incompatibility at any one locus overrides compatibility at the other 2 loci. These parallel behaviors define a gene family governing PPCC among *Zea mays* relatives. That there likely are still other members to be identified is suggested by only partial restitution of compatibility when plants in certain teosinte populations were pollinated with maize into which had been incorporated pollen-competent alleles for the 3 known loci (Supplementary Table S2).

A family of genes often serves to distinguish biological self from nonself. Vegetative compatibility among strains of filamentous fungi is a case in point (Glass et al. 2000). In *Neurospora crassa* at least 11 loci govern compatibility leading to heterokaryon formation. Remarkably, allelic difference at any one of these *het* loci causes incompatibility, in parallel to interaction between PPCC genes. That incompatibility is epistatic to compatibility also is analogous to the interaction among loci in gene-for-gene disease relations between fungi and their plant hosts. Here again, one incompatibility (resistant) relation overrides other compatible (disease) relations.

Table 5 Ability of different pollen-pistil cross-compatibility genes to cross-fertilize

	Frequency of pollen genotypes in progeny of crosses onto different pistil parents			
	Pistil parents			
Genotype of pollen mix	*ga2 tcb1 ga1*	*Ga2-s tcb1 ga1*	*ga2 Tcb1-s ga1*	*ga2 tcb1 Ga1-s*
ga2 ga1 tcb1 and *Ga2 ga1 tcb1*				
Mix 1	40 *ga2* : 227 *Ga2*	0 *ga2* : 69 *Ga2*	No seed	No seed
Mix 3	3 *ga2* : 148 *Ga2*	0 *ga2* : 155 *Ga2.*	No seed	No seed
ga2 ga1 tcb1 and *ga2 ga1 Tcb1-s*				
Mix 4	75 *tcb1* : 80 *Tcb1-s*	No seed	0 *tcb1* : 76 *Tcb1-s*	n.d.
Mix 8	8 *tcb1* : 170 *Tcb1-s*	No seed	0 *tcb1* : 123 *Tcb1-s*	n.d.
ga2 ga1 tcb1 and *ga2 Ga1-s tcb1*				
Mix 5	160 *ga1* : 20 *Ga1-s*[a]	17 *ga1* : 11 *Ga1-s*[a]	n.d.	0 *ga1* : 53 *Ga1-s*
Mix 6	18 *ga1* : 43 *Ga1-s*	No seed	n.d.	n.d.

n.d., not determined.

[a] Significantly different from one another at $P < 0.01$, Fisher Exact test.

Variation in *Ga2* Pistil Barrier Strength

Three previous studies concerning *Ga2* report genetic variation in transmission ratio distortion of maize chromosome 5 markers. Each suggests a different basis for ratio modification (Figure 8). Burnham (1936) postulated the presence in some lines of a second, linked *ga* locus, noting specifically that multiple *Ga2* alleles did not account satisfactorily for his data. Subsequent workers identified a linked *ga* gene, *Ga10* (Neuffer et al. 1997). The variant *Ga10* allele causes reduced pollen transmission. Although not shown to be specific for particular females, and therefore differing from the PPCC class of gametophyte genes, it could cause a deviation such as Burnham noted. Working concurrently to Burnham, Brieger (1937) distinguished 3 levels of ratio distortion. He attributed the variation to genetic modifier differences in both parents. Later, Longley (1961) attributed an array of pistil strengths to one or more modifiers in the female parent of his stocks. Such *trans* modifiers affecting pistil strength have been reported for the other *Zea* PPCC systems: negative modifiers for *Ga1-s* (Ashman 1975; Nelson 1994) and a linked enhancer for *Tcb1-s* (Evans and Kermicle 2001).

The procedure of producing near-isogenic lines for the present study is expected to have standardized genetic background, largely eliminating *trans* modifiers introduced with the source of *Ga2*. Rather, this procedure focuses on variation at or in the immediate region of the *ga2* locus. One allele found in ssp. *mexicana* possessed a weak pistil barrier *(Ga2-w)* corresponding to that present in certain maize genetic stocks. Another, the strong pistil barrier *Ga2-s*, was found only in teosinte. A third, *Ga2-m*, the allele lacking the pistil barrier but nevertheless competent to fertilize *Ga2-s*/— pistils, occurred both in teosinte and Mexican landrace maize. Ten US maize inbreds carried only *ga2*. This broader range in pistil strength within teosinte parallels that reported generally for single nucleotide polymorphism within teosinte

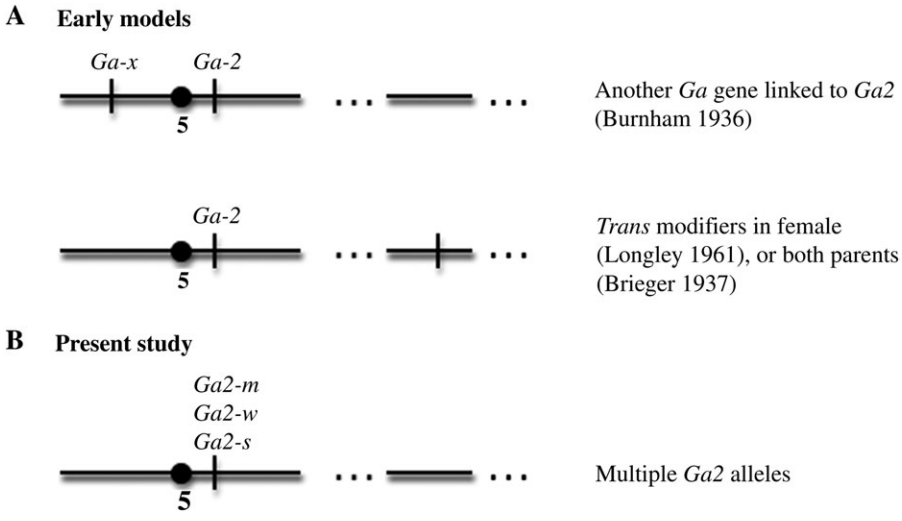

Figure 8. Genetic models of variation in strength of *Ga2* action. (**A**) Early models focusing on *trans* variation. (**B**) View based on present findings, focusing on allelic variation.

relative to maize (Matsuoka et al. 2002; Fukunaga et al. 2005). In contrast with variation in pistil strength, no difference in pollen competence was found between *Ga2-w* and *Ga2-s*. Greater variability in female than male is consistent with enhancement of prezygotic isolation having occurred by selection (Coyne and Orr 2004).

Active versus Passive Rejection of *ga2* Pollen

The basis of rejection of *ga2* pollen by *Ga2*-containing silks could result from recognition by *Ga2* silks of a factor produced by the contrasting allele, *ga2*, in the pollen and initiation of a rejection response (incompatibility). Alternatively, the matching allele, *Ga2*, in the pollen may produce a factor absent in *ga2* that leads to its ability to function on *Ga2* silks (congruity). The present experiments sought to distinguish between these possibilities genetically by determining the behavior of pollen carrying a contrasting as well as a matching allele. Pollen carrying both a contrasting allele, *ga2*, and a matching allele, *Ga2-s* or *Ga2-w*, successfully fertilized pistils containing the barrier allele, consistent with a congruity model where matching pollen is accepted rather than contrasting pollen being rejected. These findings are consistent with null activity of the *ga2* allele. This outcome is in contrast with cases of unilateral incompatibility where one parent is self-incompatible. When the *S* locus is involved in interspecific incompatibility, it is reasonable to suppose the mechanism would involve active rejection as it does in self-incompatibility (Hancock et al. 2003; Swanson et al. 2004).

Alternatively, an interaction between *Ga2* and *ga2* in heteroallelic pollen might repress *ga2* actively. For example, epigenetic cosuppression might silence *ga2*, such as has been suggested for inactivation of alleles in heteroallelic pollen in the monofactoral system of gametophytic self-incompatibility (Meyer and Saedler 1996). Were this so, silencing of homoallelic disomic *ga2 ga2* pollen would be expected, leading to acceptance of *ga2* pollen by *Ga2-s*–containing silks. Crosses to *Ga2* testers of ten $5/5/B^5$ plants carrying only *ga2* alleles were all unsuccessful, lending no support to this possibility. Additional evidence that *ga2* is a null allele comes from the lack of selection by *ga2* silks of either *ga2* or *Ga2* pollen.

Relations between *Ga2-s* and *Ga1-s* and *Tcb1-s*

The question remains as to whether the PPCC conferred by *Ga2-s*, *Ga1-s*, and *Tcb1-s* share a common biochemical mechanism. Crosses between the different systems clearly show that *Ga2* is not equivalent with *Ga1* or *Tcb1*, just as *Tcb1* and *Ga1* are not (Evans and Kermicle 2001). If the PPCC set up by these genes were identical, they would be expected to be fully compatible with one another, which is not the case. Additionally, if the difference was simply a matter of allele strength between homologous genes, one would predict unilateral cross-incompatibility between them, which is also not seen. However, there is some evidence for weak interactions between *Tcb1* and *Ga1* (Evans and Kermicle 2001) and between *Ga2* and *Ga1* (this study) suggesting that the molecular nature of the cross-incompatibility is related, perhaps impacting the same biochemical pathway.

Does *Ga2* Play a Role in Isolating Teosinte from Maize?

Maize and teosinte coexist in Mexico as sister taxa under strong divergent selection. As a cultigen, maize is subject to human selection; as a weed (most spp. *mexicana* populations) or wild plant (most spp. *parviglumis* populations), teosinte is subject to natural selection. Teosinte/maize hybrids have low fitness (e.g., Mangelsdorf 1974). As a prezygotic barrier to hybridization, *Ga2-s* is a candidate for avoiding the effects of low hybrid fitness by reinforcing reproductive isolation. However, presence of *Ga2-m* in some sympatric Mexican maize landraces, and polymorphism for various alleles within teosinte, militate against *Ga2-s* for preventing teosinte from being fertilized by maize. An analogous situation pertains to the *ga1* locus where the maize landraces sympatric to the 4 *Ga1-s* teosinte populations identified were *Ga1-m* (Kermicle et al. 2006). This distribution contrasts with *teosinte crossing barrier1* where *Tcb1-s* and *Tcb1-m* were reported only in teosinte. *Ga1-s* and *Ga2-s* could have been effective in isolating teosinte from *ga* maize in the past. Similarly, they could come back into play in the future, say as US maize is introduced into Mexico.

If not presently reinforcing reproductive isolation, what forces keep *Ga1-s* and *Ga2-s* alleles frequent in teosinte populations? Likely, another sort of selection operates, namely the strong advantage of *Ga* male gametophytes on *Ga-s /—* silks. The preference for *Ga* pollen by *Ga-s /—* silks combines features of assortative mating with distorted segregation (drive). Assortative mating in this case has a physiologic rather than morphologic basis, and drive is directed at differential pollen function rather than meiosis. The coupling of pistil and pollen effects together—whether by pleiotropic effects of a single gene or as separate, closely linked loci—confer a unique dynamic. The combination could be maintained at high frequency in the absence of ordinary fitness advantages by a "runaway process" (Muller 1930), which itself can promote reproductive isolation (Lande 1981).

Multiple independent-acting genes that promote their own propagation are not unique to *Zea*. In the flour beetle *Tribolium castaneum* any of several *Medea* genes confer maternal-effect lethality to all progeny that do not inherit a copy of the gene (Beeman et al. 1992; Chen et al. 2007). In this case, lethality is postzygotic, whereas the *Zea* factors act prezygotically.

Further Genetic and Ecological Considerations

Clearly, in a given teosinte-infested maize field, the potential for *Ga2* to protect teosinte ovules against maize pollen depends on several variables: among others, what *Ga2* allele or alleles and what modifiers are present in teosinte? How frequent is *Ga2-m* in the pollen of sympatric maize? Is the same maize variety grown year after year, and how extensive do the flowering times of these varieties overlap with that of teosinte? As seems likely with *Ga1-s*, an appropriate

combination of genotypes suitable for *Ga2-s* to confer reproduction isolation may pertain to a minority of sympatric teosinte/maize combinations in Mexico presently. Acting collectively over time, however, *Ga1-s*, *Ga2-s*, *Tcb1-s*, and possibly other members of a PPPC gene family could provide effective reproductive isolation of teosinte ovules from maize pollen in a variety of circumstances.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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