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

**JERRY L. KERMICLE AND MATTHEW M. S. EVANS**

From the Laboratory of Genetics, University of Wisconsin, Madison, WI 53706 (Kermicle); and the Department of Plant Biology, Carnegie Institution for Science, Stanford, CA (Evans).

Address correspondence to Jerry L. Kermicle at the address above, or e-mail: kermicle@wisc.edu.

**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*
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 ssp. 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/— 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 tec1, was determinative. In the terminology of Hogenboom (1975), the relation of ga1 pollen on Ga1-s/— pistils, and likewise tec1 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 F1 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 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.
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 Phenotyping of the Pistil

#### 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

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**Table 1**  

<table>
<thead>
<tr>
<th>Stock&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Source</th>
<th>Fertilizes UCIC</th>
<th>Discriminates between UCIC and non-UCIC pollen</th>
<th>Rejects non-UCIC pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCIC = Ga2-s (strong)</td>
<td>Teosinte collections 101&lt;sup&gt;b&lt;/sup&gt; and 104&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ga2-w (weak)</td>
<td>Maize genetic stocks</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Ga2-m (male)</td>
<td>Maize and teosinte</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>non-UCIC = ga2</td>
<td>Maize inbred W22</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> All are ga1/ga1; teb1/teb1.

<sup>b</sup> Zea mays ssp. mexicana, Cocotitlán, Chalco, Edo de Mexico.

<sup>c</sup> Zea mays ssp. parviglumis, Alcholoo, Teloloapan, Guerrero, Mexico.

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**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 B5 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 distal 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 B5. All 3 chromosome-5 long arms of the 5/5/B5 stock carried ga2; the 2 nontranslocated arms carried pr1, whereas B5 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 B5 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/B5 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/B5/B5, 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 B5 transmission. The frequency of function of pollen of the genotype Ga2 pr1/B5/ga2 pr1/B5 in crosses of Ga2 pr1/ga2 pr1/B5 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 B5 chromosome.

The presence of Ga2 in the 5/5/B5 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 F1 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 F1 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, F2 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 F1 plants would function, producing one-fourth compatible F2 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 F1 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
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

**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 “+.”
reciprocal translocations couples the short arm of chromosome 9 with each arm of the other 9 chromosomes. The 9S arm in each case carries the recessive allele of waxy1 (woc1) conferring high amylopectin starch to the kernel's endosperm, giving it a “waxy” appearance. If the major UCIC locus is linked with woc1 through the translocation (T), crosses of woc1 T(non-UCIC)/Woc1(UCIC) onto UCIC woc1 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 Woc1, or woc1 (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 Woc1/woc1 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 UCIC and Ga2, the UCIC-104 stock was pollinated with 2 Ga2 stocks: a colorless kernel (anthocyaninless-2) 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 UCIC-101 pollen in mixtures provided a more sensitive test of relative pollen competence. Complementary 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, F1 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-t, denoting its strong pistil barrier. Similarly, Ga2-w denotes the weak pistil action characteristic of the 2 maize Ga2 genetic stocks.

### Table 2

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Breakpoints</th>
<th>No. of kernels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
<td>Entry</td>
<td>Non-waxy</td>
</tr>
<tr>
<td>T5–9 (022-11)</td>
<td>9L27;35S30</td>
<td>P6039</td>
</tr>
<tr>
<td>T5–9 (4817)</td>
<td>9S05; 5L06</td>
<td>P6041</td>
</tr>
<tr>
<td>T5–9 (a)</td>
<td>9S17;5L09</td>
<td>P6043</td>
</tr>
<tr>
<td>T1–9 (9959)</td>
<td>9S20;1L19</td>
<td>P6046</td>
</tr>
<tr>
<td>T3–9 (8562)</td>
<td>9L22;3L65</td>
<td>P6047</td>
</tr>
<tr>
<td>T4–9 (c)</td>
<td>9L26;4S53</td>
<td>P6048</td>
</tr>
</tbody>
</table>

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

For Ga2-w and Ga2-t to be factors in reproductive isolation, they should be present either in teosinte or maize but not...
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 F1 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 Norteno. Whereas plants of the 3 collections of Cónico were all Ga2, one of Cónico Norteno 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 Zeas, 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 Tbl1 systems of cross-incompatibility, this class is designated Ga2-w, 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

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

<table>
<thead>
<tr>
<th>Pollen parent</th>
<th>UCIC</th>
<th>Ga2</th>
<th>ga2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear parent</td>
<td>Col. 104-3</td>
<td>a2</td>
<td>A2b</td>
</tr>
<tr>
<td>UCIC</td>
<td>88</td>
<td>96</td>
<td>92</td>
</tr>
<tr>
<td>Ga2/Ga2</td>
<td>96</td>
<td>92</td>
<td>96</td>
</tr>
<tr>
<td>A2b</td>
<td>92</td>
<td>98</td>
<td>96</td>
</tr>
<tr>
<td>ga2/ga2</td>
<td>96</td>
<td>92</td>
<td>96</td>
</tr>
</tbody>
</table>

* % of full seed set averaged over 5 crosses.

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

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

<table>
<thead>
<tr>
<th>Collection</th>
<th>Teosinte</th>
<th>Maize</th>
<th>ga2 constitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>State</td>
<td>ssp</td>
<td>Habitat</td>
</tr>
<tr>
<td>101</td>
<td>Mex.</td>
<td>mex.</td>
<td>weedy</td>
</tr>
<tr>
<td>102</td>
<td>Mex.</td>
<td>mex.</td>
<td>weedy</td>
</tr>
<tr>
<td>104</td>
<td>Gro.</td>
<td>par.</td>
<td>wild</td>
</tr>
<tr>
<td>105</td>
<td>Mich.</td>
<td>par.</td>
<td>wild</td>
</tr>
<tr>
<td>106</td>
<td>Mich.</td>
<td>mex.</td>
<td>weedy</td>
</tr>
<tr>
<td>107</td>
<td>Mich.</td>
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</tr>
<tr>
<td>109</td>
<td>Gto.</td>
<td>mex.</td>
<td>r/y</td>
</tr>
<tr>
<td>110</td>
<td>Mex.</td>
<td>mex.</td>
<td>weedy</td>
</tr>
<tr>
<td>201</td>
<td>Chih.</td>
<td>mex.</td>
<td>r/y</td>
</tr>
<tr>
<td>202</td>
<td>Gro.</td>
<td>par.</td>
<td>wild</td>
</tr>
<tr>
<td>203</td>
<td>Jal.</td>
<td>par.</td>
<td>wild</td>
</tr>
<tr>
<td>205</td>
<td>Dgo.</td>
<td>mex.</td>
<td>r/y</td>
</tr>
<tr>
<td>207</td>
<td>Jal.</td>
<td>mex.</td>
<td>r/y</td>
</tr>
</tbody>
</table>

*a* Subspecies: mex., mexicana; par., parviglumis.

*b* Genotype based on ability of F1 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.

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**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 5L06 and 9S07), then testcrossed onto wz1 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 was 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/B5 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/B5 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/B5 ga2 pr1 pollen grains are functional on Ga2 silks. In fact, in some crosses, the heteroallelic 5 Ga2 pr1/B5 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, 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−/− pistils and tcb1 on Tcb1−/− 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−/− and Tcb1−/− silks and vice versa. To test this model, ga1 ga2 Tcb1− or Ga1− ga2 tcb1 or ga1 Ga2− tcb1 pollen was mixed with ga1 ga2 tcb1 and applied to silks of various cross-incompatibility genotypes.

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−, Ga2−, or Tcb1− pollen when applied to the cognate silk genotype (e.g., Ga1− pollen onto Ga1− 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− tcb1 pollen was no more effective than ga1 ga2 tcb1 on Ga1− or Tcb1− silks when the barrier is strong. Similarly, Ga1− ga2 tcb1 and ga1 ga2 Tcb1− were unable to overcome a strong ga1 Ga2− tcb1 silk barrier. However, in the set of crosses involving Mix 5, in which the ga1 Ga2− 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− 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 +), another only pollen competence (−) 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.
Variation in $Ga_2$ Pistil Barrier Strength

Three previous studies concerning $Ga_2$ 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 $Ga_2$ alleles did not account satisfactorily for his data. Subsequent workers identified a linked $ga$ gene, $Ga_{10}$ (Neuffer et al. 1997). The variant $Ga_{10}$ 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 $Ga_1-s$ (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 $Ga_2$. Rather, this procedure focuses on variation at or in the immediate region of the $ga_2$ locus. One allele found in ssp. $mexicana$ possessed a weak pistil barrier ($Ga_2-w$) corresponding to that present in certain maize genetic stocks. Another, the strong pistil barrier $Ga_2-s$, was found only in teosinte. A third, $Ga_2-m$, the allele lacking the pistil barrier but nevertheless competent to fertilize $Ga_2-s/-$ pistils, occurred both in teosinte and Mexican landrace maize. Ten US maize inbreds carried only $ga_2$. This broader range in pistil strength within teosinte parallels that reported generally for single nucleotide polymorphism within teosinte.

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

<table>
<thead>
<tr>
<th>Pistil parents</th>
<th>$ga_2$ tcb1 ga1</th>
<th>$Ga_2$-s tcb1 ga1</th>
<th>$ga_2$ Tcb1-s ga1</th>
<th>$go_2$ tcb1 Ga1-s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mix 1</td>
<td>$ga_2$ : 227</td>
<td>$ga_2$ : 69</td>
<td>No seed</td>
<td>No seed</td>
</tr>
<tr>
<td>Mix 3</td>
<td>$ga_2$ : 148</td>
<td>$ga_2$ : 155</td>
<td>No seed</td>
<td>No seed</td>
</tr>
<tr>
<td>Mix 4</td>
<td>No seed</td>
<td>No seed</td>
<td>0 tcb1 : 76</td>
<td>0 tcb1 : 123</td>
</tr>
<tr>
<td>Mix 6</td>
<td>No seed</td>
<td>0 tcb1 : 170</td>
<td>0 tcb1 : 123</td>
<td>0 tcb1 : 170</td>
</tr>
<tr>
<td>Mix 8</td>
<td>0 tcb1 : 170</td>
<td>No seed</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Mix 5</td>
<td>160 ga1 : 20</td>
<td>17 ga1 : 11</td>
<td>No seed</td>
<td>0 ga1 : 53</td>
</tr>
<tr>
<td>Mix 6</td>
<td>18 ga1 : 43</td>
<td>No seed</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d., not determined.

* Significantly different from one another at $P < 0.01$, Fisher Exact test.

---

**Figure 8.** Genetic models of variation in strength of $Ga_2$ 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 monofactorial system of gametophytic self-incompatibility (Meyer and Saedler 1996). Were this so, silencing of homospecific 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/B5 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, mitigate 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—confers 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 Meda 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 Za genes 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, \( Ga1-s, Ga2-s, Tib1-s \), 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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