Kin recognition within a seed and the effect of genetic relatedness of an endosperm to its compatriot embryo on maize seed development

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As one of two sexual products resulting from double fertilization in angiosperms, the endosperm nourishes its compatriot embryo during seed development and/or germination and ultimately dies. Theoretical studies suggest that the genetic relatedness of an endosperm to its embryo in the same seed might determine the amount of resources ultimately available for the embryo during seed development. We took advantage of the phenomenon of heterofertilization in cultivated maize to empirically test whether genetic relatedness between a triploid embryo-nourishing endosperm and its compatriot diploid embryo impacts the process of resource allocation between these two sexually produced entities. We used genetically distinct maize inbred lines to perform two crossing experiments. Dry weights of dissected embryos and endosperms of mature heterofertilized and adjacent homofertilized kernels were compared. Embryo weight of heterofertilized kernels was significantly less than that of embryos of homofertilized kernels, whereas there was no significant difference in endosperm weight between the two types of kernels. Our results suggest that the degree of genetic relatedness of an endosperm to its compatriot embryo affects seed development and specifically the amount of maternal resources allocated to an endosperm that are eventually turned over to an embryo. The lower the coefficient of relatedness of an endosperm to its compatriot embryo, the smaller the embryo. Thus, the endosperm of a heterofertilized seed appears to behave less cooperatively with respect to resource transfer toward its less closely related embryo compared with the endosperm of a homofertilized seed.

inclusive fitness | interparental conflict | Zea mays

Angiosperms are characterized by a set of unique reproductive features, including double fertilization, in which two sperm cells from a single pollen tube fertilize the egg and the central cell of a female gametophyte to form an embryo and an endosperm, respectively (1). In the vast majority of angiosperms, the endosperm and embryo are genetically identical except for ploidy (2-6). The embryo is diploid, but the endosperm is triploid and is composed of one paternal gametophyte genome and two identical maternal gametophyte genomes that are derived from the two polar nuclei in the central cell (1). In contrast with the embryo, however, the endosperm does not pass its own genes directly to the next generation. Rather, it is consumed by its compatriot embryo during seed development and/or germination (7). This nourishing behavior of an endosperm raises a long-standing question: why would the second genetically biparental product of sexual reproduction “sacrifice” itself for the successful function of the embryo (2, 5, 8-10)?

Hamilton (11, 12) first modeled the evolution of altruistic behavior. His theory of inclusive fitness showed that altruistic behavior toward relatives could be favored by natural selection when the cost to the altruist is compensated by the benefit to those relatives, discounted by the coefficient of relatedness (11, 12). Subsequent theoretical studies extended Hamilton’s theory to consider the endosperms and embryos of flowering plants. Many of these analyses focused on the effects of parent-offspring conflict (conflict among sibling embryos for limited resources from the maternal sporophyte) and/or intersexual conflict (conflict between male and female parents over the investment of limited resources in the seeds of a maternal sporophyte) (2, 3, 5, 8-10, 13-23). A common metric throughout many of these theoretical analyses of conflict is the relatedness ratio, in which the coefficient of relatedness of an endosperm to its own embryo relative to its relatedness to other embryos on a maternal sporophyte is predicted to affect the relative “aggressiveness” of an endosperm to procure resources from the maternal sporophyte on behalf of its own embryo (2, 3, 5, 9, 10).

Perhaps more subtle is the notion of cooperation; that developmental and physiological integration between an endosperm and its compatriot embryo may be very much dependent on their high degree of genetic relatedness (5, 8, 10, 24). Because an endosperm is genetically identical (except for gene dosage) to its compatriot embryo, once resources from the maternal sporophyte have been allocated to a seed, the inclusive fitness of an endosperm should be maximized when it works cooperatively to allocate those resources to its compatriot embryo (5, 10).

Although the last three decades have produced a rich theoretical literature on conflicts and cooperation among the five kinds of genetic entities directly involved in angiosperm reproduction (maternal sporophyte, male gametophyte, female gametophyte, embryo, and endosperm) (3-5, 8-10, 13, 14, 16-18, 22, 23, 25-27), there have been few empirical studies (but see refs. 28, 29) that test the predictions of theoretical models of conflict within the seeds of flowering plants. Moreover, no experimental study has examined the expectation that the degree of cooperation between an endosperm and its compatriot embryo within a seed might be correlated with their degree of genetic relatedness.

Here we take advantage of the naturally occurring phenomenon of heterofertilization in maize (Zea mays ssp. mays), in which the egg and central cell of a female gametophyte within a single ovule are fertilized by sperm cells from two different pollen tubes (30), to experimentally examine the cooperation between an endosperm and its compatriot embryo in terms of resource allocation. Compared with products of homofertilization, the direct consequence of heterofertilization is to decrease the coefficient of relatedness of an endosperm to its compatriot embryo, as well as the “relatedness ratio”: the ratio of an endosperm’s genetic relatedness to its own embryo relative to that to another embryo on the same maternal sporophyte.
relatedness to its own embryo versus its genetic relatedness to other embryos on the same maternal sporophyte (Fig. 1, Table S1). At the same time, the coefficient of relatedness of an endosperm resulting from heterofertilization to embryos in other seeds on a maternal plant is unaltered (Fig. 1, Table S1). Additionally, the coefficient of relatedness of a maternal sporophyte to an endosperm in a heterofertilized seed does not change relative to endosperms in homofertilized seeds (Fig. 1, Table S1). The phenomenon of heterofertilization allowed us to experimentally examine levels of cooperation between an endosperm and its compatriparent embryo within a seed in terms of resource allocation when an endosperm and its compatriparent embryo do not share genetically identical (or even closely related) sperm as sires.

Although heterofertilization may be common among flowering plants, it remains undocumented except in maize, Zea mays ssp. mays, where the use and study of color markers in kernel formation allow for the direct visualization of embryos and endosperms that have been sired by different pollen tubes (30). We used alleles of the R1 locus, which contribute to the production of anthocyanin pigments in the endosperm and embryo (30). In our experimental crosses, a maternal inbred line homozygous for the recessive r1 allele was pollinated with a mixture of pollen from two paternal inbred lines, one of which was homozygous for the dominant R1 allele and the other homozygous for the recessive r1 allele and a line homozygous for the recessive allele. X17F and X17B are inbred lines of the L289 genetic background. X17F is homozygous for the dominant R1 allele, whereas X17B is recessive for the trait (31). W22 and X236M are inbred lines of the same genetic background: X236M is homozygous for the dominant R1-nj allele, in which purple pigment forms in the aleurone layer of the endosperm and the scutellum of the embryo, whereas X17B is recessive for the trait (31). W22 and X236M are inbred lines of the same genetic background: X236M is homozygous for the dominant R1-nj allele, in which purple pigment forms in the aleurone layer of the endosperm and the scutellum of the embryo, whereas X17B is recessive for the trait (31). B73 is homozygous for the r1 allele and was used as the maternal parent in all crosses.

To control for potential effects of the state of the R1 locus and of paternal genetic background on kernel weight, we performed two crosses (Fig. 2). In cross A, B73 plants were pollinated with a mixture of pollen from X17B (L289 carrying the r1 allele) and X236M (W22 carrying the R1-nj). In cross B, B73 plants were pollinated with a mixture of pollen from X17F (L289 carrying the R1-scm3 allele) and W22 (carrying the r1 allele). Kernels were provided by the Maize Genetics Cooperation Stock Center at the University of Illinois, Urbana (31) and grown under standard greenhouse and field conditions (details in SI Text). Standard maize pollen mixture and pollination techniques were used for crosses (details in SI Text). Measurements of Kernel, Embryo, and Endosperm Weight. Each kernel was individually numbered, identified as homofertilized or heterofertilized, and the color of the embryo and endosperm (purple or yellow) was recorded along with location on the cob. Heterofertilized and adjacent homofertilized kernels (groups as described in Fig. S1 and data analysis) were dried at 60 °C for 72 h and weighed. They were then soaked in FAA (paraformaldehyde: anhydrous acetic acid: 50% (vol/vol) alcohol = 1:1:18) for 24 h to prevent germination on rehydration. After rehydration in an ethanol series to distilled water, kernels were dissected into pericarp, embryo, and endosperm;
Results

Phenotypic Features of Kernels. A total of 103 fully developed ears were harvested over 3 y. Four types of kernels were produced in both cross A and B (Fig. 2). The majority of kernels had an endosperm and an embryo of the same color, either YY or PP (Fig. 2), indicating that they are almost certainly the products of homofertilization. YY and PP kernels (Fig. 2) are putatively the products of heterofertilization.

Chromosome Number of Yellow Embryos. In both crosses, YY kernels most likely result from heterofertilization (30). However, such kernels could result from a single fertilization event, in which only the central cell of the female gametophyte fuses with a single sperm with the R1 dominant allele while the egg cell with an r1 recessive allele develops into a haploid embryo through parthenogenesis (35). Because a haploid yellow embryo cannot be distinguished from the diploid double recessive yellow embryo visually, chromosome numbers of 23 randomly selected YY kernels were examined (the methods are described in SI Text). Seventeen seedlings were diploid (2N = 20; Fig. S2A) and resulted from heterofertilization. The remaining six seedlings (23%) were haploid (N = 10; Fig. S2B), indicating that the embryos were parthenogenetic. Because they could contain parthenogenetic embryos, we did not use YY kernels in the weight analyses. YY kernels must have a diploid embryo since the existence of purple pigment in an embryo indicates that the paternal R1 dominant allele is present.

Frequency and Distribution of Heterofertilized YP Kernels. From 103 ears, there were 40,638 kernels, 117 (0.29%) of which had the YY (heterofertilized) phenotype. Kernel number varied significantly among quarters of the cob (χ² = 1,688.27, P < 0.001), but the frequency of heterofertilized kernels did not (χ² = 3.00, P = 0.3914). Equal frequency along the length of the cob suggests that heterofertilization is independent of the length that pollen tubes grow to reach ovules.

Variation of Kernel Weight Among Years. Of the 117 YY kernels, only 82 had all neighboring kernels fully developed and were included in subsequent analyses. Mature kernel weight varied significantly among the 3 y [ANOVA followed by Tukey’s honestly significant difference (HSD); F₂, 243 = 133.33, P < 0.001]. Weights of kernels harvested in the greenhouse in 2007 (mean ± SE = 0.201 ± 0.0069 g) were greater than weights of kernels harvested from the field in 2008 (0.122 ± 0.0019 g, P < 0.05) and 2009 (0.118 ± 0.0020 g, P < 0.05). Kernel weights did not differ between 2008 and 2009 (P = 0.454).

Effects of the Paternal Genetic Background, State of R1 Locus, and Location on Kernel Weight. R1 (the state of the R1 locus) had a significant effect on kernel weight (Table 1); kernels with the dominant R1 allele expressed in the endosperm were heavier. PEn also significantly affected kernel weight, over and above the effect of the R1 allele (Table 1). The mean weight of kernels with endosperm paternal L289 background was greater than kernels with W22 background. There is no evidence for an interaction between PEn and R1 (Table 1). Kernel weight varied significantly with Loc (Table 1), decreasing with distance from the proximal end of a cob. The significant interaction of Loc with R1 (Table 1) suggests that the weight of kernels bearing the R1 allele declined with position at a greater rate than those with the r1 allele. The interaction of PEn with either R1 or Loc is not significant, however the three-way interaction of Loc, R1, and PEn was significant (Table 1). The effect of PEn on kernel weight was greater when endosperms expressed the R1 allele and

Data Analysis. Linear mixed-effects models (lme function from the nlme package of R) (32, 33) were used to examine the effects of the fertilization type (FT) (homofertilization vs. heterofertilization by sperm from pollen of different genotypes), paternal genetic background (L289 vs. W22), and the state of R1 locus (r1 vs. R1), on kernel, embryo, and endosperm weights. Because kernel weight varies with location on a cob, location of kernels (Loc) was included as a covariate in the mixed-effect models (34). A “group” was defined as a focal heterofertilized kernel, for example, with yellow endosperm and purple embryo (YP), and two adjacent homofertilized kernels, one fertilized by the same pollen parent as the embryo of the heterofertilized kernel, for example, purple endosperm and purple embryo (PP) and one fertilized by the same pollen parent as the endosperm of the heterofertilized kernel, for example, yellow endosperm and yellow embryo (YY) (Fig. S1). In a very few cases, the focal heterofertilized kernel was surrounded by only one kind of homofertilized kernel, and a nonadjacent homofertilized kernel at a similar distance from the proximal end of the same cob was selected for the group. Fertilization type (FT), paternal genetic background of an embryo (PEn), paternal genetic background of an endosperm (PEn), state of R1 locus (R1), and location of kernel (Loc) were fixed effects, and group, cob, and year were random effects in the analyses.

We ran separate models for whole kernel, embryo, and endosperm weights. For whole kernel weight, we examined the fixed effects of Loc, PEn, R1 in endosperm, and several interaction terms. The model for embryo weight analysis included Loc, FT, PEn, R1, and interactions. For endosperm weight, the model included Loc, FT, PEn, R1, and interaction terms. Data from PY kernels were excluded from analyses (see results of embryo chromosome counts for explanation); therefore, the interactions between R1 and FT were excluded from the models. Data and coding for analyses are in Dataset S1.
analyses. Degrees of freedom (PEn) on whole kernel weight. Type III sums of squares were used in the R1 in endosperm, and paternal genetic background of the endosperm *b*ryo*sm* with the L289 genetic background than for embryos with fertilized (YP) kernels, after controlling for Loc, PEn, R1, and interactions.

The effect of FT on endosperm weight was not significant for FT (0.269) divided by the intercept (8.314); Table 2. The effect of FT on endosperm weight was not significant (Table 3); mean endosperm weight of homofertilized (PP and YY) kernels was not significantly different from that of heterofertilized (YP) kernels, after controlling for Loc, PEn, R1, and interactions.

There was no evidence that the PEm affected embryo weight (Table 2), however the difference in embryo weight between homofertilized and heterofertilized kernels was greater for embryos with the L289 genetic background than for embryos with the W22 background (significant FT × PEm; Table 2). R1 and Loc had no significant effect on embryo weight and no other interactions were significant (Table 2). The paternal genetic background of the endosperm (PEn) and R1 significantly affected endosperm weight (Table 3). After controlling for other factors, the average weight of an L289 endosperm was greater than that of W22 kernels (38). The finding is consistent with previous studies showing that the expression of the R gene family results in large kernels (43). The absence of an interaction between PEn and R1 (Table 1) suggests that these two factors act additively, but independently, on mature kernel weight. In summary, differences in resource allocation to kernels are related to the specific genotypes of the parents and/or to the carrier of the R1 color marker. Our crossing design and statistical model allowed us to account for these factors (as well as kernel location and year) and isolate the effect of genetic relatedness of embryo and endosperm on resource allocation within kernels.

**Discussion**

Endosperm is typically viewed as an entity that behaves cooperatively with, and provides benefits to, its genetically identical compatriot embryo during seed development (5, 8, 10, 24). Furthermore, theoretical models suggest that the coefficient of relatedness of an endosperm to its compatriot embryo may underlie patterns of resource allocation and embryo-nourishing behavior of endosperm (10). We asked if allocation of resources within a kernel was significantly affected by decreasing the genetic relatedness of an endosperm to its compatriot embryo. We found that embryos of heterofertilized kernels, in which the embryo and endosperm do not share sires, were smaller than their counterparts in homofertilized kernels, whereas endosperm weight did not differ significantly. Hence, the endosperm of a heterofertilized kernel appears to behave less cooperatively with respect to allocation of resources to its embryo than does the endosperm of a homofertilized kernel.

**Frequency of Heterofertilization.** Two types of heterofertilized kernels were formed: YP and PY (Fig. 2). However, 25% of PY kernels had haploid embryos and were the result of parthenogenesis rather than heterofertilization; that is, only the central cell fused with a sperm cell to form a triploid endosperm (35). Assuming that the occurrence of heterofertilized PY kernels was similar to the 0.29% observed for YP kernels, the overall frequency of detectable heterofertilization events in our crosses is ∼0.58%. The frequency of heterofertilization identifiable by color marker underestimates the true frequency because kernels in which an egg and central cell fuse with sperm cells from two different pollen grains with the same state of the R1 locus are not morphologically distinguishable from homofertilized kernels. If the frequency of heterofertilization by pollen grains of the same genotype is similar to the frequency of detectable heterofertilization, then the actual frequency in our crosses may have been ∼1%. This estimate is consistent with previous reports in maize, in which the frequency of heterofertilization events ranged from 0.5 to 1%, and rarely up to 5% (31, 36–38).

**Growth Condition, Paternal Genetic Background, and the R1 Dominant Allele Affect Kernel Weight.** The mean weight of kernels harvested in the greenhouse was greater than that of kernels from the field. Kernels harvested in the greenhouse in a 3-mo grain-filling period under relatively constant conditions. Plants growing in the greenhouse in 2008 and 2009 had an approximately 2-mo grain-filling period and experienced low temperatures at the end of the growing season. Kernel weight is significantly affected by environmental conditions that alter the duration of seed development (39, 40). Because the duration of the grain-filling stage is positively correlated with mature kernel weight (41), the difference in kernel weight among years is likely due to growing conditions.

Genetic background is well known to affect mature kernel weight in maize (42). Our results show that the weight of kernels and endosperms with the L289 paternal genome was significantly greater than those carrying the W22 parental genome (Tables 1 and 3). Furthermore, we found that the R1 allele increased kernel and endosperm weight (Tables 1 and 3). This finding is consistent with previous studies showing that the expression of the R gene family results in large kernels (43). The absence of an interaction between PEn and R1 (Table 1) suggests that these two factors act additively, but independently, on mature kernel weight. In summary, differences in resource allocation to kernels are related to the specific genotypes of the parents and/or to the carrier of the R1 color marker. Our crossing design and statistical model allowed us to account for these factors (as well as kernel location and year) and isolate the effect of genetic relatedness of embryo and endosperm on resource allocation within kernels.

Table 1. Whole kernel weight

| Source | Regression coefficient ± SE | t-value | Pr > |t| |
|--------|-----------------------------|---------|------|---|
| Intercept | 129.94 ± 3.436 | 37.8 | 0.0 |
| Loc | −0.239 ± 0.070 | −3.43 | 0.0008*** |
| R1 | 1.458 ± 0.364 | 4.00 | 0.0001*** |
| PEn | −2.695 ± 1.323 | −2.04 | 0.043*** |
| R1 × PEn | 3.049 ± 2.463 | 1.23 | 0.2176 |
| Loc × R1 | −0.030 ± 0.014 | −2.13 | 0.0345* |
| Loc × PEn | −0.050 ± 0.030 | −1.68 | 0.0954 |
| Loc × R1 × PEn | 0.133 ± 0.047 | 2.84 | 0.0052** |

Mixed effects model analysis of the effects of location (Loc), the state of the R1 in endosperm, and paternal genetic background of the endosperm (PEn) on whole kernel weight. Type III sums of squares were used in the analyses. Degrees of freedom = 1, 157, calculated according to ref. 33. *P < 0.05; **P < 0.01; ***P < 0.001.

Source Regression coefficient ± SE t-value Pr > |t| |
| Intercept | 8.314 ± 0.331 | 25.12 | 0.0 |
| Loc | −0.003 ± 0.012 | −0.25 | 0.7999 |
| FT | −0.269 ± 0.111 | −2.42 | 0.0168* |
| PEm | 0.092 ± 0.056 | 1.65 | 0.1002 |
| R1 | 0.043 ± 0.056 | 0.76 | 0.4466 |
| FT × PEm | 0.241 ± 0.111 | 2.17 | 0.0314* |
| Loc × FT | 0.004 ± 0.004 | 1.02 | 0.3081 |
| Loc × PEm | 0.001 ± 0.002 | 0.46 | 0.6478 |
| Loc × R1 | −0.002 ± 0.002 | −1.12 | 0.2635 |
| Loc × FT × PEm | 0.002 ± 0.004 | 0.53 | 0.5985 |

Mixed effects model analysis of the effects of location (Loc), FT (different paternal genetic backgrounds in embryo and endosperm), state of the R1 locus, and PEm on embryo weight. *P < 0.05.
Table 3. Endosperm weight

| Source          | Regression coefficient ± SE | t-value | Pr > |t| |
|-----------------|-----------------------------|---------|------|---|
| Intercept       | 110.92 ± 3.236              | 34.28   | 0.0  |
| Loc             | -0.233 ± 0.118              | -1.96   | 0.0517 |
| FT              | -0.266 ± 1.171              | -0.23   | 0.7571 |
| Pen             | -1.594 ± 0.588              | -2.71   | 0.0074** |
| R1              | 1.991 ± 0.588               | 3.39    | 0.0009*** |
| FT x Pen        | 0.893 ± 0.170               | 0.76    | 0.4465 |
| Loc x FT        | -0.008 ± 0.046              | -0.16   | 0.8697 |
| Loc x Pen       | 0.027 ± 0.023               | 1.19    | 0.2375 |
| Loc x R1        | -0.044 ± 0.023              | -1.91   | 0.0580 |
| Loc x FT x Pen  | -0.058 ± 0.045              | -1.29   | 0.1995 |

Mixed effects model analysis of the effects of location (Loc), FT (different paternal genetic backgrounds in embryo and endosperm), state of the R1 locus of the endosperm, and paternal genetic background of the endosperm (Pen) on endosperm weight. *P < 0.05; **P < 0.01; ***P < 0.001.

Coefficient of Relatedness Affects Embryo, but Not Endosperm Weight. A maternal sporophyte is always equally related to the endosperms in each of its seeds (8, 10). Even when heterofertilization occurs, the relatedness of the maternal sporophyte to the endosperm is not altered (assuming that the pollen donors are each unrelated to the maternal sporophyte) compared with endosperms in homofertilized kernels (Fig. L4, Table S1). In our experiments, we detected no significant difference in endosperm weight between homofertilized and heterofertilized kernels, when controlling for Loc, R1, and Pen (Table 3). This indicates that allocation of maternal resources to endosperms is not affected by the altered genetic relationship of embryo and endosperm resulting from heterofertilization (Fig. L4).

In contrast, in our crosses of maize inbred lines, the coefficient of relatedness of an endosperm to its compatriot embryo decreases from 1 in homofertilized kernels to 2/3 in heterofertilized kernels (Fig. L4, Table S1). This lower coefficient of relatedness was associated with decreased embryo weight; embryos in heterofertilized kernels were significantly smaller than embryos of adjacent homofertilized kernels (Table 2). Given that the endosperms of homofertilized and heterofertilized kernels were of equal size, and presumably had equivalent resources to allocate, transfer from the endosperm to its compatriot embryo during seed development is reduced when the two entities do not share a genetically identical sire.

Taken all together, our data suggest that the endosperm in a heterofertilized kernel is less cooperative with its compatriot embryo compared with endosperms in homofertilized kernels (Fig. L4). The difference in embryo weight between homofertilized and heterofertilized kernels, coupled with no change in endosperm weight, also provides a tantalizing hint that the “dialogue” between an endosperm and maternal sporophyte is not affected by the internal dynamics of the relatedness of an endosperm to its compatriot embryo within an individual seed.

Relatedness Ratio Does Not Predict Endosperm Aggressiveness. The relatedness of an endosperm to its compatriot embryo, relative to its relatedness to other embryos on a maternal sporophyte (“kinship ratio” or “relatedness ratio”), has been viewed as indicative of the degree to which an endosperm should aggressively garner nutrients from the maternal sporophyte (Fig. 1B) (3–5, 9, 10). The relatedness ratio is calculated as \( r_{\text{En} \rightarrow \text{CEm}} / r_{\text{En} \rightarrow \text{OEm}} \), where \( r_{\text{En} \rightarrow \text{CEm}} \) is the relatedness of an endosperm (En) to its compatriot embryo (CEm) and \( r_{\text{En} \rightarrow \text{OEm}} \) is the relatedness of the same endosperm (En) to an embryo in another adjacent seed (other embryo, OEm) on the same maternal sporophyte (Fig. 1B, Table S1). The larger this ratio is, the more aggressively an endosperm is predicted to behave in procuring resources from the maternal sporophyte on behalf of its compatriot embryo within a seed (2, 3, 5, 9, 10).

For inbred maize lines, virtually all loci are homozygous (44, 45). Therefore, in our crossing experiments, the coefficient of relatedness of a homofertilized endosperm to its compatriot embryo (YY or PP), \( r_{\text{En} \rightarrow \text{CEm}} \), is 1 (Table S1). For a heterofertilized endosperm, the coefficient of relatedness to its compatriot embryo (YP or PY), \( r_{\text{En} \rightarrow \text{CEm}} \), is 2/3 (Table S1). In addition, because our experimental design used two unrelated inbred lines as simultaneous pollen sources, the coefficient of relatedness of an endosperm to embryos of adjacent kernels differs depending on the origin of their paternal contributions. For instance, the coefficient of relatedness of an endosperm (e.g., YY) to an embryo in an adjacent kernel with the same father (e.g., YY), \( r_{\text{En} \rightarrow \text{OEm}} \), is 1. The coefficient of relatedness of an endosperm (e.g., YY) to an embryo in an adjacent kernel derived from an unrelated father (e.g., PP), \( r_{\text{En} \rightarrow \text{OEm}} \), is 2/3 (Table S1).

Thus, the relatedness ratio, \( r_{\text{En} \rightarrow \text{CEm}} / r_{\text{En} \rightarrow \text{OEm}} \), of an endosperm in a homofertilized kernel to the embryo of an adjacent kernel with the same father (e.g., YY vs. YY) is 1, and to an embryo of an adjacent kernel with an unrelated father (e.g., YY vs. PP) is 3/2 (Table S1). In contrast, the relatedness ratio of an endosperm of a heterofertilized kernel to the embryo of an adjacent kernel with the same father (e.g., YP vs. PP) is 2/3, and to an embryo of an adjacent kernel with an unrelated father (e.g., YP vs. YY) is 1 (Table S1). Overall, the relatedness ratio for an endosperm derived from heterofertilization is lower than that of an endosperm derived from homofertilization (Fig. 1B, Table S1). Hence, the endosperm of a homofertilized kernel should favor its compatriot embryo (be more selfish with respect to garnering resources from the maternal sporophyte) at the expense of embryos of neighboring kernels more strongly than an endosperm in a heterofertilized kernel. Accordingly, inclusive fitness analysis suggests that endosperms in heterofertilized kernels will garner fewer resources from the maternal sporophyte, resulting in a smaller size compared with endosperms of homofertilized kernels (Fig. 1B). Our results, however, show that endosperms of heterofertilized kernels were not significantly smaller than those of adjacent homofertilized kernels (Table 3) and provide no support for the hypothesis that the endosperm relatedness ratio influences the aggressiveness of endosperms in procuring maternal resources in maize.

In fact, because heterofertilized kernels are rare, they will always be surrounded by many homofertilized kernels. Thus, the endosperm of each heterofertilized kernel is always competing against endosperms of many homofertilized kernels for maternal resources (rather than against a single one). This situation would appear to favor a finding that heterofertilized endosperms will be smaller than homofertilized endosperms if the relatedness ratio (inter-kernel effects, Fig. 1B) affected resource acquisition by endosperms in maize. Seen in this light, the lack of an effect is an important finding.

Does Embryo Size Affect Fitness? The lower genetic relatedness of an endosperm to its compatriot embryo in a heterofertilized kernel results in a smaller embryo but comparably sized endosperm, compared with the constituents of a homofertilized kernel. Whether the degree of relatedness of an endosperm and embryo truly correlates with the extent of their physiological and developmental cooperation and ultimately with the fitness of embryos/seeds that result from heterofertilized and homofertilized kernels remains unknown. Our data certainly provide an intriguing first set of insights vis-à-vis embryo size at seed/fruit maturity. However, a question yet to be resolved is whether the smaller embryos of heterofertilized kernels ultimately are less fit than the embryos resulting from homofertilization.
Due to the rarity of heterofertilization events and the need to destructively sample all heterofertilized kernels for weight analyses, we were not able to examine components of fitness (e.g., germination, growth, and/or survival rates) for embryos of heterofertilized kernels. Regrettably, we found no published information on the relationship between embryo size and fitness or yield in maize. Seed size is a critical determinant of fitness in many species, affecting germination probability, seedling performance, and survival (46–49). However, the contribution of embryo size to fitness in these studies is unclear, although many of these reports involved taxa with exalbuminous seeds, for which the embryo is the major component.

The smaller embryos of heterofertilized maize kernels might yield less fit adult plants compared with the embryos of homo-fertilized kernels. Alternatively, endosperm weights of heterofertilized and homofertilized kernels are not significantly different and embryos of both kinds of kernels might eventually gain equal access to comparable amounts of stored resources during the germination process. In this case, even though embryos of heterofertilized kernels are smaller at seed/fruit dormancy, these embryos might reach the same developmental stage (dry weight, number of leaves, or some other proxy) as embryos of homofertilized kernels by the time the seedlings became fully autotrophic. Even if the developmental difference detected in the dry weight of embryos in heterofertilized and homofertilized seeds at the time of dormancy carried through to the process of seedling establishment, the differences in weight may not have any ultimate fitness consequences.

If the fitness of heterofertilized embryos were eventually not to differ from that of homofertilized embryos, our present results would suggest that the genetic and developmental interactions between endosperm and embryo in maize are significant, but not evolutionarily meaningful. As tempting as it is to conclude that the degree of cooperation between an endosperm and its compact embryo has been shaped by the degree of genetic relatedness and selection, only further (extremely large) experiments will be able to conclusively answer the question of whether the decreased relatedness of endosperm and embryo that results from heterofertilization has a significant effect on fitness. For now, we have provided tangible data to address the longstanding predictions of inclusive fitness theory and the behaviors and interactions of maternal sporophytes, embryos, and endosperms.

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