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Genetic control of the *opaque-2* gene and background polygenes over some kernel traits in maize (*Zea mays* L.)

Xiangyang Lou[1], Jun Zhu[1,*], Quande Zhang[1], Rongchun Zang[1], Yibo Chen[2], Zhongliang Yu[2] & Yijun Zhao[2]
[1]*College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, Zhejiang 310029, People's Republic of China;* [2]*Zhejiang Dongyang Maize Research Institute, Dongyang, Zhejiang 322100, People's Republic of China; *Author for correspondence (E-mail: jzhu@zju.edu.cn)*

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Abstract

Some kernel traits of agronomical importance in maize are affected by the *opaque*-2 (*o*2) gene and background polygenes, which express in different genetic systems such as embryo, endosperm, cytoplasm and maternal plant. A genetic model for seed quantitative traits with the *o*2 gene effects and polygenic effects as well as their *GE* interactions was used for protein content, lysine content, oil content and kernel density in maize. The results suggested that the *o2* gene was involved in the traits investigated but the effects of the *o2* gene were distinctive on various traits. The effects of the *o2* gene were large on lysine content and protein content while minor on oil content. There was a substantially wide quantitative variation from polygenes expressing in different genetic systems for the traits evaluated. Significant *GE* interactions of the *o2* gene and background polygenes declared that not only the main effects but also specific expressions depending on environments were responsible for variation of the traits studied. There seemed to have strong maternal heterosis and slight embryo heterosis for kernel density.

Introduction

Maize (*Zea mays* L.) is one of the world's staple cereal crops and an important source of dietary calories and protein consumed by human and animals (Denić, 1983). However, it has a relatively low nutritional value to non-ruminant animals, because of the deficiency in the essential amino acids (lysine and tryptophan) in ordinary maize grains (Bressani, 1991). The discovery concerning the biochemical effects of the *opaque-2* (abbr. *o2*) gene (Mertz et al., 1964) aroused considerable interests among maize breeders. The *o2* mutation has been intensively investigated and utilized in breeding and genetic analysis since then. As a result, breeding programs were initiated to upgrade the protein quality of maize endosperm by enhancing the levels of lysine and tryptophan. Besides its practical importance in breeding, this finding is also of fundamental interest in the studies of gene action in protein synthesis.

It is well known that the *o2* gene has numerous pleiotropic effects and affects many traits of agronomic importance. The *o2* mutation was first described by Jones and Singleton in the early 1920s, regarding the soft texture and opaque phenotype of this mutant kernel (Emerson et al., 1935). In addition to its influence on endosperm texture and amino acid balance, the *o2* gene had been associated with many kernel physical and chemical changes (Mertz et al., 1964; Lambert et al., 1969; Misra et al., 1972; Arnold et al., 1974; Vasal et al., 1980; Denić, 1983; Damerval and Vienne, 1993; Landry et al., 2002). Some undesirable effects, e.g., low kernel density, reduced grain weight, poor kernel appearance, slow drydown, high susceptibility to insects,

pathogens and mechanical breakage, etc., had jeopardized its exploitation (Wessel-Beaver and Lambert, 1982; Ortega and Bates, 1983; Yau et al., 1999).

It was also reported that those serious drawbacks are also controlled by polygenes. Vasal et al. (1980) demonstrated that it is possible to overcome the adverse effects of the *o2* gene through selection for favorable polygenes, designated *o2* modifiers. Modifier genes that cause the formation of a hard, vitreous endosperm, first reported by Paez et al. (1969), allow the development of modified *o2* genotypes, which roughly resemble normal maize in kernel phenotype and agronomic performance while retaining the improved protein quality. These kinds of modified *o2* genotypes are generally called quality protein maize (QPM). Breeders have achieved some inspiring success in the development of hard endosperm *o2* maize. Many studies showed that the *o2* modifiers are a fairly complex genetic system with additive, dominant and recessive gene actions as well as xenia and cytoplasm effects involved in endosperm modification (Vasal et al., 1980; Wessel-Beaver and Lambert, 1982; Wessel-Beaver et al., 1985; Yau et al., 1999). In certain genetic backgrounds, endosperm modification appears to be influenced by environments although the factors responsible for the unstable phenotype are poorly understood (Lopes and Larkins, 1995; Pixley and Bjarnason, 2002). Apart from their effects on kernel appearance, other concomitant changes connected with modifiers in protein content, protein components, protein quality, kernel weight and density, etc., were also recognized (Paez et al., 1969; Vasal et al., 1980; Ortega and Bates, 1983; Wallace et al., 1990; Geetha et al., 1991; Paulis et al., 1993; Lambert and Chung, 1995). Furthermore, the actions were also observed on such kernel traits resulted from other background polygenes (Alexander, 1975; Zuber et al., 1975; Lopes and Larkins, 1995) and environmental conditions such as soil nitrogen level and irrigation (Alexander, 1975; Rendig and Broadbent, 1979; Kniep and Mason, 1991).

Most of the previously reported results concerning the inheritance mechanism of the *o2* gene or polygenes were concluded from simple comparisons between a particular set of genetic entries such as isogenic lines or reciprocal crosses and also considered only a single variation source, either maternal plant, embryo or cytoplasm. In the present experiment, we used a set of maize accessions as parents to produce an NC Design II (Comstock and Robinson, 1952), and then employed a seed genetic model to dissect phenotypic variation into the corresponding causal components due to the allele substitution at the *o2* locus, difference of polygenes and environments for 4 kernel traits in maize. This study aimed at assessing the potential capability of these factors to our QPM breeding and at providing a good understanding of genetic control over kernel traits in *o2* maize.

Materials and methods

Genetic model and statistical procedure

In the situation where the inheritances of kernel traits are controlled by a major gene as well as by polygenes, there needs a seed genetic model along with major gene effects. Assuming (1) diploid organism with triploid endosperm possessing regular gene segregation; (2) different sources of genetic variation (e.g., embryo, endosperm, maternal tissue or plant and cytoplasm) contributing independently of one another to the phenotypic variation; (3) cytoplasm genes transmitted only through female; (4) no epistases among the single gene and polygenes; and (5) no higher order interaction between alleles of endosperm polygenes, the phenotypic mean of the k-th genetic entry in replication l within environment h derived from maternal line i and paternal line j, can be represented by the following linear model:

$$y_{hijkl} = \mu + E_h + G_{ijk} + GE_{hijk} + e_{hijkl}$$

where μ is the fixed population mean, E_h is the effect of macro-environment h (e.g., year, location, etc.), fixed or random (determined by context of data) and is random in most genetic experiments, $E_h \sim (0, \sigma_E^2)$, $e_{hijkl} \sim (0, \sigma_e^2)$ is the residual effect, G_{ijk} is the total genetic main effect, and GE_{hijk} is the total interaction effect between genotype and environment.

Genotypic value G_{ijk} and GE interaction effect GE_{hijk} can be further decomposed as follows: Parent Pi ($k = 0$),

$$G_{ii0} = S_{www} + 2A_i + D_{ii} + 3Ae_i + 3De_{ii} + C_i + 2Am_i + Dm_{ii}$$

$$GE_{hii0} = SE_{hwww} + 2AE_{hi} + DE_{hii} + 3AeE_{hi} + 3DeE_{hii} + CE_{hi} + 2AmE_{hi} + DmE_{hii}$$

F_{1ij} ($k = 1$) from maternal parent i mating to paternal parent j,

$$G_{ij1} = S_{wwz} + A_i + A_j + D_{ij} + 2Ae_i + Ae_j + De_{ii} + 2De_{ij} + C_i + 2Am_i + Dm_{ii}$$

$$GE_{hij1} = SE_{hwwz} + AE_{hi} + AE_{hj} + DE_{hij} + 2AeE_{hi} + AeE_{hj} + DeE_{hii} + 2DeE_{hij} + CE_{hi} + 2AmE_{hi} + DmE_{hii}$$

F_{2ij} ($k = 2$) selfed from F_{1ij},

$$G_{ij2} = 0.25S_{www} + 0.25S_{wwz} + 0.25S_{zzw} + 0.25S_{zzz} + A_i + A_j + 0.25D_{ii} + 0.25D_{jj} + 0.5D_{ij} + 1.5Ae_i + 1.5Ae_j + De_{ii} + De_{jj} + De_{ij} + C_i + Am_i + Am_j + Dm_{ij}$$

$$GE_{hij2} = 0.25SE_{hwww} + 0.25SE_{hwwz} + 0.25SE_{hzzw} + 0.25SE_{hzzz} + AE_{hi} + AE_{hj} + 0.25DE_{hii} + 0.25DE_{hjj} + 0.5DE_{hij} + 1.5AeE_{hi} + 1.5AeE_{hj} + DeE_{hii} + DeE_{hjj} + DeE_{ij} + CE_{hi} + AmE_{hi} + AmE_{hj} + DmE_{hij}$$

BC_{1ij} ($k = 3$) ($F_{1ij} \times P_i$):

$$G_{ij3} = 0.5S_{www} + 0.5S_{zzw} + 1.5A_i + 0.5A_j + 0.5D_{ii} + 0.5D_{ij} + 2Ae_i + Ae_j + 1.5De_{ii} + 0.5De_{jj} + De_{ij} + C_i + Am_i + Am_j + Dm_{ij}$$

$$GE_{hij3} = 0.5SE_{hwww} + 0.5SE_{hzzw} + 1.5AE_{hi} + 0.5AE_{hj} + 0.5DE_{hii} + 0.5DE_{hij} + 2AeE_{hi} + AeE_{hj} + 1.5DeE_{hii} + 0.5DeE_{hjj} + DeE_{hij} + CE_{hi} + AmE_{hi} + AmE_{hj} + DmE_{hij}$$

BC_{2ij} ($k = 4$) ($F_{1ij} \times P_j$):

$$G_{ij4} = 0.5S_{zzz} + 0.5S_{wwz} + 0.5A_i + 1.5A_j + 0.5D_{jj} + 0.5D_{ij} + Ae_i + 2Ae_j + 0.5De_{ii} + 1.5De_{jj} + De_{ij} + C_i + Am_i + Am_j + Dm_{ij}$$

$$GE_{hij4} = 0.5SE_{hzzz} + 0.5SE_{hwwz} + 0.5AE_{hi} + 1.5AE_{hj} + 0.5DE_{hjj} + 0.5DE_{hij} + AeE_{hi} + 2AeE_{hj} + 0.5DeE_{hii} + 1.5DeE_{hjj} + DeE_{hij} + CE_{hi} + AmE_{hi} + AmE_{hj} + DmE_{hij}$$

where S_{wwz} is the endosperm genotypic effect at the $o2$ locus, and w is the allele carrying by maternal parent i ($w = O2$ or $o2$) and z is the allele carrying by paternal parent j at the locus ($w = z$ if $i = j$), $A_i \sim (0, \sigma_A^2)$ is the embryo additive effect, $D_{ii} \sim (0, \sigma_D^2)$ is the embryo dominance effect, $Ae_i \sim (0, \sigma_{Ae}^2)$ is the endosperm additive effect, $De_{ii} \sim (0, \sigma_{De}^2)$ is the endosperm dominance effect, $C_i \sim (0, \sigma_C^2)$ is the cytoplasm effect, $Am_i \sim (0, \sigma_{Am}^2)$ is the maternal additive effect, $Dm_i \sim (0, \sigma_{Dm}^2)$ is the maternal dominance effect, $SE_{hwww} \sim (0, \sigma_{SE}^2)$ is the $o2$ gene genotype × environment interaction, $AE_{hi} \sim (0, \sigma_{AE}^2)$ is the embryo additive × environment interaction, $DE_{hii} \sim (0, \sigma_{DE}^2)$ is the embryo dominance × environment interaction, $AeE_{hi} \sim (0, \sigma_{AeE}^2)$ is the endosperm additive × environment interaction, $DeE_{hii} \sim (0, \sigma_{DeE}^2)$ is the endosperm dominance × environment interaction, $CE_{hi} \sim (0, \sigma_{CE}^2)$ is the cytoplasm × environment interaction, $AmE_{hi} \sim (0, \sigma_{AmE}^2)$ is the maternal additive × environment interaction, $DmE_{hii} \sim (0, \sigma_{DmE}^2)$ is the maternal dominance × environment interaction.

Mixed linear model approaches were used for the statistical analysis (Lou & Zhu, 2002). MINQUE (1), a MINQUE (minimum norm quadratic unbiased estimation) (Rao, 1971) method with 1 for all prior variance components, was used for estimating variance components, the ordinary least squares method for estimating fixed gene effects while linear unbiased prediction method (LUP, Zhu and Weir, 1994) for predicting random effects. The method suggested by Zhu (1997) was used to calculate the components of phenotypic variance. The jackknife procedure (Miller, 1974) using replicate as the sampling unit was employed to estimate the sampling variances of the estimated parameters and the predicted effects, and Student's t statistics to test the significance of them. The computation was completed on an IBM Pentium III 500 computer by a piece of software written in C language.

Table 1. Parents and their origins used in this experiment

Code	Parent[a]	Endosperm texture	Origin
1	LCZ01 *o2*	Semihard	Zhejiang Maize Research Institute
2	Qi205 *o2*	Semihard	Shandong Academy of Agricultural Sciences
3	LCZ02 *o2*	Soft	Zhejiang Maize Research Institute
4	LCZ05 *o2*	Soft	Zhejiang Maize Research Institute
5	Zhongxi017 *o2*	Soft	The Chinese Academy of Agricultural Sciences
6	Qi302	Normal	Shandong Academy of Agricultural Sciences
7	92-213	Normal	Zhejiang Maize Research Institute
8	LCZ04 *o2*	Soft	Zhejiang Maize Research Institute
9	Zhongxi091 *o2*	Soft	The Chinese Academy of Agricultural Sciences
10	Va36 *o2*	Semihard	United States of America
11	*O2*-4-10	Soft	Shanxi Academy of Agricultural Sciences
12	Yin85-6 *o2*	Semihard	Zhejiang Maize Research Institute
13	LCZ03 *o2*	Soft	Zhejiang Maize Research Institute
14	92–1282	Normal	Zhejiang Maize Research Institute
15	92–1461	Normal	Zhejiang Maize Research Institute

[a] Yin85-6 *o2* was bred from tropical exotic germplasm.

Experimental design and trait measurement

Fifteen maize inbred lines (see Table 1), sampled at random from the inbred line populations of ordinary maize and high lysine (*o2*) maize in Zhejiang Province, were used as parents and planted on Zhejiang University Experimental Farm in the autumn of 1994. An NC Design II (7×8) was constructed with irregularly missing combinations to generate 26 hybrid F_1's and 24 reciprocal cross RF_1's. The parental lines and their F_1's, RF_1's were grown at two locations, Zhejiang Agricultural University Experimental Farm and Zhejiang Dongyang Maize Research Institute, in the spring of 1995. The parents and hybrids were arranged in separated blocks in which entries were placed in a completely randomized design to avoid competitive effects. Four-row plots were used with 11.5 m long and 2.67 m wide, each row containing 39 plants, thus providing a stand of approximately 50,000 plants/ha. The cultivated practice and routine field management were similar to those of the farm production in Zhejiang Province. The F_2's, RF_2's and backcrosses to both parents (i.e., BC_1's and BC_2's) were made for each F_1 or RF_1 cross during that summer. To minimize difference of seed quality, all parents, F_1's and RF_1's were also reproduced at the same season. More than 3 well pollinated and fully developed ears were harvested for each genotype at physiological maturity. After naturally dried, the ears were shelled and threshed by hand and grains collected from the middle portion of them.

Kernels were dried to a balanced and approximately uniform moisture amount by an oven. Measurements were made based on a sample of bulked 200 kernels from each genotype. Kernel density was determined using the method suggested by Wessel-Beaver et al. (1984) and expressed as the amount of gram per milliliter (g/ml). Kernels were ground in a cyclone mill to fine meal passing through a 60-mesh screen. The dye binding lysine (DBL) method (Hurrell et al., 1979) was used for measuring lysine content. Protein content was tested by Lowry method (Lowry et al., 1951). Oil content was scored by the Soxhlet's extraction procedure (AOAC, 1984). Amounts of protein, lysine and oil were measured on a basis of whole kernel. Protein and oil contents were expressed as percentage and lysine content as permillage. Two replicates were measured for all experimental entries.

Results

Population mean, the genotype effects and GE interaction of the o2 gene

The estimation results of population mean and four endosperm genotypic effects at the *o2* locus

Table 2. Estimates of population mean and the *o2* gene genotype effects for lysine content, protein content, oil content and density

Genetic parameter[a]	Lysine content (‰)[b]	Protein content (%)	Oil content (%)	Density
μ	3.052**	11.262**	4.184**	1.062**
+ + +	−0.279**	0.965**	0.051*	−0.017
+ + −	−0.319*	−0.155	0.067	0.025
+ − −	−0.068	−0.182	−0.233**	0.036**
− − −	0.666**	−0.629**	0.114*	−0.045*

[a] μ, + + +, + + −, + − − and − − − are population mean, triplex (wild type, *O2O2O2*) effect, duplex (*O2O2o2*) effect, simplex (*O2o2o2*) effect and nulliplex (*o2o2o2*) effect, respectively.
[b] * and ** are significant at 0.05 and 0.01 levels, respectively.

Table 3. Estimates of phenotypic variance and its components for lysine content, protein content, oil content and density

Variance[a]	Lysine content	Protein content	Oil content	Density ($\times 10^{-2}$)
V_E	0.000	0.001	0.094	0.000
V_A	0.614**	1.042*	0.000	0.039
V_D	0.000	0.000	0.000	0.199**
V_{Ae}	0.000	0.000	0.699	0.000
V_{De}	0.410**	0.105	0.153	0.000
V_{Am}	0.148**	1.071*	0.000	0.120
V_{Dm}	0.017	0.000	0.000	0.229**
V_C	0.000	0.000	0.000	0.000
V_{SE}	0.002	0.362**	0.000	0.004
V_{AE}	0.000	3.344**	1.727**	0.446**
V_{DE}	0.116*	0.515	0.000	0.025
V_{AeE}	0.004	0.000	0.000	0.000
V_{DeE}	0.000	0.219*	1.067*	0.293*
V_{AmE}	0.083*	0.704**	1.052**	0.191**
V_{DmE}	0.046**	2.647**	0.161	0.210**
V_{CE}	0.209**	1.551**	0.378*	0.201**
V_e	0.078**	0.883*	0.283	0.078**
V_P	1.727**	12.443**	5.613**	2.034**

[a] Variances represent: V_E = environment, V_A = embryo additive, V_D = embryo dominance, V_{Ae} = endosperm additive, V_{De} = endosperm dominance, V_{Am} = maternal additive, V_{Dm} = maternal dominance, V_C = cytoplasm, V_{SE} = interaction between the *o2* gene genotype and environment, V_{AE} = embryo additive interaction, V_{DE} = embryo dominance interaction, V_{AeE} = endosperm additive interaction, V_{DeE} = endosperm dominance interaction, V_{AmE} = maternal additive interaction, V_{DmE} = maternal dominance interaction, V_{CE} = cytoplasm interaction, V_e = error and V_P = phenotype.
[b] * and ** are significant at 0.05 and 0.01 levels, respectively.

are summarized in Table 2 and the estimated variance components in Table 3 for the traits studied. The effect of the nulliplex genotype or mutant type (*o2o2o2*) on lysine content was significantly larger than zero at $\alpha = 0.01$, while that of the triplex or ordinary (*O2O2O2*) significantly smaller than zero. Homozygotic mutant considerably increased the lysine content and its average performance is 0.37%, near 0.10% higher than that of ordinary type. The duplex (*O2O2o2*) was similar to the triplex and the *o2* gene exhibiting a pattern of entirely single recessive gene inheritance. A little different result from the duplex occurred in this study that the simplex (*O2o2o2*) stood between the nulliplex and the triplex, but close to the triplex. This indicated that the second dominance was inherited in an incomplete fashion. The minor interaction between the *o2* gene and environment suggests that the effects of the *o2* gene were stable across different environments.

On the contrary for protein content, the genotypic effect of the nulliplex was the lowest while that of the triplex the highest. The effects of the heterozygous genotypes were intermediate between those of two homozygotes, but close to that of the nulliplex. This showed that protein content displayed incomplete dominance inheritance. The variance of *GE* interaction of the *o2* gene (V_{SE}) was highly significant, impling that the *o2* action on protein content largely changed across different environments.

Significant genotypic effects were detected on oil content. There displayed a little different mode of inheritance from those of lysine and protein contents. Only the simplex genotype had decremental effect while the others had incremental effects. Both mutant and normal types slightly enhanced oil percentage. There was the highest value in the mutant homozygote. As a whole, the influence of the *o2* gene on oil content seemed to be relatively weak since the variation coefficient of oil content affected by the *o2* gene was small as compared to those of lysine and protein contents. The utilization of the *o2* gene for improving protein quality appears to have a little effect on oil content. However, it can be concluded that gene substitution of the *o2* locus affects not only metabolism of amino acid and protein but lipoidal synthesis as well. There was no interaction between the *o2* gene and environment, indicating

that four genotypes responded similarly to environments for oil content.

The nulliplex had about 3% less kernel density than the ordinary counterpart. The heterozygote tended to have larger density while the homozygote to have less density (see Table 2), suggesting that there was some heterosis at the *o2* locus, even over the normal type, for kernel density. The relatively small *GE* interaction variance showed that the genetic effects of the *o2* locus in density might remain stable across environments.

Phenotypic variance and its components of polygenes

The estimated variances are presented in Table 3. There was a considerably wide quantitative variation among the assessed breeding population resulted from polygenes for all traits studied. And there also existed a strong variation due to *GE* interactions although V_E's were trivial, suggesting that the expression of polygenes is susceptible to external environments and exhibits a pattern of differential rather than parallel responses.

Estimation results show that the variation of lysine content affected by polygenes was mostly controlled by genetic main effects such as embryo additive, endosperm dominance and maternal additive. They accounted for a proportion of more than two thirds to the total variance. It was implied that the expression of polygenes was relatively stable across environments. Among genetic main effects, additive effects including embryo additive and maternal additive were large. Since the two portions of phenotypic variation can be transmitted from parents to their offspring, there will be a high degree of resemblance between relative individuals. Early generation selection or sib indirect selection based on the performance in a particular environment could result in a large selection progress for lysine content. It was suggested, by the fact that the variances of seed direct effects (embryo and endosperm) were the principal components, that an obvious xenia effect emerged and the genetic constitution of seed should be firstly taken into consideration in the utilization of polygenes. Cytoplasm by environment interaction variance occupied a little share of the phenotypic variance. Genetic response could be gained to a certain extent through exploiting elite cytoplasmic sources but such a response was environment-specific.

For protein percentage, significant embryo additive, maternal additive, embryo additive interaction, maternal additive and dominance interactions occupied large portions of the total variance. Only dominance interaction was significant among endosperm components and its proportion was small. It can be concluded that protein content was mainly controlled by embryo and maternal plant. Of all variance components, embryo additive was the largest component while maternal dominance interaction the second. This suggested that the selection on maternal plants with an elite source of polygenes was important for obtaining breeding materials with high protein content. *GE* interaction effects of the polygenes were predominant as compared to genetic main effects. It was implied, by the fact of *CE* interaction variance holding more than 10% share of the total variance, that cytoplasm genome is a valuable source for enhancing protein level restricted to specific environments.

For oil content, some of the variance components of *GE* interaction effects were large as compared with those of genetic main effects. It was suggested that the expression of polygenes depended on specific environments to a great extent. The *GE* interactions of embryo additive, endosperm dominance and maternal additive were more important than the other effects. It was shown that four genetic systems were involved in the genetic control of polygenes for oil content. Large embryo additive and maternal additive interactions revealed that there existed high resemblance between relative individuals at a particular location.

Along with the decreased volume, the less kernel density of the mutant types is regarded as the main factor that contributes to lower grain weight and reduced yield of the *o2* maize. The results for kernel density (see Table 3) suggested that it might be feasible to capitalize the background genes existing in our population to remedy this defects. The estimated variances of embryo dominance, maternal dominance, embryo additive interaction, endosperm dominance interaction, maternal additive and dominance interactions as well as cytoplasmic interaction were highly significant. Significant xenia effects (due to the nuclear genes of embryo or endosperm) indicated that the expression of polygenes in seeds played an important role in kernel modification. Although

xenia and cytoplasm effects were also involved in determining density, maternal control appeared to be more predominant than the other genetic systems. Trivial endosperm components suggested that dosage effects in an endosperm contributed little to reciprocal differences. Large *GE* interaction variances showed that kernel density was also subject to the impact of environments. It was suggested, by the results observed in the present study with respect to kernel density, that the inheritance was under complex polygenic control.

Genetic main effects of parents attributable to polygenes

The predicted values for genetic main effects in parents can provide a guide to evaluating genetic merits ascribable to polygenes in selection programs over different environments and to the effective utilization of those polygenes. Predicted effects are listed in Table 4 for those with significant estimated genetic main variances.

For lysine content, there seemed to be no obvious heterosis, as the sum of the predicted endosperm dominance effects of all parents was close to zero. Parents 9 (Zhongxi091 *o2*) and 12 (Yin85-6 *o2*) had highly significant positive maternal additive but negative embryo additive effects, whereas parent 2 (Qi205 *o2*) had the reversed ones. The opposite effects in the same parent gave rise to less variation among parents but more in segregating hybrid progeny between those parents and also suggested that much improvement could be achieved over lysine content by breaking the undesirable association between maternal and embryo genomes.

The significant negative embryo and maternal additive effects of parent 5 (Zhongxi017 *o2*) indicated that the expression of polygenes in both embryo and maternal plant might decrease protein content, while maternal and embryo additive effects appeared to be antagonistic in most of parents for protein content. Parent 9 and its hybrids might be prone to high performance, and thus superior to the others in increasing protein content, but parents 7 (92–213) and 5 as well as their hybrids prone to low one. The predicted results indicated that the recombination of the polygenes in different genetic systems could be helpful to enhancing protein content and that the utilization of favorable xenia was also a remarkable source.

All predicted maternal dominance effects of parents and the sum of embryo dominance values were negative, suggesting that strong maternal

Table 4. Predicted genetic main effects of parents for lysine content, protein content and density

Parent	Lysine content			Protein content		Density	
	A_i[a]	De_{ii}	Am_i	A_i	Am_i	D_{ii}	Dm_{ii}
LCZ01 *o2*	0.004	0.007**	−0.002	−0.08	0.06	0.004*	−0.030**
Qi205 *o2*	0.016**	0.000	−0.021**	0.54**	−0.53*	−0.009**	−0.048**
LCZ02 *o2*	−0.014*	0.005	0.014	−0.17	0.09	0.008**	−0.042**
LCZ05 *o2*	0.012	0.009**	−0.015	0.44*	0.20	−0.004**	−0.016**
Zhongxi 017 *o2*	0.009	0.006*	−0.017*	−0.25*	−0.27*	−0.016**	−0.025**
Qi 302	0.010*	0.002	−0.005	0.04	0.92**	−0.005**	−0.043**
92-213	−0.004	0.008*	0.002	−0.22	−1.06**	−0.007*	−0.014
LCZ04 *o2*	0.016**	−0.005	−0.019*	−0.54**	0.09	0.008**	−0.036**
Zhongxi 091 *o2*	−0.020**	0.016**	0.027**	0.80**	0.31	−0.007**	−0.038**
Va36 *o2*	−0.013*	−0.006*	0.018*	−0.71**	0.74**	0.002**	−0.027**
O2-4–10	−0.002	−0.001	−0.003	0.35**	−0.44**	0.001	−0.026**
Yin85-6 *o2*	−0.015**	0.006**	0.025**	−0.35**	−0.15	0.010**	−0.043**
LCZ03 *o2*	0.008	0.008	−0.006	−0.02	−0.11	−0.014**	−0.028**
92-1282	−0.009*	0.001	0.010*	0.20	−0.03	0.000	−0.046**
92-1461	0.003*	−0.004	−0.007*	0.06	0.17*	0.007**	−0.045**

[a] * and ** are significant at 0.05 and 0.01 levels, respectively.

heterosis and somewhat embryo heterosis were expected in most hybrids for kernel density. The crosses mating with 3 semi-hardness parents such as parents 2 (Qi205 *o2*), 3 (LCZ02 *o2*) and 12 (Yin85-6 *o2*), or 3 normal parents such as parents 6 (Qi302), 14 (92–1282) and 15 (92–1461) probably possessed higher density on the whole. This was compatible with the phenotypic data and that crosses 3×12 (LCZ02 *o2*× Yin85-6 *o2*), 2×9 (Qi205 *o2* × Zhongxi091 *o2*) and 2×10 (Qi205 *o2*× Va36 *o2*) had the highest predicted values (data not shown).

Discussion

Several mutations, e.g., *brittle-1* (*bt1*), *brittle-2* (*bt2*), *floury-2* (*fl2*), *o2*, *opaque-7* (*o7*), *shrunken-1* (*sh1*), *shrunken-2* (*sh2*), *shrunken-4* (*sh4*), *sugary-1* (*su1*), etc., cause dramatic decreases in zein content accompanied by increases in the proportion of other protein fractions and the relative amount of essential amino acids (Mertz et al., 1964; Nelson et al., 1965; Misra et al., 1972; Tsai and Dalby 1974). Incorporation of any one and more of these mutant genes into the traditional maize can substantially enhance the biological value of cereal grains. The *o2* mutant is the earliest found and most widely studied of these, and a very logical source for genetic improvement of the nutritional value of poor maize proteins. Great enthusiasm was quite evident in many breeding programs and the mutant allele has also been introduced into many genetic backgrounds. Some intrinsic problems limited significant utilization of the *o2* mutation have been putting spurs to probing into its action mechanism and improving measures. Breeders started a feasible projection to introduce modifier genes into an *o2* background though the mechanism by which *o2* modifiers convert the starchy endosperm of *o2* mutants to a hard, vitreous phenotype is not well understood. The presence of other background genes responsible for the difference of kernel traits has also been reported by many studies (Lambert et al., 1969; Zuber et al., 1975; Dudley et al., 1977; Denić 1983; Cavanaugh et al., 1995; Lambert & Chung 1995; Moro et al., 1995; Pratt et al., 1995). The large polygenic variability, not only due to *o2* modifiers, was revealed by dissecting phenotypic variation in terms of the *o2* gene and background polygenes as well as their *GE* interactions in the present study. It was suggested that the utilization of polygenes in the breeding population could greatly improve all the traits studied and remedy some undesirable effects of the *o2* gene.

Pleiotropic phenomena may be ubiquitous, e.g., some of the genes such as *brittle-1* (*bt1*), *brittle-2* (*bt2*), *shrunken-1* (*sh1*), *shrunken-2* (*sh2*), *shrunken-4* (*sh4*), *sugary-1* (*su1*) are known to affect starch synthesis in maize endosperm, but at the same time they also alter the amino acid patterns (Misra et al., 1972; Tsai & Dalby 1974). Investigating the inheritance of traits pleiotropically related is of importance not only for evaluating the future utilization of a specific germplasm in multipurpose genetic improvement, but also for shedding light on the physiological relationships between different effects. As revealed by many previous studies (Mertz et al., 1964; Misra et al., 1972; Damerval & Vienne 1993), the *o2* gene displayed multiple effects and intricate action patterns in this study, and the tendency was in good agreement with the previous studies. It has been shown that *O2* of maize is a regulatory gene of zein synthesis and its product belongs to basic region-leucine zipper (bZip) family of transcription activators (Schmidt et al., 1990). The regulatory properties of the *O2* protein probably explain the multiple effects and also the different impacts of *o2* already documented. On the basis of the current available studies it seems that most differences between normal and *o2* genotypes at the level of amino acids are a secondary consequence of this important physiological change variation. Far reaching effects of the *o2* gene could occur through metabolic rather than transcriptional control. It is therefore reasonable that all the examined traits are affected by the *o2* gene, which has influences different from one trait to another, e.g., strong on lysine and protein contents but relatively weak on oil content. The *o2* gene exhibits not only pleiotropic effects but also distinct inheritance modes for various traits, e.g., the nearly recessive single gene inheritance for lysine content whereas incomplete dominance for protein content. Additionally, the expression of the *o2* gene is conditioned by environments and the magnitude of dependence on environments varies with the traits. The degree is relatively small for lysine content while large for protein content and oil content. This provides a convincing example that

pleiotropically related characters display different inheritance.

Seed traits usually exhibit miscellaneous genetic behaviors with the morphologic and physiological complexity of seed. Maize seed is composed of diploid maternal tissue (testa and pericarp), diploid offspring tissue (embryo) and triploid offspring tissue (endosperm). Maternal plant also bears its physiological impacts on seed traits via supplies of nourishment and other physiological activity substances (e.g., longevity mRNA, hormones, etc.). Furthermore, cytoplasm genomes may play a part in the development of seed as a consequence of some metabolic pathways controlled by cytoplasm genes. The results of the present study indicated that the polygenes involved in the kernel traits may not only express in different parts of organism, tissues and places of cell but also have function in distinct manners. Moreover, the kernel traits may be subject to *GE* interaction modification. Similar results have been widely reported about nuclear gene effects of embryo and endosperm (e.g., xenia effects), cytoplasm and maternally controlled genetic effects as well as environmental effects on seed traits in maize (Garwood et al., 1970; Rao & Fleming 1979; Tsai & Tsai 1990; Weiland 1992; Pratt et al., 1995; Seka & Cross 1995). These facts require us to simultaneously consider all possible sources rather than a single source in genetic studies on seed traits for exposing the whole genetic architecture. Some noticeable and interesting results in the present study further lay stress on the needs. For instance, since oil in maize is mostly found in germ (embryo plus scutellum), oil content is traditionally taken as an embryo trait influenced by maternal plant (Garwood et al., 1970). However, it is in contrast to this notion that oil content is controlled not only by embryo effects but also by endosperm and maternal plant effects. It is therefore necessary considering the influence of endosperm on embryo traits and vice versa.

As pointed out by numerous authors (reviewed by Mackay, 2001), epistatic interaction between major genes, polygenes or both is a potential source of genetic variation. Given the complexity of the genetic behavior of seed traits, this may be true in the present study. However, epistasis seems to be elusive and is difficult to detect due to its very high sampling variance (Mackay, 2001), especially with such a complicated seed model. Moreover, the current mating design is not optimal to do so either. Based on that knowledge of significant epistasis is not terribly enlightening regarding the nature of the individual effects (Mackay, 2001), we do not take epistatic interaction into consideration in this article. But we think that a systematic investigation into epistatic effects is advisable and also judicious to unravel the inheritance mechanism of *o2* related traits. Such an investigation requires one to adopt an *ad hoc* mating design, e.g., simultaneously using isogenic ordinary and *o2* lines as parents, to obtain reliable estimation of epistatic components.

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