

Pleiotropic Effects of the Duplicate Maize *FLORICAULA/LEAFY* Genes *zfl1* and *zfl2* on Traits Under Selection During Maize Domestication

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ABSTRACT

Phenotypic variation on which selection can act during evolution may be caused by variation in activity level of developmental regulatory genes. In many cases, however, such genes affect multiple traits. This situation can lead to co-evolution of traits, or evolutionary constraint if some pleiotropic effects are detrimental. Here, we present an analysis of quantitative traits associated with gene copy number of two important maize regulatory genes, the duplicate *FLORICAULA/LEAFY* orthologs *zfl1* and *zfl2*. We found statistically significant associations between several quantitative traits and copy number of both *zfl* genes in several maize genetic backgrounds. Despite overlap in traits associated with these duplicate genes, *zfl1* showed stronger associations with flowering time, while *zfl2* associated more strongly with branching and inflorescence structure traits, suggesting some divergence of function. Since *zfl2* associates with quantitative variation for ear rank and also maps near a quantitative trait locus (QTL) on chromosome 2 controlling ear rank differences between maize and teosinte, we tested whether *zfl2* might have been involved in the evolution of this trait using a QTL complementation test. The results suggest that *zfl2* activity is important for the QTL effect, supporting *zfl2* as a candidate gene for a role in morphological evolution of maize.

THE identification of genes involved in morphological evolution in both plants and animals has benefited greatly from quantitative trait locus (QTL) studies. Genes underlying QTL causing trait differences in various plant and animal species have been identified, providing an opportunity to study the mechanisms of morphological change under selection. Two important questions for better understanding the mechanisms of the evolution of development are (1) What role do developmental regulatory genes play in the control of quantitative traits?, and (2) Do these genes underlie QTL?

Several genes responsible for QTL play important roles in normal development (MACKAY 2001). Interestingly, some of the genes identified as QTL have complex or pleiotropic effects on development. For example, the *teosinte branched1* (*tb1*) gene, underlying a QTL involved in maize domestication, affects inflorescence sex, ear size, branch length and tiller number (DOEBLEY *et al.* 1997), while in tomato, the *fw2.2* locus, which controls changes in fruit shape and size during domestication (CONG *et al.* 2002; LIU *et al.* 2003), also plays a quantitative role in fruit number and photosynthate distribution (NESBITT and TANKSLEY 2001). Identifying pleiotropic quantitative effects of regulatory genes pu-

tatively involved in evolution will help us understand the possibilities and limitations for morphological evolution in populations by shedding light on how trait correlations may arise.

In this study, we examine phenotypic variation of several traits associated with maize domestication for association with varying wild-type copy number of the paralogous maize regulatory genes *zfl1* and *zfl2* (BOMBLIES *et al.* 2003). These genes are orthologous to the *FLORICAULA(FLO)/LEAFY(LFY)* genes of *Antirrhinum* and *Arabidopsis* (COEN *et al.* 1990; WEIGEL *et al.* 1992). As with *FLO* and *LFY*, *zfl1* and *zfl2* control essential aspects of reproductive development, such as flower identity and patterning, that are required for fertility (BOMBLIES *et al.* 2003). On the basis of a preliminary finding that mutations at the *zfl2* locus are associated with quantitative effects on inflorescence phyllotaxy, we proposed *zfl2* as a candidate locus for a major-effect QTL for inflorescence phyllotaxy differences between maize (*Zea mays* ssp. *mays*) and its wild ancestor, teosinte (*Z. mays* ssp. *parviglumis*; BOMBLIES *et al.* 2003), that maps to the same region of maize chromosome 2 (DOEBLEY and STEC 1991; DOEBLEY and STEC 1993).

This study addresses the following questions:

1. What are the pleiotropic quantitative effects associated with *zfl1* and *zfl2* gene dosage in different maize genetic backgrounds?
2. Have these duplicate genes diverged in their pleiotropic effects?

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3. Is *zfl* activity (rather than that of a linked gene) important for the observed associations?
4. Is *zfl2* activity important for the large-effect chromosome 2 domestication QTL for ear phyllotaxy?

MATERIALS AND METHODS

Plant materials and breeding: To analyze the effects of diverse maize *zfl* alleles relative to mRNA null alleles, we generated 10 maize F₂ populations (H95a, H95b, IL, OH43a, OH43b, M01, M02-1, M02-2, W00, and W03) that segregated for mRNA null alleles of *zfl1* and *zfl2* and wild-type alleles from five different maize genetic backgrounds (inbred lines H95, IL101, OH43 and W22, and the “Mu-Killer” background) (MARTIENSSSEN and BARON 1994). Each F₂ population was generated from a cross between a maize plant that carried mRNA null alleles at both *zfl1* and *zfl2* and individuals from the respective “wild-type” maize line. The mRNA null alleles used were the previously described *Mutator* transposon insertion alleles *zfl1-mum1* and *zfl2-mum1*, or *zfl1-mum2* and *zfl2-mum3* (BOMBLIES *et al.* 2003). All of the populations segregated for the alleles *zfl1-mum1* and *zfl2-mum1* except the W03 population, which segregated *zfl1-mum2* and *zfl2-mum3*.

F₂ populations used to score phenotypes were generated as follows. The breeding schemes used to generate the M01 and W00 populations were previously described (BOMBLIES *et al.* 2003). The M02-1 and M02-2 F₂ populations (157 and 158 plants, respectively, “Mu-killer” background) were derived as described for M01 (BOMBLIES *et al.* 2003). The H95a and H95b F₂ populations (234 and 72 plants, respectively, H95 background) were derived from a cross between a plant from the M02-1 population that carried *zfl1-mum1* and *zfl2-mum1* and inbred line H95. OH43a and OH43b (193 and 105 plants, respectively, OH43 background) were derived from a cross between a plant from the M02-1 population that carried *zfl1-mum1* and *zfl2-mum1* and inbred line OH43. The W03 F₂ population (99 plants, W22 background) was derived from a cross between two lines, one that carried *zfl1-mum2* and the other *zfl2-mum3*, each backcrossed four generations to inbred line W22. The IL population (262 plants, IL101 background) was derived from a cross between a plant that carried *zfl1-mum1* and *zfl2-mum1* (from an F₂ population generated as described for the M01 population) and the IL101 inbred maize line.

Quantitative phenotypic variation was scored in field-grown populations cultivated and analyzed during summer (May–September) in an outdoor field plot at the University of Wisconsin West Madison Agricultural Research Station (Madison, WI). All plants were phenotyped and subsequently genotyped to test for associations (see *Genotyping* below). The W00 population was grown in summer 2000; M01 in summer 2001; M02-1, M02-2, and IL in summer 2002; and H95a/b, OH43a/b, and W03 in summer 2003.

To test whether *zfl2* gene activity is necessary for a QTL for ear traits differentiating maize and teosinte that maps to the same chromosomal region, we performed a QTL complementation test. To obtain maize/teosinte hybrid F₂ families for the QTL complementation tests, we crossed a maize plant that was heterozygous for *zfl2-mum1* (backcrossed six times into the W22 background) to teosinte (*Z. mays* ssp. *parviglumis* collection Ittis and Cochrane 81). We genotyped F₁ plants at *zfl2* (see *Genotyping* below) and selected the following four F₁ individuals: (1) plant 1, which carried wild type maize *zfl2* and a teosinte *zfl2* allele we named “T1”; (2) plant 2, which carried *zfl2-mum1* and T1; (3) plant 3, which carried wild-type maize *zfl2* and a different teosinte *zfl2* allele we named “T2”; and (4) plant 4, which carried *zfl2-mum1* and T2. The T1 and T2 teosinte

alleles were defined by restriction fragment length polymorphism (RFLP; see *Genotyping* below). F₁ plants were grown in isolation and allowed to self-fertilize in the University of Wisconsin greenhouse in winter 2002–2003 to generate the following four parallel F₂ populations: (1) T1W, 193 plants derived from F₁ plant 1; (2) T1m, 178 plants derived from F₁ plant 2; (3) T2W, 168 plants derived from F₁ plant 3; and (4) T2m, 150 plants derived from F₁ plant 4. F₂ populations were grown and phenotyped at Hawaiian Research in Kaunakakai, Molokai Island, HI, in a field plot in winter 2003–2004.

Independent *zfl1* mutations: Two of the 10 maize F₂ populations (M02-1 and IL) carried independent mutations at the *zfl1* locus, and thus were used as “control” populations to ask whether associated effects require *zfl1* gene activity, as opposed to being caused by segregation of linked genes. The maize inbred line IL101 carries a 220-bp deletion in exon 2, which leads to a frame shift and a premature stop codon in the mRNA (Ed Buckler, personal communication; sequence available at: http://www.panzea.org/db/searches/polyexp_search). The M02-1 population segregated phenotypically *zfl* double-mutant plants at a ratio of 1/4 ($P > \chi^2 = 0.98$), while the related M02-2 population segregated phenotypically mutant plants as expected for the 1/16 segregation of recessive mutations in two redundant genes ($P > \chi^2 = 0.65$). We sequenced *zfl1* and *zfl2* from the M02-1 population and found that the *zfl1* locus derived from the *Mu-killer* parent had a *Ds* transposon insertion 30 bases upstream of intron 1. Sequencing RT-PCR products showed that the resulting message is misspliced from the *Ds* element to exon 2. This generates a message with a frameshift, which truncates exon 1, encodes eight amino acids not normally found in *zfl*, followed by a premature stop codon in exon 2. Thus, at the *zfl1* locus both the IL and M02-1 populations segregated only *zfl1-mum1* and independently derived putative null *zfl1* alleles (*zfl1-IL101* and *zfl1-Ds*). The M02-1 line, which was generated from an F₁ parent sibling to the M02-2 F₁ parent, thus provides a genetically similar “control” population for M02-2, which inherited a *zfl1* allele with a normal sequence from the *Mu-Killer* parent.

Genotyping: Plants in F₂ populations were genotyped using a combination of PCR and RFLP approaches. The IL, M01, M02-1, and M02-2 F₂ populations were genotyped using RFLP markers with previously described hybridization conditions (DOEBLEY and STEC 1991). M01 genotyping was previously described (BOMBLIES *et al.* 2003). For the M02-1 and M02-2 populations, we performed southern hybridization on genomic DNA restricted with *Xba*I, while for the IL population, we used *Hind*III/*Xba*I double digests. The probe used for the blots was generated from the *zfl1* cDNA (BOMBLIES *et al.* 2003) using random primers supplemented with *zfl*-specific primers (5' CCAACGACGCCCTTCTCGG 3' and 5' ACATCGACGACGC AGCTAGA 3'). The remaining populations (H95a/b, OH43a/b, and W03) were genotyped using a PCR approach. First, we assayed for the presence of the *zfl1-mum1*, *zfl1-mum2*, and *zfl2-mum1* *Mutator* alleles as described previously (BOMBLIES *et al.* 2003). We genotyped for the presence of the *zfl2-mum3* allele (W03 population) using the primers: 5' ATTTGTGGCC GCCAGCTTAGCGA 3' and 5' AGAGAAGCCAACGCCA(A/T) CGCCTC(C/T)ATTTTCGTC 3'.

We then tested for the presence of wild-type alleles using a *zfl2* specific primer set (5' AGCCTCGCCGTGTCTTCT 3' and 5' CCCGTGGACTTGCGAGAC 3') and a *zfl1* specific primer set (5' GCATTGGAAAACAGTTAC 3' and 5' GTCTGCCGTT TGTATAT 3') spanning the transposon insertion sites. Double-mutant control DNA was included to verify that wild-type bands did not amplify from DNA homozygous for *Mutator* alleles. This information was combined to assign genotypes for both *zfl1* and *zfl2* to each individual.

Maize/teosinte hybrids used in the QTL complementation test were genotyped as follows. To initially identify F₁ maize/teosinte hybrid plants that carried the same teosinte alleles in combination with wild-type and mutant maize *zfl2* alleles, genomic DNA from F₁ plants was restricted with *Hind*III and hybridized by Southern blotting with a 392-bp *zfl2*-specific 5' region probe generated by PCR using the primers 5' AGCC TCGCCGTGTCCTTCT 3' and 5' AAGGCGTCGTTGGGATCC AT 3'. The probe matches the first 372-bp 5' of *zfl2* and did not cross-hybridize with *zfl1* under our conditions. DNA from maize line A682 was used as the PCR template. Hybrid F₂ populations were genotyped by PCR for segregating simple sequence repeat (SSR) markers located on chromosome 2. SSR map locations and primer sequences were obtained from the maize mapping project (<http://www.maizegdb.org/ssr.php>) and tested for segregation in our populations. For the T1W and T1m populations, we used markers umc1165, mmc0231, and bnlgl1175, while for T2W and T2m we used umc1227 instead of umc1165 and umc1026 in place of bnlgl1175. We genotyped plants at *zfl2* using a size polymorphism that results from a 307-bp MITE transposon insertion 329 bp 5' of the translation start present in the W22 and *zfl2-mum1* alleles, but not in the teosinte T1 and T2 *zfl2* alleles.

Phenotyping: To ask whether the *zfl* genes might associate with quantitative variation for traits important in maize domestication, we scored our maize F₂ populations for 11 traits for flowering time and plant and inflorescence architecture. We measured flowering time by counting vegetative leaf number (LN; with seed leaf as leaf one), days to pollen (DTP; number of days after planting that anthers exerted on the tassel), days to silk (DTS; number of days after planting that silks are visible on ears), and husk leaf number (HLN; number of husk leaves enclosing the uppermost ear). To assay plant architecture, we scored the number of lateral branches, or ears (LBN; number of ear shoots or lateral branches in leaf axils), the node with the lowermost developed ear (LDE; "developed" was defined as any ear whose husk extended beyond the subtending leaf sheath), the node with the uppermost developed ear (UDE), and the number of blank vegetative nodes (producing no ears) between the uppermost ear and the terminal tassel (BLN). We analyzed inflorescence architecture by scoring the number of long branches on the terminal male inflorescence or tassel (TBN), ear phyllotaxy (KRN; the number of rows of kernels around the circumference of the ear averaged over three counts taken 1 cm from the base, at the middle, and ~2 cm from the end of the ear), and tassel phyllotaxy or rank (TRNK; average of three measurements of the number of rows, or ranks, of spikelet pairs around the rachis circumference taken along the central spike of the tassel).

For the maize/teosinte hybrid families used in the QTL complementation test, we measured ear phyllotaxy and blank node number (BLN). Ear phyllotaxy was scored as ear rank (RNK), the number of grain-bearing cupules around the circumference, rather than as KRN, because maize/teosinte hybrid populations independently segregate for paired *vs.* single kernels, making rank a more consistent measure of phyllotaxy in these groups. We report two RNK measurements for these families: average ear rank for the terminal ear of the uppermost lateral branch (uRNK) and the terminal ear of the branch below the uppermost branch (sRNK).

Data analysis: We tested for associations between plant phenotypes and *zfl1* and *zfl2* genotypes using two-way analysis of variance (ANOVA) in JMP-IN (SAS Institute) and report least-square mean (LSM) trait values for each genotype category for each *zfl* gene individually. Two-way ANOVA was performed in JMP using the "fit model" function with the model $y = zfl1 + zfl2 + zfl \times zfl2$. Percentage of phenotypic variance explained is reported as an r^2 value calculated from the sums of squares

(SS) for each gene and the whole model in JMP. We tested for effects associated with *zfl1* or *zfl2* individually using ANOVA by analyzing one subset for each gene in each of the three largest populations (H95a, OH43a, M01) that segregated for one of the *zfl* genes, but was homozygous for mutant alleles at the paralogous locus. Dominance/additivity (d/a) ratios were calculated using the LSM trait values where a = wild-type trait value/2 - mutant trait value/2 and d = heterozygote trait value - (mutant trait value/2 + wild-type trait value/2). For maize/teosinte hybrid populations, we performed ANOVA for each marker individually for the traits scored and used the resulting trait means to calculate d/a ratios with maize as "wild type" and teosinte as "mutant" in the d/a equations.

As a first step in QTL mapping analysis, we generated genetic linkage maps for the chromosome 2 SSR markers and *zfl2* in Mapmaker/EXP v 3.0 (LANDER *et al.* 1987) using the Kosambi mapping function. Windows QTL Cartographer v 2.0 (WANG *et al.* 2001–2004) was used for QTL mapping in the maize/teosinte hybrid populations by composite interval mapping (CIM) using model 6 (which selects certain control markers to correct for background effects) and the forward regression method with five control markers and a window size of 10 cM. LOD score significance thresholds were estimated for each trait in each population with 1000 permutations of the data.

RESULTS

Maize F₂ populations—developmental timing: We estimated the timing of reproductive development by counting leaf number (LN), days to pollen shed (DTP; male maturity), days to silk (DTS; female maturity) and the number of husk leaves (HLN) on the ear shoot. LN consistently showed stronger associations with *zfl1* than with *zfl2* (Table 1). For *zfl1*, we observed a statistically significant LN decrease associated with increasing number of wild-type *zfl1* alleles in all seven populations assayed, (Tables 1 and 2) with plants homozygous wild type at *zfl1* averaging 4–13% fewer leaves than the homozygous *zfl1* mutant class. *zfl2* showed a significant association with LN in five of the seven populations (Table 2). HLN decreased significantly with increasing active *zfl1* and *zfl2* copies in all four populations scored (Tables 1 and 2), with the homozygous wild-type *zfl1* class averaging 17–37% fewer husk leaves than the homozygous *zfl1* mutant class. *zfl1* explained more of the HLN variance than *zfl2* in all four populations.

We observed a modest but statistically significant decrease in DTP and DTS with increasing active *zfl1* copies in all six populations examined, while for *zfl2* DTP and DTS trends were each statistically significant in five of the six populations (Tables 1 and 2). Plants homozygous for mutant *zfl1* alleles averaged 2–4% higher DTP and 3–5% higher DTS than plants carrying two wild-type alleles, while plants that had two mutant *zfl2* alleles averaged 2–4% higher DTP and 1–5% higher DTS than plants with two wild-type *zfl2* alleles. *zfl1* explained more variance for DTP and DTS than *zfl2* in most of the populations (Table 2).

Plant architecture: We observed significant associations between the *zfl* genes and several plant architecture

TABLE 1
Summary of significant trends

Gene	Population	Trait category									
		Developmental timing				Plant architecture				Inflor. arch.	
		LN	DTP	DTS	HLN	LDE	UDE	LBN	BLN	TBN	KRN
<i>zfl1</i>	H95a	▼	▼	▼		▼	▼	▼	▲	○	▲
	H95b	▼	▼	▼		▼	▼	○	○	○	
	OH43a	▼	▼	▼	▼	▼	▼	○	○	○	○
	OH43b		▼	▼	▼			▼	○	○	○
	W03	▼	▼	▼	▼	○	○	▲	▼	○	○
	W00	▼						○		OD	○
	M02-2	▼				▼	▼	○	○	○	○
	M01	▼	▼	▼	▼			▼		▲	▲
<i>zfl2</i>	H95a	▼	▼	○		▼	▼	▼	▲	▲	▲
	H95b	▼	▼	▼		▼	▼	○	○	▲	
	OH43a	▼	▼	▼	▼	▼	▼	▼	○	▲	▲
	OH43b		▼	▼	▼			▼	▲	▲	▲
	W03	○	○	▼	▼	○	▲	▲	▼	▲	▲
	W00	○						▼		▲	○
	M02-2	▼				▼	▼	○	▲	▲	▲
	M01	▼	▼	▼	▼			▼		▲	▲

Significance of traits analyzed in the different maize populations studied. ▲, $P < 0.01$; trait value increases (▲) or decreases (▼) with higher *zfl1* or *zfl2* wild-type copy number; ▲, $0.01 < P < 0.05$; trait value increases (▲) or decreases (▼) with higher *zfl1* or *zfl2* wild-type copy number; ○, not significant; OD, overdominant; blank, not determined. Infl. arch, inflorescence architecture. Traits: LN, leaf number; DTP, days from planting to pollen shed; DTS, equals; days from planting to silk emergence from husk of most developed ear; HLN, husk leaf number on uppermost ear shoot; LDE, lowest node with developed ear; UDE, uppermost node with developed ear; LBN, lateral branch (ear) number; BLN, blank node number between uppermost ear and tassel; TBN, tassel branch number; KRN, kernel row number ($2 \times$ ear rank) of uppermost ear.

traits: the number of ears or lateral branches (LBN), the lowermost and uppermost nodes bearing developed ears (LDE and UDE, respectively), and the number of blank nodes (BLN). A decrease in LBN was significantly associated with increasing wild-type *zfl2* copy number in six of eight populations tested and with increasing *zfl1* copy number in four populations (Tables 1 and 3). *zfl2* explained more of the trait variance in all of these populations than *zfl1*. Plants homozygous for wild-type *zfl2* alleles averaged 23–44% lower LBN than plants carrying two mutant *zfl2* alleles in the populations with significant trends, while plants homozygous for two wild-type *zfl1* alleles averaged 7–29% lower LBN. LDE was significantly lower in plants with higher active *zfl1* or *zfl2* copy number in four of the five populations scored. *zfl1* explained more of the LDE variance in all four of these with plants that carried two mutant alleles averaging 11–17% lower LDE than plants that carried two wild-type *zfl1* alleles. Plants that carried two mutant *zfl2* alleles averaged 5–12% lower LDE than plants that carried two wild-type *zfl2* alleles (Table 3). UDE was significantly lower in plants with higher active copy numbers of either *zfl1* or *zfl2* in four of the five populations scored, while the reverse trend was observed for *zfl2* in one population (W03; Table 3). The UDE variance explained by each gene in these groups was similar and UDE averaged 9–13% higher in plants with two wild-type copies of either

zfl gene (Table 3). BLN was significantly higher (24–30%) in plants with higher active *zfl2* copy numbers in three of six populations examined and with higher *zfl1* in one population (9% increase with two wild-type copies of *zfl1*). *zfl2* explained more of the BLN variation in all three lines (Table 3). In contrast, in the W03 population, increasing wild-type copy number from zero to two of either *zfl1* or *zfl2* was significantly associated with a decrease in BLN (17 and 12%, respectively).

Inflorescence architecture: We examined variation for inflorescence structure by measuring the number of long tassel branches (TBN), ear phyllotaxy as the number of kernel rows around the ear (KRN), and phyllotaxy or rank of the central tassel spike (TRNK).

TBN showed statistically significant quantitative associations with *zfl2* in all eight populations scored for this trait (Tables 1 and 4). In these populations, increasing active *zfl2* copy number from zero to two was associated with an increase in TBN of 12–73%, while increasing active *zfl1* copy number was associated with a significant increase in TBN (of 5%) in one population (M01) and a statistically significant but strongly overdominant trend in the W00 population (Table 4). Correspondingly, *zfl2* explained more of the TBN variance than *zfl1* in all populations.

KRN was significantly associated with *zfl2* in six of seven populations assayed, while *zfl1* genotype showed a

TABLE 2
Reproductive timing traits associated with *zfl1* and *zfl2*

Trait	Pop.	Gene	<i>n</i>	<i>mum/mum</i>	WT/WT	<i>P</i>	<i>d/a</i>	<i>r</i> ²	tot. <i>r</i> ²	
LN	H95a	<i>zfl1</i>	223	23.9 ± 0.18	22.1 ± 0.24	***	0.27	0.16	0.26	
		<i>zfl2</i>	223	23.5 ± 0.23	22.5 ± 0.20	**	0.44	0.04		
	H95b	<i>zfl1</i>	72	23.4 ± 0.28	21.8 ± 0.29	**	0.53	0.17	0.44	
		<i>zfl2</i>	72	22.9 ± 0.26	21.9 ± 0.32	*	-0.38	0.06		
	OH43a	<i>zfl1</i>	183	22.2 ± 0.19	20.2 ± 0.19	***	0.68	0.21	0.47	
		<i>zfl2</i>	183	21.9 ± 0.16	20.5 ± 0.22	***	0.89	0.14		
	W03	<i>zfl1</i>	89	21.6 ± 0.19	20.7 ± 0.18	**	1.11	0.20	0.23	
		<i>zfl2</i>	89	21.3 ± 0.18	20.8 ± 0.18	—	—	0.05		
	W00	<i>zfl1</i>	115	11.9 ± 0.25 ^a	10.6 ± 0.20 ^a	**	0.09	0.13	0.19	
		<i>zfl2</i>	115	11.3 ± 0.20 ^a	11.2 ± 0.24 ^a	—	—	0		
	M02-2	<i>zfl1</i>	127	22.4 ± 0.23	19.5 ± 0.28	***	0.34	0.35	0.43	
		<i>zfl2</i>	127	21.4 ± 0.28	20.3 ± 0.24	*	0.38	0.04		
	M01	<i>zfl1</i>	294	21.4 ± 0.14	18.6 ± 0.14	***	0.51	0.37	0.52	
		<i>zfl2</i>	294	21.0 ± 0.16	19.0 ± 0.13	***	0.88	0.20		
DTP	H95a	<i>zfl1</i>	221	76.8 ± 0.31	75.0 ± 0.41	**	0.76	0.07	0.17	
		<i>zfl2</i>	221	76.9 ± 0.41	74.9 ± 0.34	**	0.58	0.06		
	H95b	<i>zfl1</i>	69	78.7 ± 0.53	76.9 ± 0.51	*	1.12	0.10	0.40	
		<i>zfl2</i>	69	79.3 ± 0.52	76.3 ± 0.55	**	0.71	0.20		
	OH43a	<i>zfl1</i>	181	72.9 ± 0.31	71.2 ± 0.32	**	0.71	0.09	0.18	
		<i>zfl2</i>	181	72.8 ± 0.27	71.2 ± 0.35	**	0.51	0.08		
	OH43b	<i>zfl1</i>	101	73.0 ± 0.44	70.7 ± 0.57	**	0.80	0.10	0.40	
		<i>zfl2</i>	101	73.6 ± 0.45	70.6 ± 0.54	***	1.02	0.19		
	W03	<i>zfl1</i>	96	75.9 ± 0.65	73.7 ± 0.44	*	0.69	0.09	0.12	
		<i>zfl2</i>	96	75.4 ± 0.64	74.0 ± 0.43	—	—	0.04		
	M01	<i>zfl1</i>	286	77.4 ± 0.58	74.0 ± 0.35	***	0.13	0.08	0.16	
		<i>zfl2</i>	286	77.2 ± 0.60	74.9 ± 0.30	**	1.01	0.04		
	DTS	H95a	<i>zfl1</i>	219	78.1 ± 0.40	76.2 ± 0.52	**	0.90	0.07	0.11
			<i>zfl2</i>	219	77.5 ± 0.52	76.7 ± 0.43	—	—	0.02	
H95b		<i>zfl1</i>	72	81.6 ± 0.62	77.5 ± 0.65	***	0.57	0.21	0.46	
		<i>zfl2</i>	72	81.0 ± 0.59	78.3 ± 0.71	**	1.14	0.14		
OH43a		<i>zfl1</i>	181	73.9 ± 0.36	71.8 ± 0.37	***	0.86	0.10	0.23	
		<i>zfl2</i>	181	73.8 ± 0.32	71.6 ± 0.42	***	0.45	0.10		
OH43b		<i>zfl1</i>	100	74.9 ± 0.41	70.8 ± 0.51	***	0.80	0.28	0.57	
		<i>zfl2</i>	100	74.7 ± 0.43	70.8 ± 0.48	***	0.72	0.23		
W03		<i>zfl1</i>	96	78.1 ± 0.50	75.1 ± 0.44	***	0.82	0.21	0.35	
		<i>zfl2</i>	96	78.0 ± 0.49	74.9 ± 0.43	***	0.54	0.18		
M01		<i>zfl1</i>	291	82.1 ± 0.47	77.7 ± 0.39	***	0.20	0.15	0.19	
		<i>zfl2</i>	291	81.2 ± 0.50	79.4 ± 0.34	***	1.85	0.06		
HLN		OH43a	<i>zfl1</i>	180	13.2 ± 0.37	10.0 ± 0.39	***	0.99	0.20	0.35
			<i>zfl2</i>	180	12.0 ± 0.33	10.6 ± 0.44	**	0.90	0.04	
	OH43b	<i>zfl1</i>	99	16.3 ± 0.51	10.2 ± 0.70	***	0.61	0.31	0.61	
		<i>zfl2</i>	99	14.4 ± 0.57	11.8 ± 0.64	**	1.06	0.07		
	W03	<i>zfl1</i>	94	9.6 ± 0.33	7.4 ± 0.29	***	0.46	0.22	0.31	
		<i>zfl2</i>	94	9.4 ± 0.32	7.7 ± 0.28	**	0.71	0.15		
	M01	<i>zfl1</i>	282	14.1 ± 0.40	11.7 ± 0.38	***	0.36	0.07	0.09	
		<i>zfl2</i>	282	13.5 ± 0.43	12.4 ± 0.34	*	1.27	0.02		

Traits: LN, leaf number; DTP, days to pollen shed; DTS, days to silk emergence; HLN, husk leaf number; Pop, population analyzed; *n*, number of plants in analysis; *mum/mum*, least-square mean (± standard error) for plants homozygous for mutant alleles at the gene analyzed; WT/WT, least-square mean (± standard error) for plants homozygous for wild-type alleles at the gene analyzed (heterozygous trait values reported in supplemental material at <http://www.genetics.org/supplemental/>); *P*, *F*-test *P*-value from two-way ANOVA; ****P* < 0.0001; ***P* = 0.0001 < *P* < 0.01; **P* = 0.01 < *P* < 0.05; —, not significant (*P* > 0.05); *d/a*, dominance/additivity ratio (reported for statistically significant trends only); *r*², percentage trait variance explained (single gene effect from two-way ANOVA); tot. *r*², whole model percentage trait variance explained (from two-way ANOVA).

^aLeaf number (LN) for W00 population was counted at maturity from soil level node as “node 1.”

TABLE 3
Plant branching traits associated with *zfl1* and *zfl2*

Trait	Pop.	Gene	<i>n</i>	<i>mum/mum</i>	WT/WT	<i>P</i>	<i>d/a</i>	<i>r</i> ²	tot. <i>r</i> ²
LBN	H95a	<i>zfl1</i>	222	4.0 ± 0.16	3.2 ± 0.22	*	-0.04	0.03	0.18
		<i>zfl2</i>	222	4.4 ± 0.21	2.8 ± 0.18	***	0.02	0.13	
	H95b	<i>zfl1</i>	72	3.5 ± 0.22	3.6 ± 0.23	—		0.01	0.09
		<i>zfl2</i>	72	3.6 ± 0.21	3.6 ± 0.25	—		0.03	
	OH43a	<i>zfl1</i>	183	3.5 ± 0.16	3.2 ± 0.16	—		0.02	0.23
		<i>zfl2</i>	183	3.9 ± 0.13	3.0 ± 0.18	***	1.02	0.16	
	OH43b	<i>zfl1</i>	103	3.6 ± 0.16	3.4 ± 0.22	**	4.19	0.08	0.36
		<i>zfl2</i>	103	4.0 ± 0.17	2.7 ± 0.20	***	0.22	0.19	
	W03	<i>zfl1</i>	96	3.1 ± 0.26	3.9 ± 0.23	*	0.74	0.06	0.15
		<i>zfl2</i>	96	3.0 ± 0.25	3.9 ± 0.22	**	1.01	0.12	
	W00	<i>zfl1</i>	115	2.5 ± 0.26	2.7 ± 0.21	—		0.02	0.17
		<i>zfl2</i>	115	3.2 ± 0.22	1.8 ± 0.26	**	-0.10	0.14	
	M02-2	<i>zfl1</i>	125	1.7 ± 0.10	1.5 ± 0.12	—		0.01	0.09
		<i>zfl2</i>	125	1.8 ± 0.12	1.4 ± 0.10	—		0.04	
M01	<i>zfl1</i>	297	2.8 ± 0.11	2.0 ± 0.10	***	0.73	0.11	0.23	
	<i>zfl2</i>	297	2.9 ± 0.12	1.9 ± 0.09	***	0.66	0.15		
LDE	H95a	<i>zfl1</i>	222	15.2 ± 0.16	13.6 ± 0.22	***	0.57	0.17	0.27
		<i>zfl2</i>	222	14.8 ± 0.21	13.8 ± 0.18	**	0.43	0.05	
	H95b	<i>zfl1</i>	72	14.6 ± 0.25	13.1 ± 0.26	**	-0.07	0.14	0.49
		<i>zfl2</i>	72	14.4 ± 0.24	13.0 ± 0.28	**	-0.62	0.13	
	OH43a	<i>zfl1</i>	183	14.0 ± 0.18	12.4 ± 0.19	***	0.56	0.19	0.28
		<i>zfl2</i>	183	13.5 ± 0.15	12.8 ± 0.21	**	0.68	0.05	
	W03	<i>zfl1</i>	87	11.7 ± 0.24	11.4 ± 0.20	—		0.02	0.13
		<i>zfl2</i>	87	11.8 ± 0.23	11.2 ± 0.20	—		0.05	
	M02-2	<i>zfl1</i>	125	16.0 ± 0.27	13.2 ± 0.30	***	0.13	0.25	0.47
		<i>zfl2</i>	125	15.6 ± 0.30	13.8 ± 0.25	***	0.38	0.10	
UDE	H95a	<i>zfl1</i>	223	18.1 ± 0.18	15.8 ± 0.24	***	0.37	0.20	0.44
		<i>zfl2</i>	223	18.2 ± 0.24	15.6 ± 0.20	***	0.18	0.19	
	H95b	<i>zfl1</i>	72	17.1 ± 0.24	15.6 ± 0.25	**	0.15	0.15	0.45
		<i>zfl2</i>	72	17.0 ± 0.23	15.5 ± 0.27	**	-0.15	0.15	
	OH43a	<i>zfl1</i>	183	16.5 ± 0.15	14.6 ± 0.15	***	0.70	0.24	0.61
		<i>zfl2</i>	183	16.5 ± 0.13	14.7 ± 0.17	***	0.89	0.26	
	W03	<i>zfl1</i>	88	14.0 ± 0.20	14.3 ± 0.17	—		0.02	0.23
		<i>zfl2</i>	88	13.8 ± 0.20	14.2 ± 0.17	**	2.51	0.12	
	M02-2	<i>zfl1</i>	125	16.7 ± 0.24	13.8 ± 0.30	***	0.15	0.26	0.53
		<i>zfl2</i>	125	16.4 ± 0.30	14.2 ± 0.24	***	0.36	0.13	
BLN	H95a	<i>zfl1</i>	223	5.8 ± 0.13	6.3 ± 0.18	*	0.73	0.03	0.25
		<i>zfl2</i>	223	5.3 ± 0.17	6.9 ± 0.14	***	0.01	0.19	
	H95b	<i>zfl1</i>	72	6.4 ± 0.18	6.2 ± 0.19	—		0.05	0.16
		<i>zfl2</i>	72	5.9 ± 0.17	6.3 ± 0.13	—		0.04	
	OH43a	<i>zfl1</i>	183	5.7 ± 0.13	5.6 ± 0.14	—		0	0.06
		<i>zfl2</i>	183	5.4 ± 0.11	5.8 ± 0.15	—		0.03	
	OH43b	<i>zfl1</i>	101	6.3 ± 0.15	6.0 ± 0.20	—		0.02	0.38
		<i>zfl2</i>	101	5.3 ± 0.16	6.8 ± 0.19	***	0.39	0.29	
	W03	<i>zfl1</i>	97	7.6 ± 0.19	6.3 ± 0.17	***	1.00	0.26	0.42
		<i>zfl2</i>	97	7.5 ± 0.19	6.6 ± 0.16	***	1.85	0.26	
M02-2	<i>zfl1</i>	125	5.7 ± 0.14	5.7 ± 0.17	—		0.01	0.32	
	<i>zfl2</i>	125	4.9 ± 0.17	6.1 ± 0.14	***	0.41	0.17		

Traits: LBN, lateral branch (ear shoot) number; LDE, node number of lowest developed ear; UDE, node number of uppermost ear; BLN, blank vegetative node number above top ear. For remaining definitions, see Table 2 legend.

significant association with KRN in only two of these (Tables 1 and 4). In the six populations that showed significant KRN associations with *zfl2*, plants homozygous for wild-type *zfl2* alleles averaged 6–13% higher

KRN than plants that had two mutant *zfl2* alleles. Plants that were homozygous wild type for *zfl1* in the H95 and M01 populations averaged 4 and 18% higher KRN, respectively, than plants homozygous mutant for *zfl1*. In

TABLE 4
Inflorescence structure traits associated with *zfl1* and *zfl2*

Trait	Pop.	Gene	<i>n</i>	<i>mum/mum</i>	WT/WT	<i>P</i>	<i>d/a</i>	<i>r</i> ²	tot. <i>r</i> ²	
TBN	H95a	<i>zfl1</i>	223	14.1 ± 0.70	15.9 ± 0.94	—		0.02	0.13	
		<i>zfl2</i>	223	13.6 ± 0.93	17.0 ± 0.78	*	0.05	0.03		
	H95b	<i>zfl1</i>	71	14.1 ± 0.95	14.2 ± 0.97	—		0.02	0.32	
		<i>zfl2</i>	71	11.7 ± 0.91	15.1 ± 1.06	**	2.03	0.21		
	OH43a	<i>zfl1</i>	182	11.4 ± 0.54	10.3 ± 0.56	—		0.02	0.36	
		<i>zfl2</i>	182	8.7 ± 0.48	12.0 ± 0.63	***	1.39	0.15		
	OH43b	<i>zfl1</i>	101	14.8 ± 0.74	13.4 ± 0.97	—		0.02	0.48	
		<i>zfl2</i>	101	10.0 ± 0.77	17.3 ± 0.91	***	0.74	0.28		
	W03	<i>zfl1</i>	99	13.5 ± 0.73	14.3 ± 0.80	—		0.05	0.24	
		<i>zfl2</i>	99	13.0 ± 0.72	14.6 ± 0.79	*	2.46	0.08		
	W00	<i>zfl1</i>	116	8.0 ± 0.56	7.7 ± 0.46	**	-12.5	0.06	0.39	
		<i>zfl2</i>	116	6.5 ± 0.47	9.0 ± 0.56	***	1.44	0.15		
	M02-2	<i>zfl1</i>	146	12.0 ± 0.52	13.4 ± 0.70	—		0.02	0.50	
		<i>zfl2</i>	146	9.1 ± 0.69	15.4 ± 0.51	***	0.65	0.21		
	M01	<i>zfl1</i>	295	7.3 ± 0.39	9.2 ± 0.38	***	1.06	0.05	0.29	
		<i>zfl2</i>	295	6.0 ± 0.43	9.7 ± 0.33	***	1.15	0.16		
KRN (2 × RNK)	H95a	<i>zfl1</i>	208	16.0 ± 0.33	16.7 ± 0.42	*	2.00	0.03	0.15	
		<i>zfl2</i>	208	15.4 ± 0.43	17.7 ± 0.34	**	0.09	0.08		
	OH43a	<i>zfl1</i>	179	16.0 ± 0.25	16.3 ± 0.26	—		0	0.21	
		<i>zfl2</i>	179	15.1 ± 0.22	16.9 ± 0.29	***	0.73	0.18		
	OH43b	<i>zfl1</i>	100	15.7 ± 0.32	16.0 ± 0.42	—		0.01	0.32	
		<i>zfl2</i>	100	14.3 ± 0.34	17.5 ± 0.40	***	0.16	0.29		
	W03	<i>zfl1</i>	94	13.1 ± 0.31	13.7 ± 0.27	—		0.05	0.18	
		<i>zfl2</i>	94	13.0 ± 0.30	13.8 ± 0.27	*	0.93	0.06		
	W00	<i>zfl1</i>	72	13.4 ± 0.51	13.1 ± 0.50	—		0	0.10	
		<i>zfl2</i>	72	12.8 ± 0.44	13.2 ± 0.55	—		0.03		
	M02-2	<i>zfl1</i>	121	16.5 ± 0.42	17.4 ± 0.51	—		0.03	0.12	
		<i>zfl2</i>	121	16.2 ± 0.54	17.2 ± 0.39	*	2.18	0.05		
	M01	<i>zfl1</i>	280	14.0 ± 0.24	16.5 ± 0.22	***	0.03	0.17	0.27	
		<i>zfl2</i>	280	14.2 ± 0.26	16.0 ± 0.20	***	0.62	0.09		
	TRNK	M01	<i>zfl1</i>	294	5.6 ± 0.11	6.3 ± 0.11	***	-0.22	0.07	0.14
			<i>zfl2</i>	294	5.7 ± 0.12	6.1 ± 0.08	*	1.22	0.03	

Traits: TBN, tassel branch number; KRN, kernel row number (2 × ear rank [RNK]); TRNK, tassel rank. For other definitions, see Table 2 legend.

all populations except M01, *zfl2* explained more KRN variance than *zfl1*. TRNK was scored only in one population (M01), in which a statistically significant association with both *zfl1* and *zfl2* was observed. TRNK was more strongly associated with *zfl1*, corresponding to 12% higher TRNK in plants that carried two wild-type *zfl1* alleles than in plants with two mutant alleles (Table 4).

***zfl1* and *zfl2* in isolation:** To more specifically analyze the effects associated with each of the two *zfl* genes, we analyzed two subsets each (one for each gene) from the three largest populations (H95a, OH43a, M01). These subsets contained only individuals homozygous for mutant alleles at one of the two genes, while segregating for the paralog. Analyzing the effects of each of the two *zfl* genes in absence of gene product from the paralog allowed us to compare the effects of the two genes and to analyze whether either gene alone is sufficient to rescue specific aspects of the double-mutant phenotype.

For LN, DTP, and DTS we observed significant associations with both *zfl1* and *zfl2* genotype in all six subpopulations. HLN, which was scored for only two of the subpopulations showed a similar trend (Table 5). In keeping with trends observed in whole populations, *zfl1* genotype explained more phenotypic variance for these reproductive timing traits than *zfl2* did in most of the subpopulations. LBN strongly associated with both genes in the OH43a and M01 subpopulations, and with only *zfl2* in the H95a subpopulation (Table 5). *zfl2* genotype consistently explained more LBN variance than *zfl1*. LDE showed a stronger association with *zfl1* than with *zfl2* in both sets of subpopulations scored, while UDE associated with both *zfl1* and *zfl2* (Table 5). BLN showed statistically significant associations with *zfl1* and *zfl2* only in the H95 subpopulations (Table 5).

zfl2 associated with KRN in all three *zfl2* subsets (Table 5). Plants homozygous wild type at *zfl2* had 24–30%

TABLE 5
Traits associated with *zfl1* or *zfl2* when paralog is mutant

Trait	Pop.	Gene	<i>n</i>	<i>mum/mum</i>	WT/WT	<i>P</i> value	<i>r</i> ²
LN	H95a	<i>zfl1</i>	39	25.2 ± 0.43	22.5 ± 0.55	***	0.40
		<i>zfl2</i>	69	25.2 ± 0.48	23.0 ± 0.35	**	0.18
	OH43a	<i>zfl1</i>	64	24.0 ± 0.31	20.4 ± 0.32	***	0.55
		<i>zfl2</i>	51	24.0 ± 0.37	21.3 ± 0.53	***	0.41
	M01	<i>zfl1</i>	66	23.8 ± 0.34	19.2 ± 0.30	***	0.66
		<i>zfl2</i>	80	23.8 ± 0.34	20.0 ± 0.23	***	0.55
DTP	H95a	<i>zfl1</i>	38	78.9 ± 0.60	76.0 ± 0.74	**	0.37
		<i>zfl2</i>	67	78.9 ± 0.74	75.1 ± 0.51	**	0.22
	OH43a	<i>zfl1</i>	61	74.3 ± 0.58	72.1 ± 0.55	**	0.15
		<i>zfl2</i>	48	74.3 ± 0.53	72.0 ± 0.64	*	0.17
	M01	<i>zfl1</i>	57	80.0 ± 1.56	75.2 ± 0.70	*	0.13
		<i>zfl2</i>	72	80.0 ± 1.46	75.6 ± 0.49	*	0.10
DTS	H95a	<i>zfl1</i>	38	80.1 ± 0.98	76.3 ± 1.19	**	0.26
		<i>zfl2</i>	66	80.1 ± 1.03	76.7 ± 0.71	*	0.10
	OH43a	<i>zfl1</i>	61	76.1 ± 0.70	72.7 ± 0.66	**	0.24
		<i>zfl2</i>	49	76.1 ± 0.69	72.3 ± 0.84	**	0.25
	M01	<i>zfl1</i>	62	86.6 ± 1.36	77.3 ± 0.93	***	0.35
		<i>zfl2</i>	75	86.6 ± 1.28	79.7 ± 0.67	***	0.26
HLN	OH43a	<i>zfl1</i>	61	16.1 ± 0.67	9.65 ± 0.63	***	0.53
		<i>zfl2</i>	49	16.1 ± 0.76	11.5 ± 0.93	***	0.33
	M01	<i>zfl1</i>	64	16.3 ± 1.13	11.4 ± 0.97	**	0.15
		<i>zfl2</i>	76	16.3 ± 0.98	12.9 ± 0.66	*	0.12
LBN	H95a	<i>zfl1</i>	39	4.90 ± 0.39	4.17 ± 0.51	—	0.07
		<i>zfl2</i>	69	4.90 ± 0.47	3.05 ± 0.34	**	0.14
	OH43a	<i>zfl1</i>	64	4.61 ± 0.28	3.53 ± 0.29	*	0.14
		<i>zfl2</i>	51	4.61 ± 0.21	3.00 ± 0.30	***	0.46
	M01	<i>zfl1</i>	66	4.18 ± 0.41	2.27 ± 0.35	**	0.21
		<i>zfl2</i>	80	4.18 ± 0.36	2.04 ± 0.23	***	0.26
LDE	H95a	<i>zfl1</i>	39	16.7 ± 0.40	13.7 ± 0.52	***	0.49
		<i>zfl2</i>	69	16.7 ± 0.41	14.1 ± 0.30	***	0.29
	OH43a	<i>zfl1</i>	64	14.7 ± 0.33	12.7 ± 0.34	**	0.24
		<i>zfl2</i>	51	14.7 ± 0.27	13.8 ± 0.38	*	0.16
UDE	H95a	<i>zfl1</i>	39	20.6 ± 0.48	16.8 ± 0.62	***	0.52
		<i>zfl2</i>	69	20.6 ± 0.52	16.2 ± 0.37	***	0.42
	OH43a	<i>zfl1</i>	64	18.3 ± 0.26	15.2 ± 0.27	***	0.57
		<i>zfl2</i>	51	18.3 ± 0.23	15.8 ± 0.33	***	0.64
BLN	H95a	<i>zfl1</i>	39	4.60 ± 0.35	5.67 ± 0.46	*	0.16
		<i>zfl2</i>	69	4.60 ± 0.39	6.79 ± 0.29	**	0.24
	OH43a	<i>zfl1</i>	64	5.72 ± 0.24	5.24 ± 0.25	—	0.03
		<i>zfl2</i>	51	5.72 ± 0.24	5.56 ± 0.34	—	0.01
TBN	H95a	<i>zfl1</i>	39	9.30 ± 1.30	16.8 ± 1.68	**	0.32
		<i>zfl2</i>	69	9.30 ± 1.77	18.9 ± 1.28	**	0.24
	OH43a	<i>zfl1</i>	62	5.00 ± 0.91	10.4 ± 0.89	***	0.33
		<i>zfl2</i>	49	5.00 ± 1.07	13.8 ± 1.36	***	0.56
	M01	<i>zfl1</i>	65	0.64 ± 0.75	9.33 ± 0.65	***	0.60
		<i>zfl2</i>	80	0.64 ± 0.85	10.9 ± 0.54	***	0.61
KRN	H95a	<i>zfl1</i>	34	14.3 ± 0.95	15.5 ± 1.12	—	0.10
		<i>zfl2</i>	61	14.3 ± 0.87	17.9 ± 0.53	**	0.23
	OH43a	<i>zfl1</i>	60	14.4 ± 0.46	15.3 ± 0.42	—	0.07
		<i>zfl2</i>	47	14.4 ± 0.43	17.0 ± 0.51	**	0.33
	M01	<i>zfl1</i>	63	11.9 ± 0.57	16.1 ± 0.44	***	0.37
		<i>zfl2</i>	74	11.9 ± 0.57	15.5 ± 0.35	***	0.30
TRNK	M01	<i>zfl1</i>	66	5.33 ± 0.26	6.13 ± 0.24	—	0.08
		<i>zfl2</i>	80	5.33 ± 0.23	5.99 ± 0.16	*	0.09

Definitions are as for Tables 2–4.

TABLE 6
Traits associated with *zfl1* and *zfl2* in populations carrying independent *zfl1* mutations

Trait	Pop.	Gene	<i>n</i>	<i>mum/mum</i>	WT/WT	<i>P</i> value	<i>r</i> ²
LN	IL	<i>zfl1</i>	221	16.7 ± 0.17	16.7 ± 0.19	—	0
		<i>zfl2</i>	221	18.0 ± 0.19	15.6 ± 0.16	***	0.28
	M02-1	<i>zfl1</i>	151	22.6 ± 0.28	22.6 ± 0.22	—	0
		<i>zfl2</i>	151	24.5 ± 0.28	21.7 ± 0.24	***	0.29
DTP	IL	<i>zfl1</i>	206	63.0 ± 0.47	63.2 ± 0.51	—	0
		<i>zfl2</i>	206	64.0 ± 0.58	62.0 ± 0.38	*	0.04
DTS	IL	<i>zfl1</i>	200	66.2 ± 0.46	67.0 ± 0.65	—	0.01
		<i>zfl2</i>	200	69.9 ± 0.70	64.3 ± 0.40	***	0.19
LBN	IL	<i>zfl1</i>	221	2.2 ± 0.10	2.1 ± 0.11	—	0
		<i>zfl2</i>	221	2.5 ± 0.11	1.8 ± 0.10	***	0.12
	M02-1	<i>zfl1</i>	151	2.4 ± 0.20	2.4 ± 0.16	—	0.01
		<i>zfl2</i>	151	3.1 ± 0.20	1.8 ± 0.17	***	0.15
LDE	IL	<i>zfl1</i>	221	10.3 ± 0.15	10.6 ± 0.17	—	0.01
		<i>zfl2</i>	221	11.3 ± 0.18	9.6 ± 0.14	***	0.21
	M02-1	<i>zfl1</i>	151	16.3 ± 0.27	16.4 ± 0.22	—	0
		<i>zfl2</i>	151	18.4 ± 0.27	15.1 ± 0.24	***	0.36
UDE	IL	<i>zfl1</i>	221	11.5 ± 0.14	11.6 ± 0.16	—	0
		<i>zfl2</i>	221	12.9 ± 0.17	10.4 ± 0.14	***	0.38
	M02-1	<i>zfl1</i>	151	17.7 ± 0.30	17.8 ± 0.24	—	0.01
		<i>zfl2</i>	151	20.5 ± 0.30	15.9 ± 0.26	***	0.47
TBN	IL	<i>zfl1</i>	220	8.6 ± 0.36	7.3 ± 0.40	*	0.01
		<i>zfl2</i>	220	2.7 ± 0.42	10.9 ± 0.34	***	0.50
	M02-1	<i>zfl1</i>	151	11.4 ± 0.62	11.3 ± 0.51	—	0
		<i>zfl2</i>	151	2.8 ± 0.63	15.5 ± 0.54	***	0.54
KRN	IL	<i>zfl1</i>	185	13.7 ± 0.32	13.9 ± 0.35	—	0
		<i>zfl2</i>	185	13.4 ± 0.42	13.5 ± 0.25	**	0.05
	M02-1	<i>zfl1</i>	119	15.9 ± 0.42	15.9 ± 0.33	—	0
		<i>zfl2</i>	119	14.1 ± 0.47	17.6 ± 0.31	***	0.25

All definitions are as for Tables 2–4.

higher KRN than double-mutant plants. KRN showed significant association with *zfl1* only in the M01 *zfl1* subset, where plants carrying two wild-type *zfl1* copies averaged 35% higher KRN than double-mutant plants. TRNK was significantly associated with *zfl2* in the M01 subset, where homozygous wild-type plants had 11% higher TRNK than double mutants. *zfl1* was associated with a similar trend that was not statistically significant, although this is likely due to higher variance because fewer plants were in this subset. Plants homozygous wild type for *zfl1* had 13% higher TRNK than double-mutant plants, a slightly larger effect than that observed for *zfl2*.

Lines carrying independent *zfl1* mutations: Results from two populations, IL and M02-1, shed light on the important question whether *zfl* activity itself, as opposed to segregation of a linked gene or genes, was responsible for the observed trait associations. The IL and M02-1 populations carried independent putative null alleles of *zfl1* (see MATERIALS AND METHODS). As expected, in both populations, phenotypically double-mutant plants segregated at 1/4 instead of 1/16 as observed in populations with functional *zfl1* alleles.

In both the IL and M02-1 populations, significant associations were observed with *zfl2* genotype for numerous traits (Table 6). In contrast, only one trait, DTS, showed a statistically significant trend for *zfl1*. The DTS trend observed, however, was only marginally significant, and was opposite to that observed in wild-type populations (lower DTS was associated with increasing *zfl1*–*mum* alleles; Table 6). Overall, these results are consistent with the hypothesis that the trends associated with *zfl1* genotype in the segregating populations require *zfl1* activity rather than being caused by segregation of a linked gene or genes.

Maize/teosinte hybrid populations

To determine whether *zfl2* activity is required for the chromosome 2 ear rank QTL that differentiates maize and teosinte (DOEBLEY and STEC 1991, 1993), we generated four parallel maize/teosinte hybrid populations: two of the four populations (T1W and T2W) segregated wild-type maize *zfl2* alleles, and each was paired with a parallel population (T1m and T2m) that

TABLE 7
Significant associations in maize/teosinte hybrid populations

Trait	Group	Marker	Bin	<i>n</i>	<i>P</i>	<i>r</i> ²	<i>d/a</i>	High class
uRNK	T1W	<i>zfl2</i>	2.02	192	*	0.04	-0.40	M
		<i>bnlg1175</i>	2.04	192	**	0.04	-0.07	M
	T2W	<i>zfl2</i>	2.02	166	**	0.07	0.32	M
		<i>mmc0231</i>	2.03	167	*	0.04	-0.60	M
sRNK	T1W	<i>umc1165</i>	2.01	193	*	0.03	0.24	M
		<i>zfl2</i>	2.02	193	***	0.09	0.14	M
		<i>mmc0231</i>	2.03	191	**	0.05	0.23	M
		<i>bnlg1175</i>	2.04	193	***	0.10	-0.05	M
	T2W	<i>zfl2</i>	2.02	166	**	0.09	-0.06	M
		<i>mmc0231</i>	2.03	167	*	0.04	-0.61	M
	T2m	<i>zfl2</i>	2.02	145	*	0.04	17.5	H
		<i>mmc0231</i>	2.03	145	*	0.05	7.60	H
BLN	T1W	<i>umc1165</i>	2.01	193	*	0.04	0.90	M
		<i>zfl2</i>	2.02	193	***	0.10	0.29	M
		<i>mmc0231</i>	2.03	193	**	0.08	0.55	M
		<i>bnlg1175</i>	2.04	193	*	0.04	0.76	M
	T1m	<i>zfl2</i>	2.02	178	**	0.05	-0.45	T
		<i>mmc0231</i>	2.03	175	*	0.04	-0.54	T
	T2m	<i>mmc0231</i>	2.03	148	**	0.08	-1.91	T

Marker, genetic marker used; bin, approximate region of maize genetic map; *n*, number of individuals in analysis; high class, genotype class with highest trait value: M, homozygous maize; T, homozygous teosinte; H, heterozygous; uRNK, ear rank of terminal ear of uppermost branch; sRNK, ear rank of terminal ear of second branch from top (below uppermost); BLN, blank vegetative nodes between uppermost lateral branch and tassel. For other definitions see Table 2 legend. Only significant trends are shown. For trait values and remaining traits, see supplemental materials at <http://www.genetics.org/supplemental/>.

carried the same teosinte allele (T1 or T2), but segregated the *zfl2-mum1* allele from maize (BOMBLIES *et al.* 2003). The *zfl2-mum1* line used had been backcrossed for six generations into the W22 inbred line to maximize similarity between maize *zfl2* wild type and *zfl2-mum1* carrying chromosomes used in parallel populations. However, since the extent of the introgression is unknown, the degree of genetic differences between these chromosomes is not known.

Trait associations: We tested for associations between ear rank of both the terminal ear of the uppermost lateral branch (uRNK) and the terminal ear of the second branch from the top (the branch below the uppermost branch; sRNK), with markers in the previously described QTL regions on chromosome 2. We observed statistically significant associations between chromosome 2 markers, including *zfl2* and flanking markers, in the T1W and T2W populations (Table 7). In contrast, no significant ear rank associations were observed in population T1m. In T2m, two markers on chromosome 2 showed statistically significant ear rank associations, but these were associated with overdominant trends and the *zfl2-mum1* homozygous maize class did not significantly differ from the teosinte homozygous class (Table 7). This effect may suggest a more complex interaction. For example, there is perhaps a *trans*-effect on ear rank due to a factor or factors linked

to *zfl2-mum1* on the maize chromosome that require *zfl2* activity, but can act through the teosinte *zfl2* allele when it is present.

BLN also showed statistically significant associations with chromosome 2 marker genotypes in populations T1W and T1m. In T1W, BLN was higher in plants homozygous for maize alleles at chromosome 2 markers, but in T1m, the teosinte homozygous class averaged higher BLN (Table 7). This suggests that the teosinte *zfl2* allele may also promote higher BLN, but to a lesser degree than the maize allele. T2m showed a similar BLN association trend as T1m, but no significant BLN association was observed in T2W, suggesting that this trait may be sensitive to differences in genetic background or linked genes on chromosome 2 itself.

QTL analysis: We used QTL mapping to further analyze the genetic effects associated with chromosome 2 in the maize-teosinte hybrid populations. We detected statistically significant sRNK QTL in populations T1W and T2W (Table 8). In each case maize alleles were associated with higher ear rank (the more maize-like phenotype). We did not detect QTL for ear rank in either of the populations carrying mutant maize *zfl2* alleles, consistent with the hypothesis that *zfl2* activity is important for the chromosome 2 ear rank effect.

The T2W QTL peak for sRNK is centered over the *zfl2* locus as expected, but in T1W, the QTL is centered over

TABLE 8
Significant QTL identified in maize/teosinte hybrid populations

Trait	Group	Position	Near marker	LOD	Cutoff	Add.	r^2
BLN	T1W	26.01	<i>zfl2</i>	2.96	2.66	3.17	0.05
sRNK	T1W	48.51	bnlg1175	4.63	2.74	3.86	0.10
	T2W	19.51	<i>zfl2</i>	3.32	2.89	3.33	0.10

Traits: BLN, blank vegetative nodes between uppermost lateral branch and tassel; sRNK, ear rank of terminal ear of second branch from top (below uppermost); Position, position in centimorgans of center of QTL peak on linkage map generated for SSR markers; near marker, closest genetic marker; LOD, LOD score of QTL; cutoff, significance established by 1000 permutations of the data for each trait; Add, additive QTL effect. T1W marker locations: umc1165, 0cM; *zfl2*, 27.4 cM; mmc0231, 32.5 cM; bnlg1175, 50.3 cM. T2W marker locations: umc1227, 0 cM; *zfl2*, 19.5 cM; mmc0231, 31.4 cM; umc1026, 48.3 cM.

bnlg1175 instead (Table 8). However, when marker-genotype associations were examined for each of the markers individually, we observed that in T1W *zfl2* was associated with sRNK more strongly ($r^2 = 0.09$) than the flanking markers, umc1165 ($r^2 = 0.03$) and mmc0231 ($r^2 = 0.05$), while bnlg1175 was more strongly associated with sRNK ($r^2 = 0.10$) than its neighboring locus mmc0231. This observation suggests that the location of the single QTL peak may be a statistical artifact, and suggests that two or more chromosome 2 factors may affect ear rank in this population. However, since no significant associations or QTL were observed in the parallel population, T1m, the chromosome 2 ear rank effect in this population, even if genetically complex, appears to be influenced by maize *zfl2* activity.

A significant QTL for BLN was detected in population T1W (Table 8), but not in the parallel population lacking *zfl2* activity (T1m). This result is consistent with association effects that suggest that *zfl2* activity is important for the BLN effect on chromosome 2 in population T1W. However, no BLN QTL were detected in populations T2W or T2m, suggesting genetic variability for this trait.

DISCUSSION

Associations suggest partially conserved pleiotropic *zfl* functions: In this study we analyzed associations between quantitative phenotypic variation in maize F₂ populations and varying wild-type copy number of the duplicate maize *FLO/LFY* orthologs *zfl1* and *zfl2*. We observed associations with multiple traits, suggesting that these genes have pleiotropic functions. We caution, however, that while the lack of associations with *zfl1* in populations carrying independent *zfl1* mutations strongly suggests that the observed associations are indeed due to activity of the *zfl* genes themselves, further experiments will be necessary to ascertain that the pleiotropic functions suggested by the *zfl*-associated trends actually reside within the *zfl1* and *zfl2* loci.

We observed associations of *zfl1* (and *zfl2* to a lesser degree) genotype with flowering time. While the effect is relatively weak, this may be explained by the fact that

many genes appear to affect flowering time in maize (CHARDON *et al.* 2004). A role in flowering time has also been demonstrated for *LFY* in Arabidopsis with a dosage series experiment that clearly demonstrated that increasing functional *LFY* copies results in a quantitative dosage-dependent acceleration of flowering time analogous to what we observed in maize (BLÁZQUEZ *et al.* 1997). Recently, a meta-analysis of maize flowering time QTL has implicated the *zfl1* region in controlling flowering time variation among maize lines, suggesting *zfl1* as a candidate locus for this trait (CHARDON *et al.* 2004).

We also observed quantitative associations that implicate *zfl1* and *zfl2* in various aspects of branching. Lateral branch or ear shoot number and tassel branch number were primarily associated with *zfl2*, while branch placement was associated with both genes. The *zfl* genes are associated with increased lateral branch number in the tassel, but decreased lateral branch number during vegetative phases. This suggests that the effects of the *zfl* genes on branching are complex and depend on other genes. This is not surprising, as *FLO/LFY* orthologs from other species are associated with similarly complex effects on branching: the flower to shoot conversion observed in *flo/lfy* mutants in numerous species suggests that the wild-type role of these proteins is to repress branching by conferring floral identity onto lateral structures (COEN *et al.* 1990; WEIGEL *et al.* 1992; HOFER *et al.* 1997; SOUER *et al.* 1998; MOLINERO-ROSALES *et al.* 1999; AHEARN *et al.* 2001). In rice, *RFL* has been proposed to be involved in suppression of inflorescence branch formation (KYOZUKA *et al.* 1998), while in Arabidopsis, *LFY* appears to be required for inflorescence branch initiation in a *filamentous flower* mutant background (SAWA *et al.* 1999).

Our data showed an association between the *zfl* genes, especially *zfl2*, and increased numbers of grain rows around the circumference of the ear. A role for *FLO/LFY*-like genes in controlling inflorescence phyllotaxy has not been reported in other species. However, *FLO/LFY* orthologs are involved in promoting whorled organ phyllotaxy during flower development in diverse species (COEN *et al.* 1990; WEIGEL *et al.* 1992; HOFER *et al.*

1997; SOUER *et al.* 1998; MOLINERO-ROSALES *et al.* 1999; AHEARN *et al.* 2001), and in tobacco the *FLO/LFY* ortholog *NFL1* is also involved in controlling leaf phyllotaxy (AHEARN *et al.* 2001). Thus, our results suggest that a basic role of *FLO/LFY* orthologs in phyllotaxy may have been appropriated for a novel role in maize inflorescence phyllotaxy, perhaps through an expression pattern change.

Pleiotropic functions for *FLO/LFY* orthologs have been previously described in other species. For example, in addition to their roles in flower development and reproductive timing, *FLO* and *LFY* orthologs in some species also play roles in leaf compounding (HOFER *et al.* 1997; DEMASON and SCHMIDT 2001), branching (SAWA *et al.* 1999; BOMBLIES *et al.* 2003), and shoot meristem organization (AHEARN *et al.* 2001). Pleiotropy of this sort has long been recognized as a common feature of developmental regulatory genes (CASPARI 1952; LANDE 1980). It has previously been suggested that selection pressure acting on traits controlled by pleiotropic genes may cause neutral or even detrimental traits to be selected due to correlation with beneficial traits (MITCHELL-OLDS 1996a,b; CONNER 2002; ALBERTSON *et al.* 2003). This situation may place limits on the degree to which a particular gene can be selected for its effect on a beneficial trait (LANDE 1980; MAYNARD SMITH *et al.* 1985). In the case of *zfl2*, the quantitative pleiotropic associations we observe suggest that lines selected for higher kernel row number via *zfl2* would likely also flower slightly earlier, average fewer ears placed lower on the plant, and have more blank vegetative nodes. Thus, some domestication-associated traits, such as an increase in blank node number in maize, may be by-products of selection for resource allocation to fewer and larger ears that may have acted in part through *zfl2*. If any of the secondary effects associated with *zfl2* were undesirable to early agriculturalists, these would limit the degree to which *zfl2* might have been selected for its useful effects on ear rank or reduced ear number.

Functional divergence of *zfl1* and *zfl2*: Duplicate genes play a potentially important role in evolution since one or both redundant paralogs may be released from selective constraint for essential functions and thus provide potential “raw material” for evolution (OHNO 1970). Several models of duplicate gene evolution posit that most duplicates are likely to be lost through deleterious mutations, while some gene pairs may be retained via evolution of novel functions or subdivision of a complex ancestral function (FORCE *et al.* 1999; LYNCH and CONERY 2000).

The maize genome contains many duplicate regions (AHN and TANKSLEY 1993; BERHAN *et al.* 1993; DEVOS and GALE 1997; GALE and DEVOS 1998) due to a genome duplication thought to have occurred ~11 million years ago in the lineage leading to maize and its relatives (GAUT and DOEBLEY 1997). The maize *zfl* genes fall into a class of retained duplicates with largely redundant functions, as severe morphological defects are observed

only in *zfl1/zfl2* double-mutant plants (BOMBLIES *et al.* 2003). Despite their functional redundancy in flower development, we observed several differences in quantitative trends associated with the *zfl* genes. For example, *zfl1* was more strongly associated with aspects of flowering time, while *zfl2* was more strongly associated with trends affecting lateral branch number and ear phyllotaxy. These results suggest that *zfl1* and *zfl2* may be evolving subtle differences in function, perhaps through partitioning of a modular ancestral function as predicted by the sub-functionalization model for duplicate gene evolution (FORCE *et al.* 1999).

***zfl2* and maize domestication:** One of the major differences between maize and teosinte is the phyllotaxy of the ear; teosinte ears are exclusively two-ranked, while maize ears have four or more ranks (MANGELSDORF and REEVES 1939). LANGHAM (1940) argued that a single gene, *two-ranked* (*tr*), controls the difference between the two-ranked condition in teosinte and the higher-ranked condition of maize. A major effect region controlling ear rank was later localized to the short arm of chromosome 2 by introgressing this region from *Tripsacum dactyloides* (MAGIURE 1961; GALINAT 1973), a species more distantly related to maize than teosinte, but also having two-ranked ears. Plants homozygous for the *Tripsacum* chromosome 2 introgression had (among other traits) fewer tassel branches, fewer total nodes, fewer rows of ovules (lower ear rank), and more ear shoots than siblings heterozygous or homozygous for the maize chromosome 2 (MAGIURE 1961). Interestingly, many of these trends are similar to trends we observed in plants carrying mutations in *zfl2*. More recently, QTL mapping experiments have also strongly implicated the chromosome 2 region within which *zfl2* maps in the increase in ear rank associated with maize domestication (DOEBLEY and STEC 1991, 1993). Together, these observations suggest the hypothesis that an increase in *zfl2* activity during maize domestication may be responsible for multiple traits associated with chromosome 2, including the ear rank effect. As we have previously suggested (BOMBLIES *et al.* 2003), *zfl* may control phyllotaxy through effects on inflorescence meristem size and patterning.

To test the hypothesis that *zfl2* activity might underlie this domestication QTL, we performed a QTL complementation test (DOEBLEY *et al.* 1995; MACKAY 2001). Ear rank showed associations with chromosome 2 only in hybrid populations that segregated for wild-type maize *zfl2* alleles, and not in populations that segregated only teosinte and mutant maize *zfl2* alleles, suggesting that the teosinte *zfl2* alleles in these populations may be equivalent to loss-of-function maize *zfl2* alleles in terms of their effect on ear rank. The association and QTL mapping results support the hypothesis that a functional maize *zfl2* allele is important for the chromosome 2 ear rank QTL effect. We caution that these results do not necessarily indicate that the *zfl2* gene itself was under selection during maize domestication, as they do

not preclude the possibility that a linked gene acting through *zfl2* was selected. An important implication, however, is that activity of *zfl2*- or a *zfl2*-dependent developmental pathway appears to be important for a large-effect maize domestication QTL.

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