

GBS identification of genomic loci associated with polyembryony in *ig1* mutants



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SUMMARY

The *LBD6* or *ASYMMETRIC LEAVES2* gene in maize is an important regulator in the transition from proliferation to differentiation in embryo sac development (Evans, 2007). The mutant, also known as *indeterminate gametophyte1* (*ig1*), produces embryo sacs which lack the ability to limit proliferation, leading to structural defects. These defects include embryo sacs with extra egg cells, extra central cells, and extra polar nuclei, which give rise to abnormal seed phenotypes post-fertilization. Extra egg cells in an embryo sac can be fertilized producing kernels with multiple viable embryos.

The *ig1-O* mutation results in different frequencies of multiple embryos, or 'twinning,' in different maize inbred backgrounds. Confocal microscopy confirms that Mo17 embryo sacs carrying the *ig1-O* allele have a significantly higher frequency of multiple egg cells than *ig1-O* embryo sacs in a B73 inbred background. The frequency of twinning is intermediate in the *ig1-O/+* Mo17/B73 hybrid compared to *ig1-O* inbred lines. This suggests that a genetic modifier(s) may be responsible for the difference in phenotypic frequency between these inbred lines.

Genotyping By Sequencing (GBS) data analysis and initial SSR marker fine mapping indicate broad enhancers of twinning on chromosomes 3 and 9, as well as narrow regions elsewhere.

Carrying the Mo17 allele appears to be sufficient to increase the frequency of twinning caused by the *ig1-O* mutation.

BACKGROUND

The *indeterminate gametophyte* mutation was found in W23 inbreds and named by Kermicle (1969).

Matt Evans (2007) found that the incidence of twinning varies with background; in particular, *ig1* Mo17 has a much higher incidence compared to *ig1* B73 and others.

Table 1. Phenotypic Severity of <i>ig1-O</i> and <i>ig1-mum</i>							
	Normal Endosperm		Absent or Abnormal Embryo		Miniature Endosperm		Homozygous Phenotype
	One Embryo	Two or More Embryos	One Embryo	Two or More Embryos	Aborted/Collapsed Endosperm	Ovules without Seeds	
<i>ig1-O</i>	24	3	1	13	1	12	46 Male-fertile
W23 (656)	34	2	3	9	0	25	27 Male-sterile
W64a (793)	46	6	2	7	1	18	20 Male-sterile
Mo17 (948)	53	11	2	6	2	11	15 Male-sterile
B73 (762)	62	1	2	1	0	7	27 ND
W23 (760)	72	0	0	2	1	2	24 Male-fertile
<i>ig1-mum</i>	70	4	3	3	2	3	15 Variable male sterility
Mo17 (775)	70	4	3	3	2	3	15 Variable male sterility

Values shown are percentages of mutant ovules. Values shown in boldface represent normal seeds. Numbers in parentheses indicate total ovules (with and without seeds) examined. Inbred lines are ordered from the most to the least severe based on lowest to highest frequency of normal seed production (i.e., highest to lowest penetrance of combined ovule failure and seed defects). ND, not determined.

Phenotypes

The *ig1* mutant produces many seeds with twin embryos (as well as triplets and other abnormalities).



Twin seedlings grow from an *ig1* mutant twin-embryo seed.

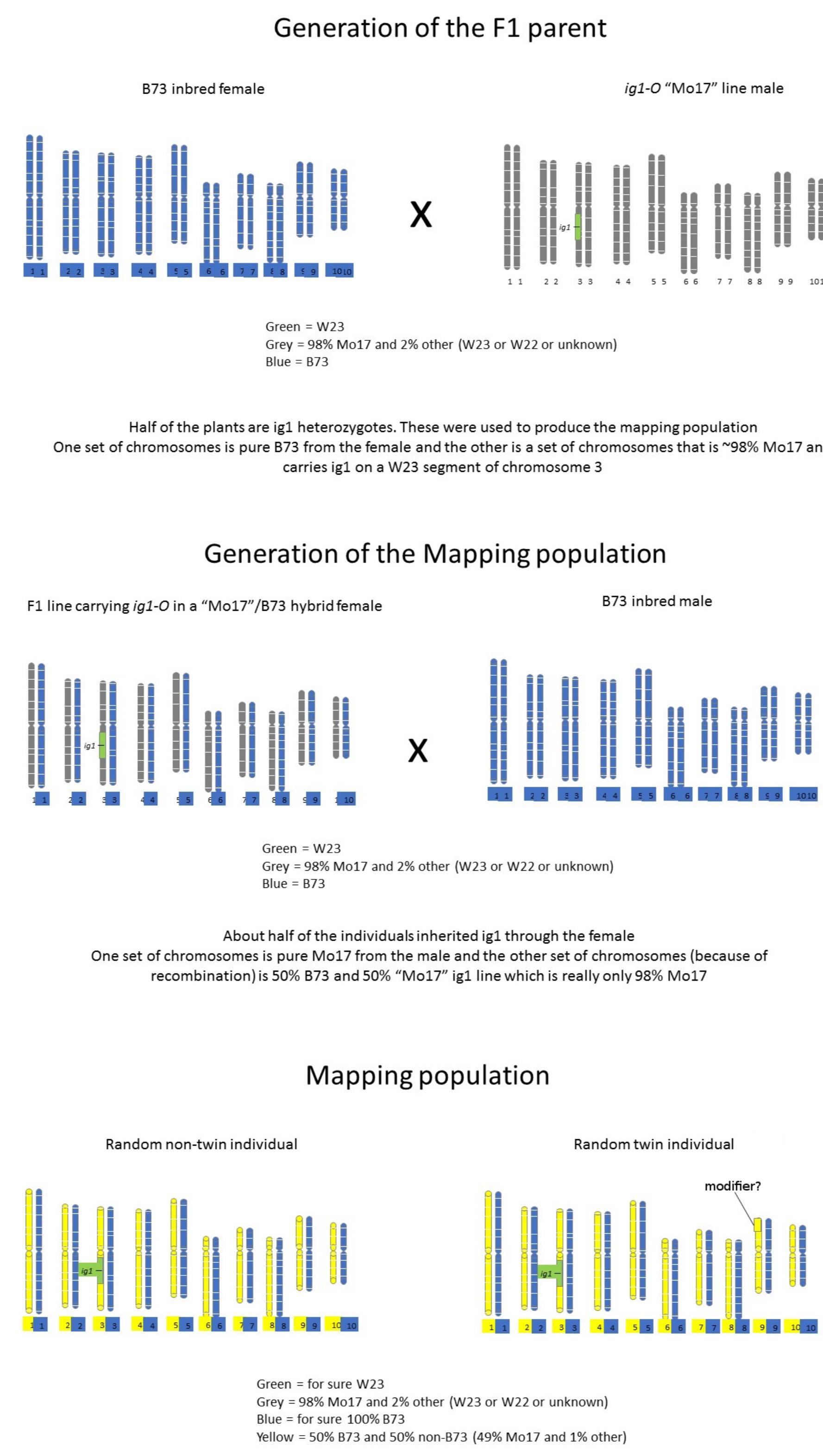
Rate of occurrence

The Mo17/B73 hybrid *ig1* mutants exhibit a twinning phenotype at a frequency intermediate between the frequency of twinning in individual inbred lines.

<i>ig1</i> inbred lines	# seeds	twins
B73	1372	0.5%
Mo17/B73 hybrid	5217	4.2%
Mo17	840	8.5%

This suggests a model in which B73 and Mo17 differ for a single modifier with a major effect on twinning (the Mo17 allele of this modifier promoting twinning in *ig1* mutants).

GBS MAPPING POPULATION



GBS RESULTS

Mapping and SNP statistics

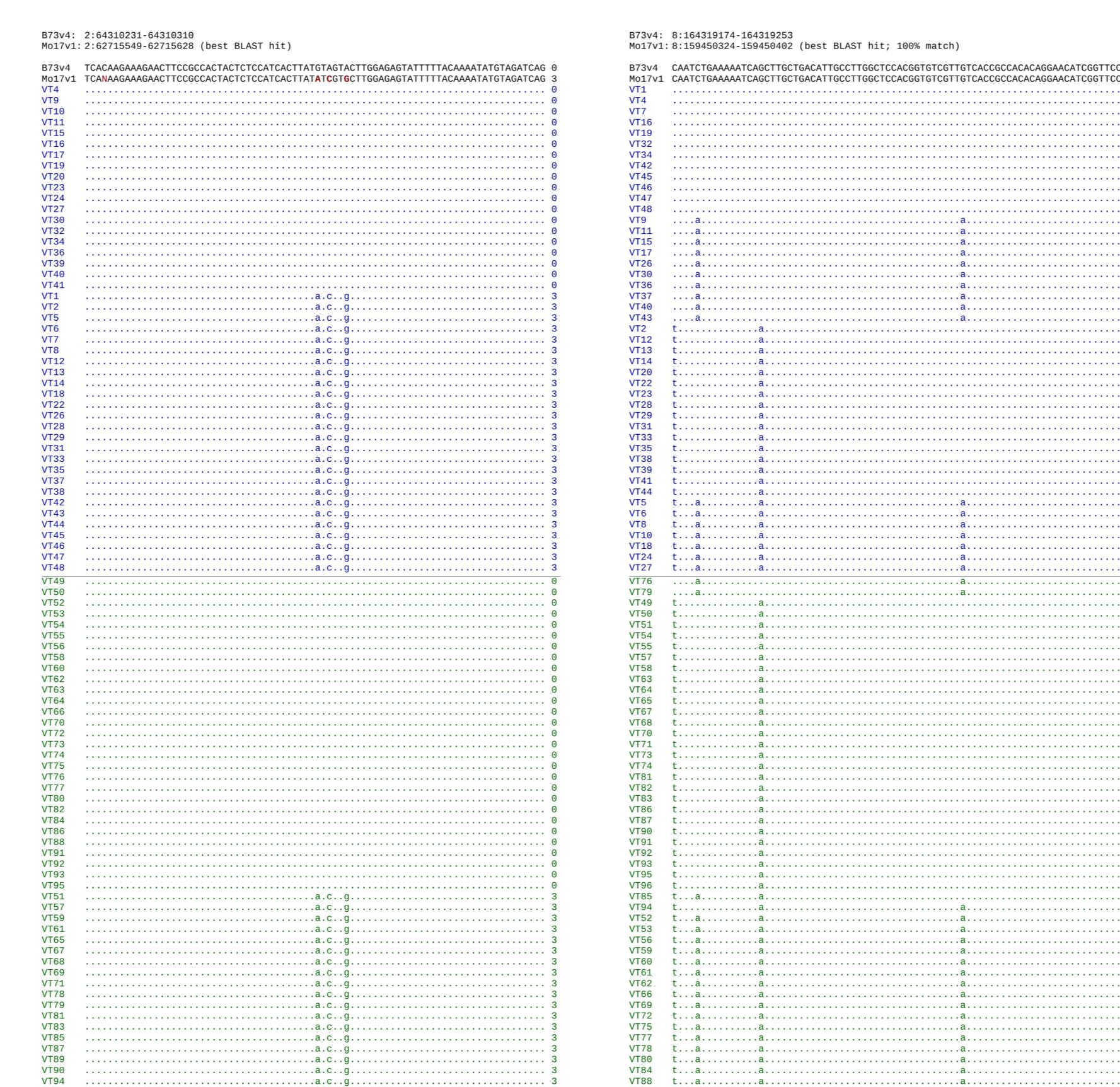
45 sequenced samples had non-twin phenotype, 48 had twin phenotype.

93 samples	mean	s.d.	max	min
mapped reads	3,633,114	676,410	5,204,340	1,956,272
# SNPs	85,118	16,892	132,620	54,132
avg. depth	8.79	1.06	11.02	6.04
# > 1x	2.86%	0.24	3.61%	2.27%
# > 4x	1.62%	0.15	1.96%	1.10%
Het rate	0.026%	0.005	0.039%	0.017%

The GBS sequencing was done by Novogene. The reads were mapped against the B73v4 genome. Positions with at least four reads qualified for SNP calls.

Example non-segregated and segregated loci

In these regions roughly half of the samples have heterozygous SNP calls:



Twin samples (lower, green) do not segregate from non-twin samples (upper, blue) in this region. The three SNPs match the corresponding Mo17v1 region.

Twin samples (lower, green) segregate significantly from non-twin samples (upper, blue) in this region, with more twins carrying three SNPs (which don't match Mo17v1).

Fisher's exact test

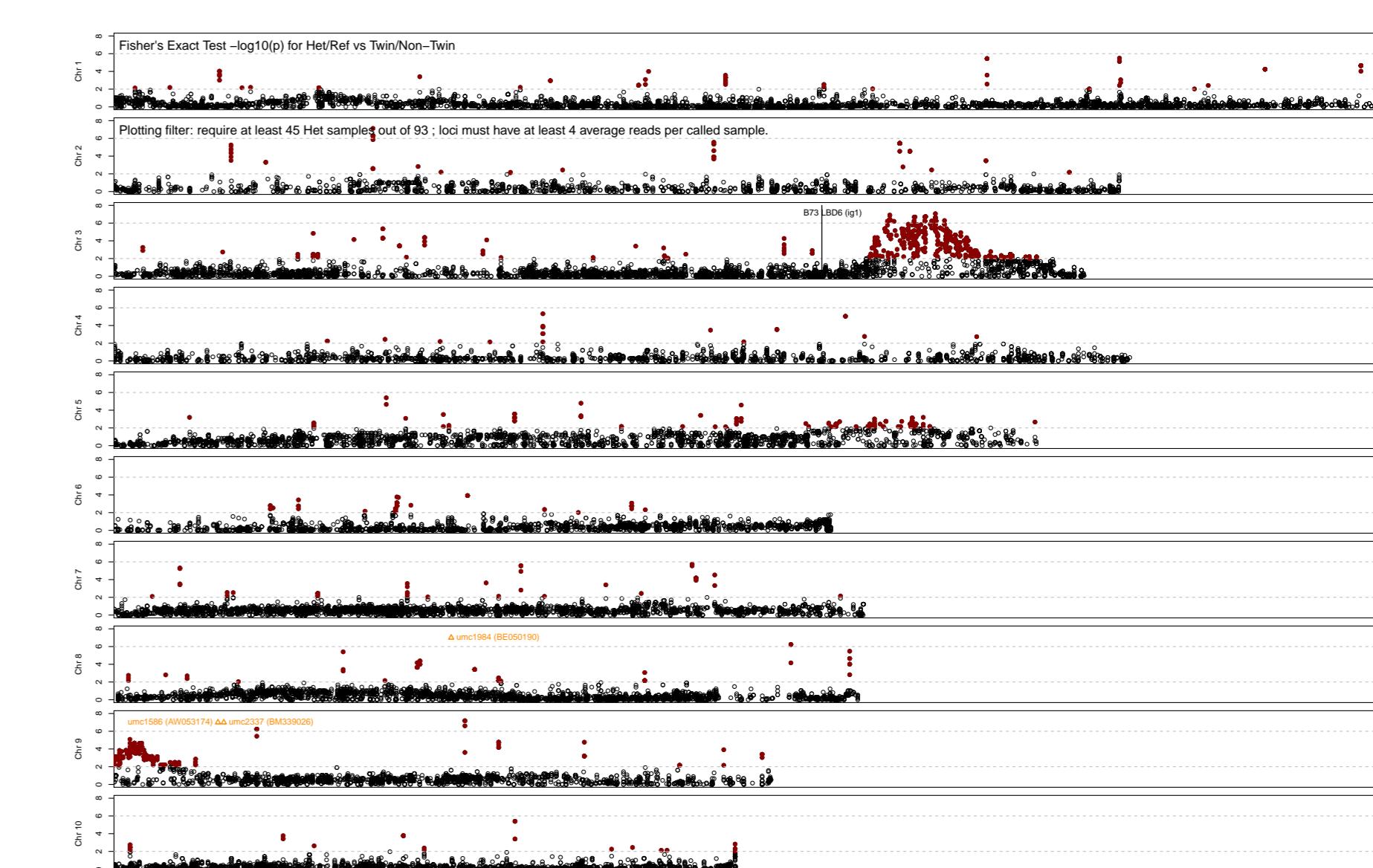
Each SNP location on the genome provides a 2 X 2 contingency table, appropriate for Fisher's Exact Test for the null hypothesis that the number of Het calls does not differ between the twin and non-twin groups. Fisher's Exact Test was applied at every location, generating a *p*-value each time. For example:

2:28422677	reads	no call	het call
non-twin	417	27	18
twin	773	7	41
total	1190	34	59

Fisher's exact test on calls at this locus gives $p = 5.5 \times 10^{-6}$. The number of called samples, 59, and the average reads per called sample, $1190/59 = 20.2$, pass the filter used for the whole-genome Manhattan plot below.

Regions of segregation

$-\log_{10}(p)$ from Fisher's Exact Test was plotted where there were at least 4 reads per called sample and at least 45 Het calls out of 93 samples:



The position of the LBD6 (*ig1*) gene on chr 3 is indicated, as well as locations of three SSR markers that exhibit increased heterozygosity in twin samples. Red dots indicate significance at $p < 10^{-2}$. Dashed lines are drawn at $p = 10^{-2}$ and 10^{-6} .

Broad regions of segregation are seen on:

▪ **3:183,000,000..210,000,000** above the LBD6 locus, this broad region contains W23 and other contributions in addition to Mo17;

▪ **9:1..10,000,000** this region contains 223 annotated genes on B73v4, 158 with functional descriptions. The best-fit segregation peak is at 5,130,500;

as well as many narrow regions throughout the genome.

SSR MARKERS

For comparison, SSR markers on chromosomes 8 and 9 were applied to 73 DNA samples of *ig1* twins from different plants and progenitor wild-type inbred lines to identify the parental alleles for each locus. The primers for **umc1984** on chromosome 8, and **umc1586** and **umc2337** on chromosome 9 were polymorphic between B73 and Mo17 and showed the clearest bands. All three showed a significantly higher number of heterozygous samples than expected (χ^2 test: $p < 0.05, 0.01, 0.05$, respectively).

CONCLUSIONS

- An unusual use of GBS provides clues to the location of a modifier enhancing twinning in Mo17 *ig1* mutants.
- The Mo17 modification is very likely to at least have a component within 9:1..10,000,000.

References

- M. M. Evans. The *indeterminate gametophyte1* gene of maize encodes a LOB domain protein required for embryo Sac and leaf development. *Plant Cell*, 19(1):46–62, Jan 2007.
J. L. Kermicle. Androgenesis conditioned by a mutation in maize. *Science*, 166(3911):1422–1424, Dec 1969.
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