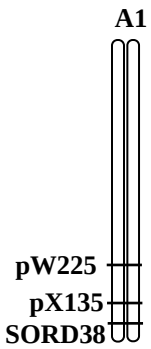
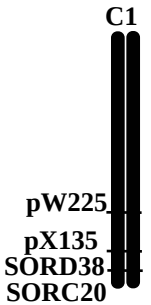
Supplemental Data. Gaeta et al. (2007). Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype.

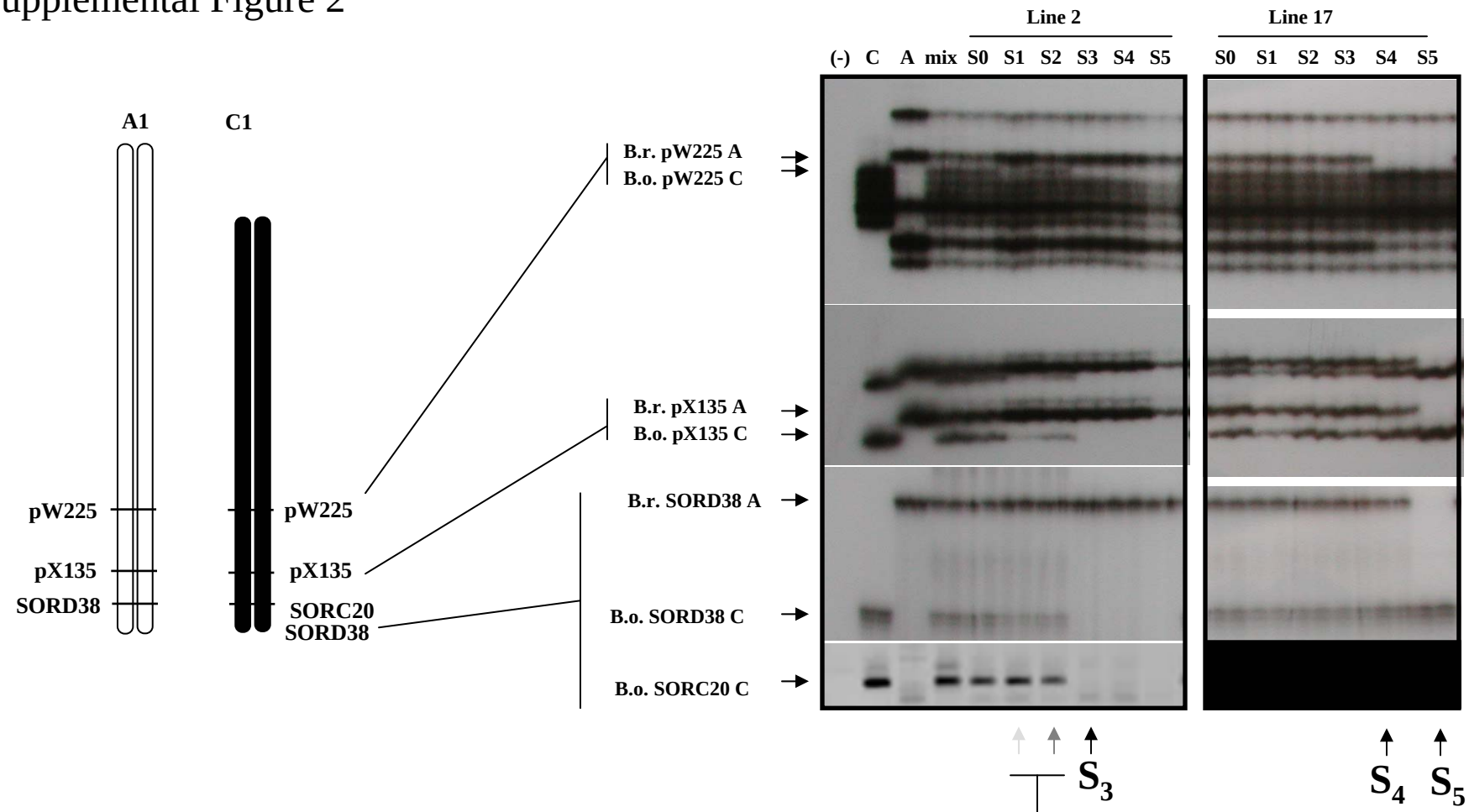




	Line 2						Line 26						Line 8						Line 22						Line 17						Line 48					
A1 Marker	**S0**	**S1**	**S2**	**S3**	**S4**	**S5**	**S0**	**S1**	**S2**	**S3**	**S4**	**S5**	**S0**	**S1**	**S2**	**S3**	**S4**	**S5**	**S0**	**S1**	**S2**	**S3**	**S4**	**S5**	**S0**	**S1**	**S2**	**S3**	**S4**	**S5**	**S0**	**S1**	**S2**	**S3**	**S4**	**S5**
pW225 A	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+
pX135 A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
SORD38 A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
C1 Marker																																				
pW225 C	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
pX135 C	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
SORD38 C	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
SORC20 C	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+

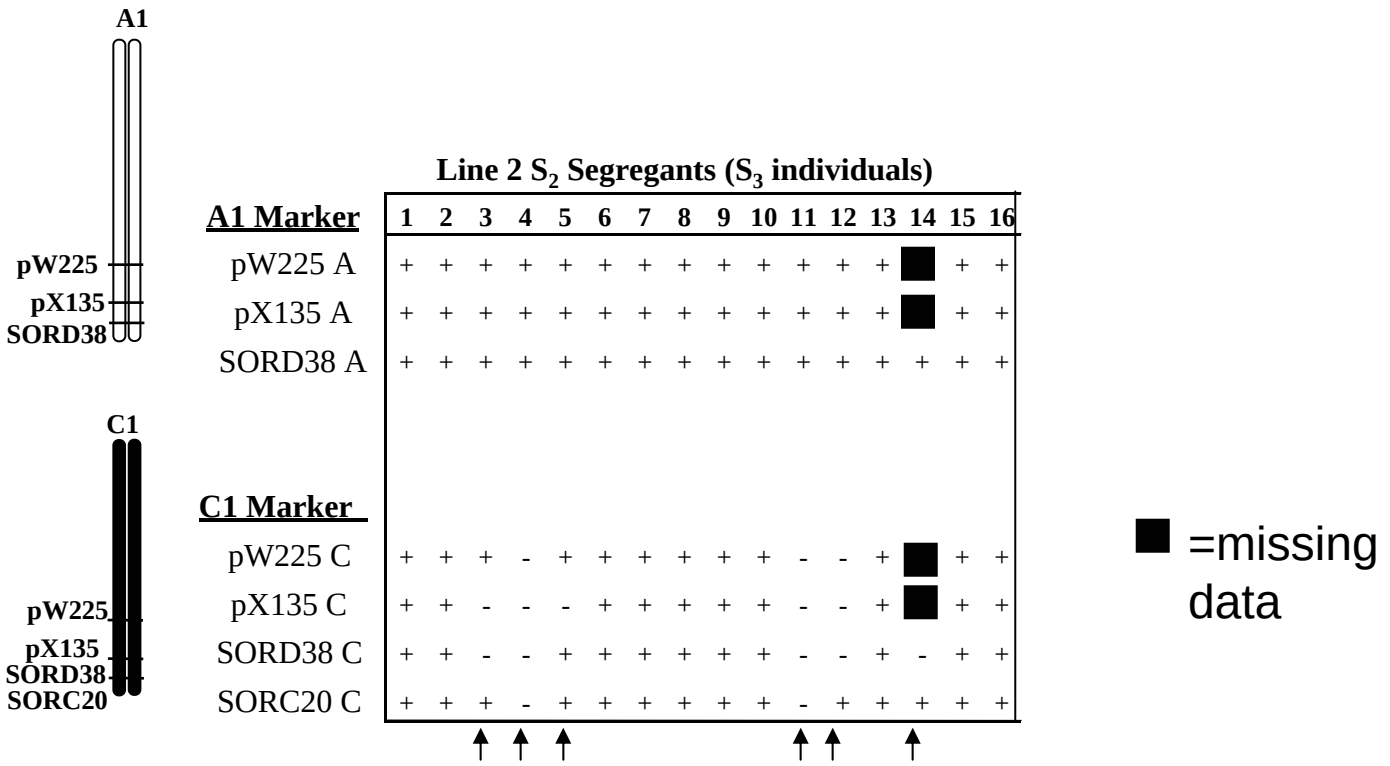
Supplemental Figure 1: Analysis of DNA samples across generations. Bulked DNA samples of 16 individuals/ line were genotyped. (+) and (-) refer to the presence or absence of expected fragments, respectively. Arrows indicate the generation in which a rearrangement was fixed in the marker analysis of bulked DNA. *pW225* and *pX135* were SSCP markers designed from RFLP probe sequences, and SORD38 and SORC20 are SSRs. *pW225* and *pX135* both showed loss/intensification patterns in southern blot analysis. PCR-SSCP of *pW225* detects the same loss/intensification patterns among homoeologous amplicons that RFLPs detect (see Figure 4 and Supplemental Figure 2). Note: Lines 17 and 22 both showed successive rearrangement over multiple generations. We tracked the rearrangements by following the loss of these PCR-based markers that are known to undergo HNRT based on RFLPs. Rearrangements can be detected as genetically fixed homoeologous non-reciprocal transpositions as early as the S_2 generation (bulked S_3 plants).

Supplemental Figure 2



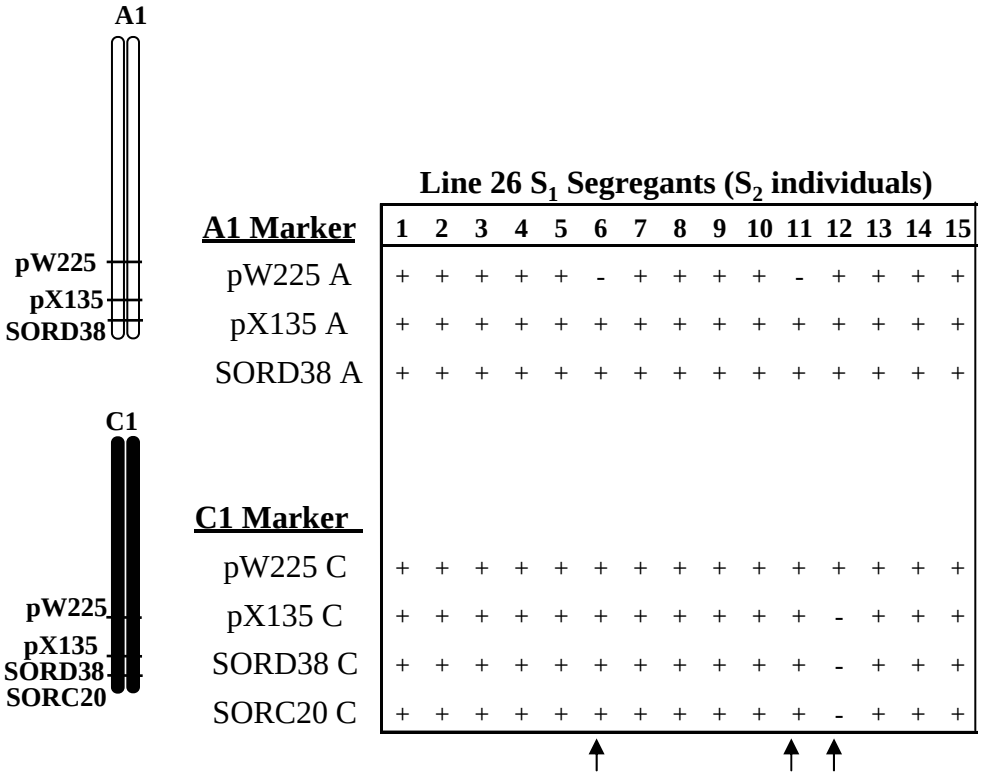
Supplemental Figure 2: DNA SSCP and SSR gel analyses of DNA samples across generations for two lineages. DNA from the diploid parents *Brassica rapa* (line IMB218; A genome) and *B. oleracea* (line TO1000; C genome) and a synthetic mix of parental DNAs were analyzed and compared to allopolyploid lineages across all generations (S_0-S_5). The example shown in the figure is for lineages 2 (S_0-S_5) and 17 (S_0-S_5). Arrows and generation designations at the bottom indicate the generations in which rearrangements became fixed in these lineages. See Supplemental Figure 1 for summary of markers used for screening and transgenerational genotyping for all lines analyzed.

Supplemental Figure 3



Supplemental Figure 3: DNA SSCP and SSR analysis of line 2 (S_2) segregants. Arrows indicate individual S_3 plants that have rearrangements. This line showed a fixed C1 rearrangement in the S_3 generation (analysis of bulked S_4 individuals). Note different length rearrangements segregating, suggesting either frequent double recombination or exchanges over multiple generations. See Supplemental Figure 1 for bulked DNA marker analysis of this line.

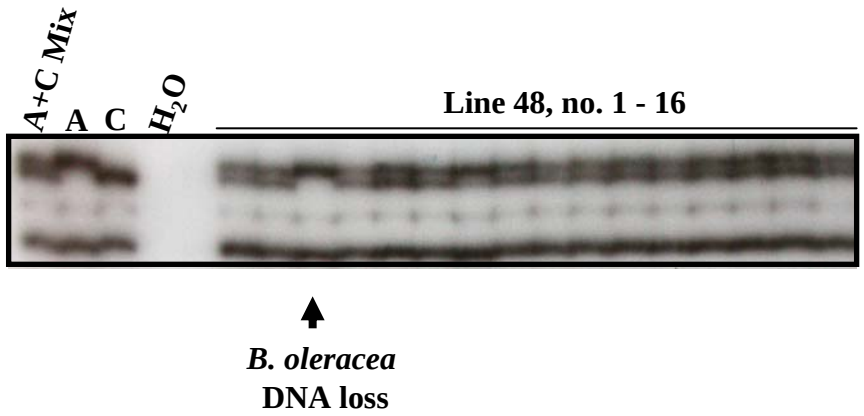
Supplemental Figure 4



Supplemental Figure 4: DNA SSCP and SSR analysis of line 26 (S_1) segregants. Arrows indicate individual S_2 plants carrying rearrangements. This line showed a fixed interstitial A1 rearrangement in the S_2 generation (analysis of bulked S_3 individuals). Note that we detected two individuals with the expected rearrangement and 1 with a partial reciprocal rearrangement. The appearance of a partial reciprocal rearrangement suggests multiple rounds of homoeologous exchange over successive generations. See Supplemental Figure 1 for bulked DNA marker analysis of this line.

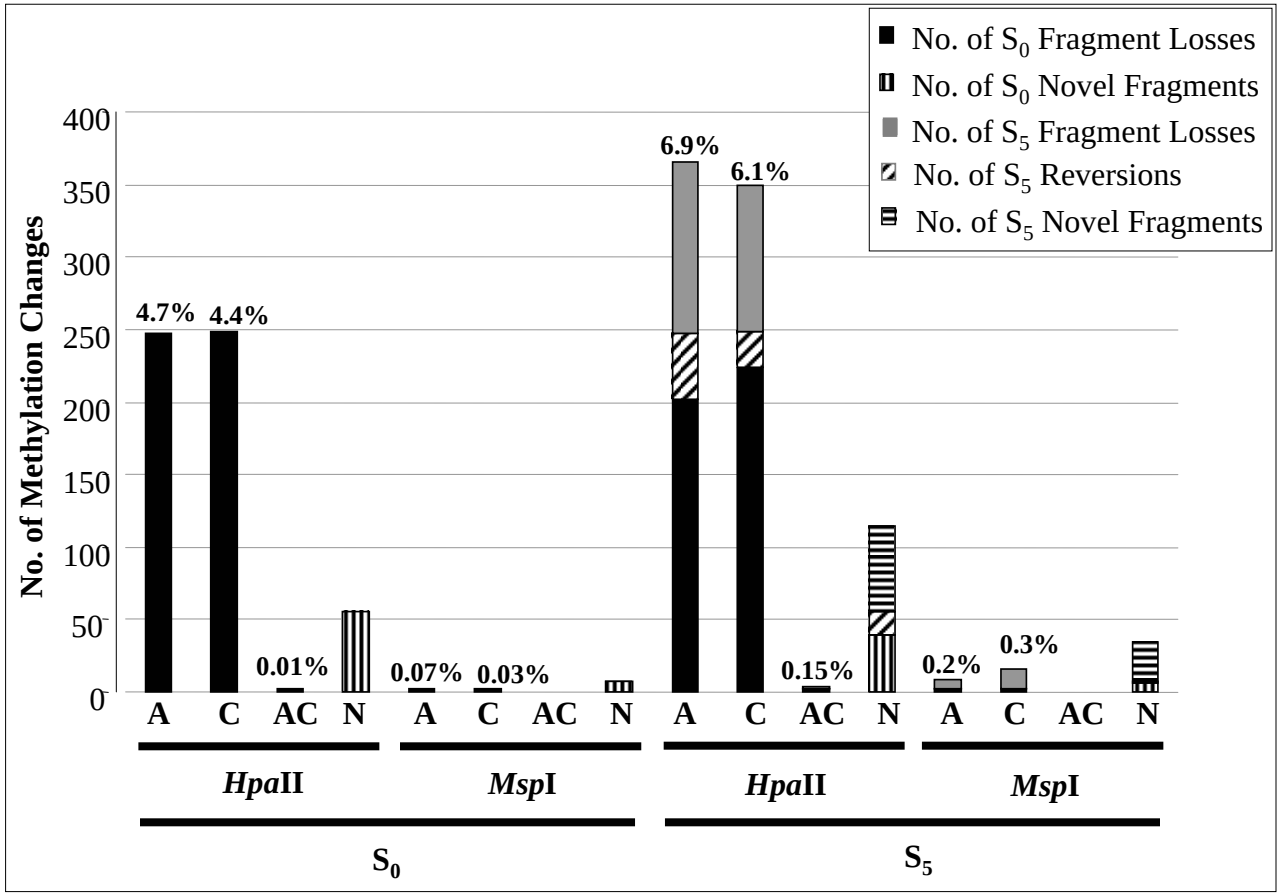
Supplemental Figure 5

<u>*FLC-3* SSCP Example</u>: Line 48 S_0 Segregants (S_1 individuals)



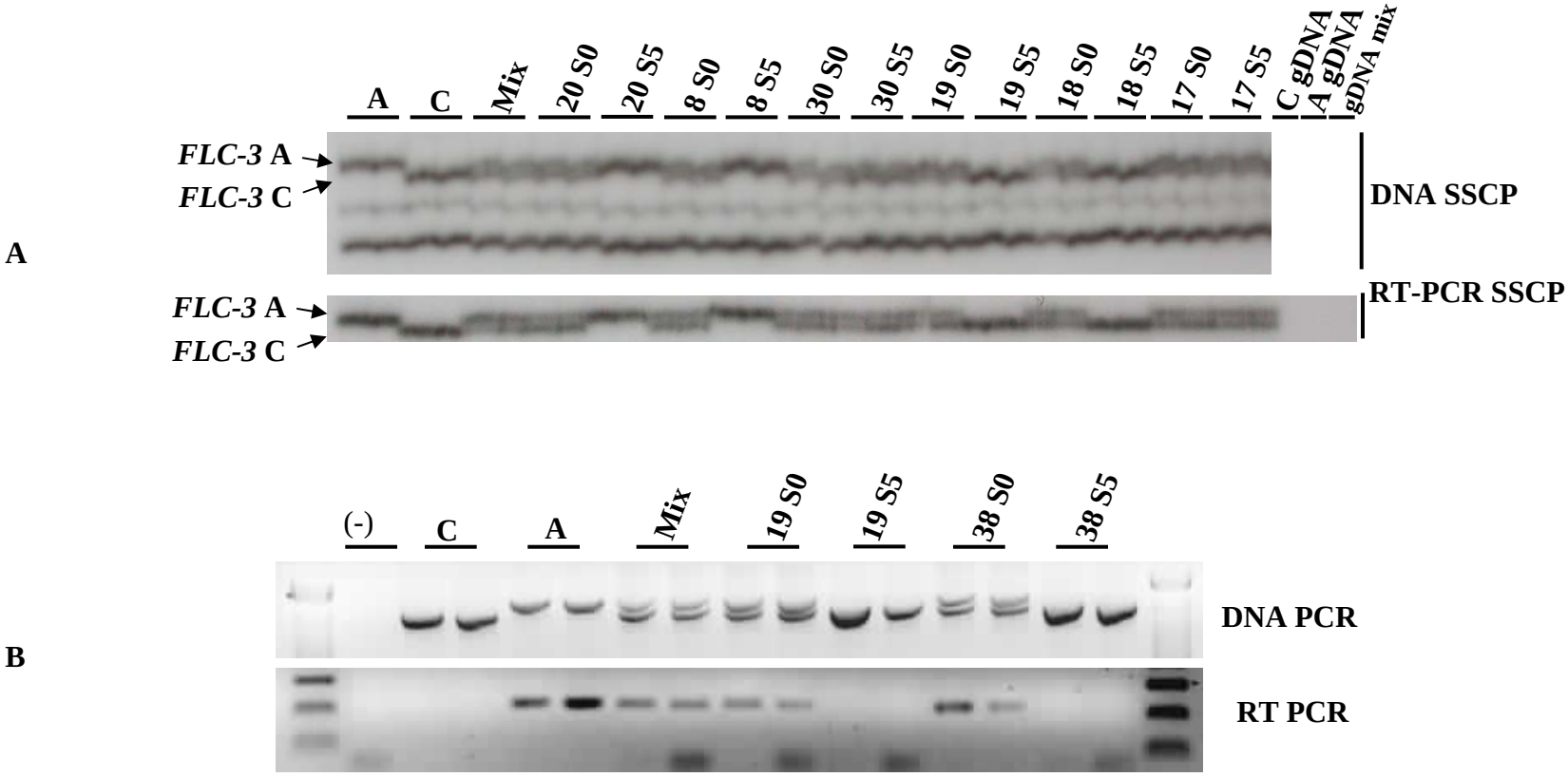
Supplemental Figure 5: DNA SSCP analysis of *FLC-3* in line 48 (S_0) segregants. If a single exchange occurred among homoeologs, and homologs underwent proper disjunction, 1/16 of the progeny would be expected to be homozygous for one homoeolog and 1/16 would be expected to be fixed for the other homoeolog. In 16 segregants analyzed here, 1/16 carried a HNRT.

Supplemental Figure 6



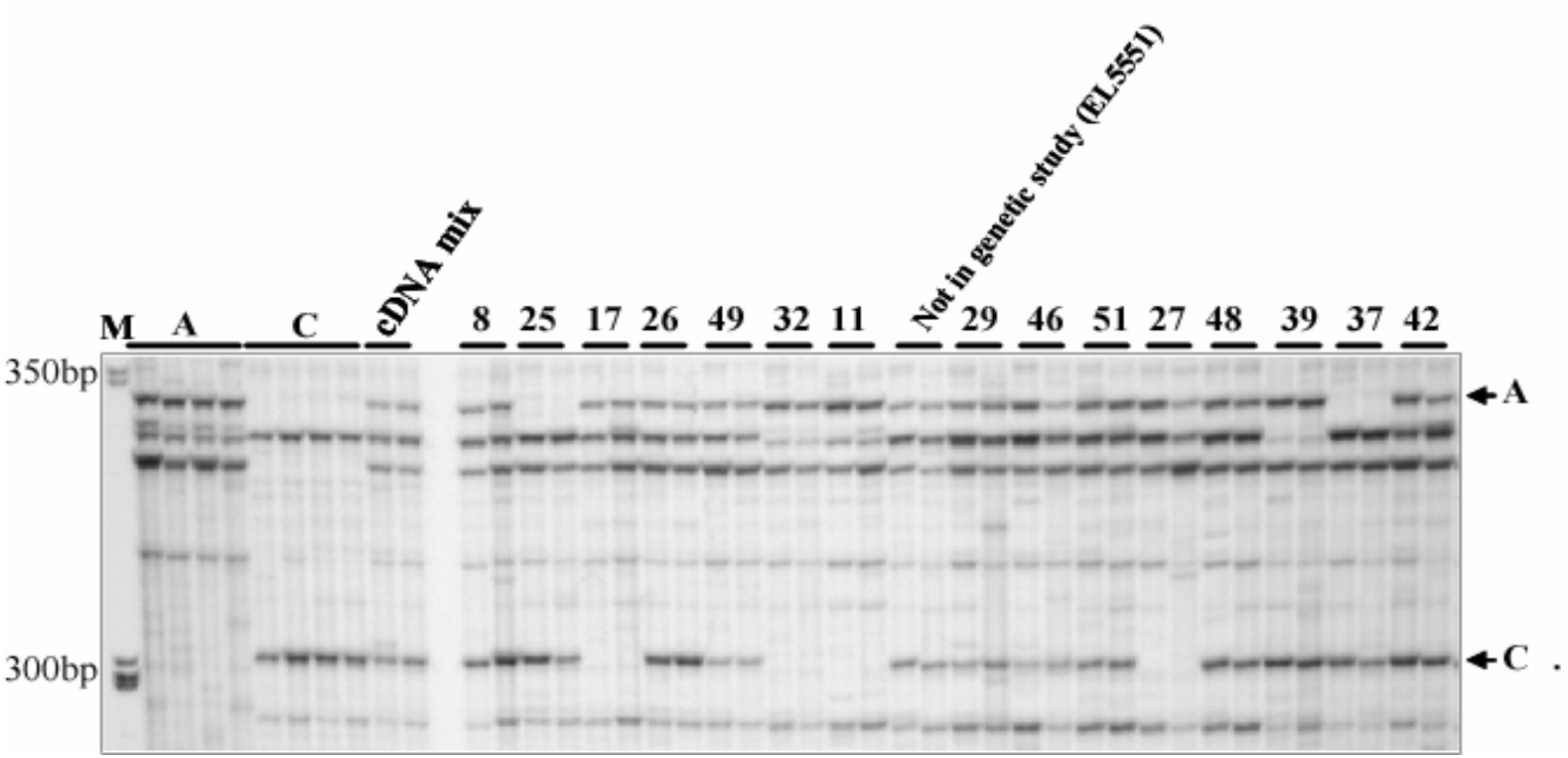
Supplemental Figure 6. Summary of methylation-sensitive RFLP changes detected among S_0 and S_5 polyploids relative to the parents. The total number of marker-fragments that changed among the polyploids in the S_0 and S_5 generations relative to the parents is indicated by bar height, and data is further categorized by *Hpa*II or *Msp*I fragments and by the genome of origin of fragments (A = *B. rapa*, C = *B. oleracea*, AC = monomorphic, and N = novel). The value over each bar represents the number of fragment changes as a percentage of total expected fragments assuming complete additivity of diploid parental fragments. S_0 estimates differ slightly from those reported by Lukens et al., 2006, because here we summarize data based on lines and probes that could be scored in both the S_0 and the S_5 analyses. Thirty-two S_5 fragment losses corresponded with the gain of a novel fragment. In 29/32 of these cases, the novel fragment gained was larger than the one lost, suggesting *de novo* methylation.

Supplemental Figure 7



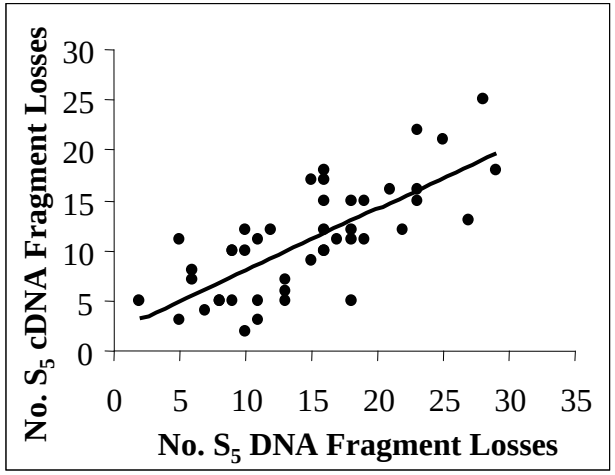
Supplemental Figure 7: RT-PCR analysis of *FLC-3* and *FLC-5*. Loss of homoeologous *FLC-3* and *FLC-5* genomic fragments (N3/N13) by HNRT was detected by Southern blot and PCR analyses. The figure panels shows both the S_0 and S_5 generation for several polyploid lineages, which were compared to parental genotypes (IMB218; A genome) and (TO1000; C genome) and a synthetic mix of the parents. Two biological replicates for each RT-PCR sample and two technical replicates of each DNA PCR sample were resolved in adjacent lanes. **A)** Loss of parental *FLC-3* DNA fragments was perfectly correlated with the loss of homoeologous *FLC-3* RNA transcripts among all lines. Two lines show evidence for the loss of *FLC-3*-A DNA and transcripts (lines 18 and 19). Two lines show evidence for the loss of *FLC-3*-C DNA and transcripts (lines 8 and 20). **B)** Loss of *FLC-5*-A cDNA was detected in lines 19 and 38 and correlated perfectly with the loss of gDNA. *FLC-5*-C is not expressed in the diploid *oleracea* parent or the polyploids. Experiments were repeated twice.

Supplemental Figure 8



Supplemental Figure 8: cDNA-AFLP display of resynthesized *Brassica napus* polyploids: This section of a gel demonstrates the qualitative loss of *B. rapa* and *B. oleracea*-specific cDNA AFLP markers, as well as additivity, among 16 different S_5 *B. napus* polyploid lines. Two biological replicates of RNA for each line were resolved in adjacent lanes. Polyploid lines are shown compared to parental lines of *B. rapa* (IMB218; A genome) and *B. oleracea* (TO1000; C genome), and a 1:1 mixture of parental cDNAs.

Supplemental Figure 9



Supplemental Figure 9: Relationship between total number of genome fragment losses and total number of cDNA fragment losses in resynthesized *Brassica napus* polyploids: Forty-five polyploid lines were compared for the total numbers of DNA and cDNA-AFLP fragment losses that occurred by the S_5 generation ($R^2 = 0.55$, $P < 0.0001$). Significant linear relationships were also detected between A-DNA and A-cDNA fragment losses ($R^2 = 0.34$, $P < 0.0001$), and C-DNA and C-cDNA fragment losses ($R^2 = 0.64$, $P < 0.0001$).

Supplemental Table 1: Summary of the distribution of marker changes among *Brassica* linkage groups.

LG	No. mapped[a] markers	No. markers showing fragment losses	No. expected[b] fragments	No. lost[c] fragments	%[d] lost
A1	9	7	418	33	7.9%
C1	10	9	467	97	20.8%
A2	11	10	516	40	7.8%
C2	11	7	510	40	7.8%
A3	10	9	468	33	7.1%
C3	14	8	640	30	4.7%
A4	0	0	ND[f]	ND[f]	ND[f]
C4	8	7	376	30	8.0%
A5	2	2	94	7	7.4%
C5	5	1	235	3	1.3%
A6	9	6	414	14	3.4%
C6	10	4	469	14	3.0%
A7	8	4	372	7	1.9%
C7	4	2	188	6	3.2%
A8	2	0	94	0	0.0%
C8	7	6	328	24	7.3%
A9	1	1	47	7	14.9%
C9	7	5	314	25	8.0%
A10	5	3	235	6	2.6%

[a]Mapped markers are those for which we could assign a map location.
[b]No. of expected fragments across the 47 independent S_5 lines assuming complete additivity of diploid parental fragments.
[c]No. of fragments lost represents both losses and loss/duplications.
[d]The proportion of fragment losses across linkage groups with mapped markers was not equal (Wald X^2 test; $P<0.0001$). Pairwise comparisons among linkage groups with Bonferroni correction found inequality among many groups. Because A1/C1, A2/C2 and A3/C3 all share extensive homoeology, we tested the null hypothesis that the frequency of changes was equal and found that A2/C2, A3/C3 were equal, but A1/C1 were significantly different ($P<0.0001$).
[f]ND=not determined because no markers could be assigned to this linkage group.

Supplemental Table 2: F-tests for equal phenotypic variance among lines of resynthesized *Brassica napus* polyploids in the S_0 and S_5 generations.

Trait Measured	S_0 σ^2	S_5 σ^2	F	P > F
Plant Height	9.27	35.46[a]	3.83	<0.0001
Raceme Height	1.36[a]	1.09	1.25	0.4455
Days Till Flowering	3.06	19.72	6.44	<0.0001
No. Open Flowers at Flowering	0.42[a]	0.42[a]	1.01	0.9797
Flower Size	0.12	0.33	2.77	0.0007
No. Leaves at Flowering	1.14	7.81	6.88	<0.0001
Total Leaf Length[b]	1.14	4.59	4.03	<0.0001
Leaf Width	0.32	1.13[a]	3.55	<0.0001
Petiole Length	0.42	1.81	4.34	<0.0001
Leaf Blade Length	0.51	1.33	2.62	0.0013
Length of Winged Petiole	0.37	2.35	6.26	<0.0001
No. Serration Leaf 4	2.87	14.68	5.12	<0.0001
No. Seconday Branches	0.55[a]	1.36[a]	2.48	0.0024
No. Siliques With ≥ 1 Seed	18.81	32.62	1.73	0.0633

[a]The distribution of this data did not pass a test for normality, thus results for this comparison should be interpreted with caution.

[b]Total leaf length was calculated for this table by adding petiole length and leaf blade length, but was not included in statistical analyses of phenotypes.