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Genetic basis for dosage sensitivity in Arabidopsis thaliana.

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Aneuploidy, the relative excess or deficiency of specific chromosome types, results in gene dosage imbalance. Plants can produce viable and fertile aneuploid individuals, while most animal aneuploids are inviable or developmentally abnormal. The swarms of aneuploid progeny produced by *Arabidopsis* triploids constitute an excellent model to investigate the mechanisms governing dosage sensitivity and aneuploid syndromes. Indeed, genotype alters the frequency of aneuploid types within these swarms. Recombinant inbred lines that were derived from a triploid hybrid segregated into diploid and tetraploid individuals. In these recombinant inbred lines, a single locus, which we call **SDI** (Sensitive to Dosage Imbalance), exhibited segregation distortion in the tetraploid subpopulation only. Recent progress in quantitative genotyping now allows molecular karyotyping and genetic analysis of aneuploid populations. In this study, we investigated the causes of the ploidy-specific distortion at **SDI**. Allele frequency was distorted in the aneuploid swarms produced by the triploid hybrid. We developed a simple quantitative measure for aneuploidy lethality and using this measure demonstrated that distortion was greatest in the aneuploids facing the strongest viability selection. When triploids were crossed to euploids, the progeny, which lack severe aneuploids, exhibited no lethality and using this measure demonstrated that distortion was greatest in the aneuploids facing the strongest viability selection. When triploids were crossed to euploids, the progeny, which lack severe aneuploids, exhibited no distortion at **SDI**. Genetic characterization of **SDI** in the aneuploid swarm identified a mechanism governing aneuploid survival, perhaps by buffering the effects of dosage imbalance. As such, **SDI** could increase the likelihood of retaining genomic rearrangements such as segmental duplications. Additionally, in species where triploids are fertile, aneuploid survival would facilitate gene flow between diploid and tetraploid populations via a triploid bridge and prevent polyploid speciation. Our results demonstrate that positional cloning of loci affecting traits in populations containing ploidy and chromosome number variants is now feasible using quantitative genotyping approaches.

**Introduction**

Most eukaryotic genomes maintain genes in a one-to-one relationship by their syntenic organization on chromosomes. This normal stoichiometry between chromosomes of a set can sometimes be disrupted, resulting in altered dosage of both genes and their encoded products. Such disruptions can arise via the nondisjunction of chromatids and chromosomes during mitosis and meiosis and result in uneven chromosome numbers, a condition called aneuploidy. Trisomy, the most common form of viable aneuploidy is characterized by the presence of one extra chromosome in an otherwise diploid background. The observation of stereotypical phenotypes for trisomies of each chromosome type illustrated that genetic factors are sensitive to dosage [1–5]. Indeed, the proper functioning of cells and organisms relies on molecular complexes, which require a delicate balance between components for proper operation [6]. Even a slight departure from this balance can have dramatic phenotypic or developmental consequences [6,7] as exemplified by the many haplo-insufficient genes identified in human as tumor suppressors [8] and as essential or regulatory genes in yeast [7,9] and *Drosophila* [10,11]. In aneuploids, where dosage variations affect whole chromosomes rather than single genes, the consequences can be severe when the copy numbers of many dosage-sensitive genes are altered at once. Therefore, an alteration of gene dosage such as it occurs in aneuploids typically has unfavorable consequences.

Interestingly, aneuploidy is not always deleterious and can be persistent. For example, aneuploid cells are normally found in certain tissues such as the brain and the placenta, where they appear to play a functional role [12–15]. Aneuploidy has been associated with invasive cancer [16,17] and controversially proposed to play a causal role in malignancy [18]. Although cancer is obviously deleterious to the affected organism, somatic selection of aneuploid sectors underscores the fact that dosage imbalance can be advantageous to cells. Finally, aneuploid individuals are common in plants and in yeast and provide a pool of phenotypic variation not present in the euploid population. In specific conditions, these phenotypes can be advantageous, and the corresponding aneuploid karyotypes selected. Such successful aneuploids have been observed both in nature and in industry [19–22]. Thus, although dramatic alterations of...
Author Summary

Each eukaryotic genome is subdivided into a specific number of chromosome types, which in turn are present in a characteristic number of copies, usually the same for all chromosomes. In the condition called aneuploidy, copy number differs among chromosome types, disrupting their balance and that of their encoded factors. As a result, aneuploidy is associated with developmental defects and death. For example, most types of human aneuploids are inviable: the only autosomal aneuploidy compatible with protracted survival, Down syndrome, is caused by the presence of three copies, instead of two, of the very small Chromosome 21. In plants, aneuploidy is more common and less deleterious. This suggests that plants can more easily tolerate the effects of aneuploidy and can be used to investigate them. Here, we used the model plant Arabidopsis thaliana to produce and investigate populations of aneuploid individuals. By comparing genetically distinct aneuploid populations, we identified a chromosomal region that is associated with greater aneuploid survival. Characterizing the genetic mechanism modulating the response to changes in chromosomal dosage and aneuploid survival will help understand how genome organization affects biological processes and why aneuploidy results in such severe developmental defects.

phenotype are associated with aneuploidy, this condition can be compatible with efficient function and even fitness.

There is also tremendous variation for aneuploidy tolerance between different organisms. For reasons that remain unknown, plants are generally less sensitive than animals to the type of dosage imbalance caused by aneuploidy [17]. Indeed, in humans, most aneuploids are embryo-lethal, and the few that are viable are associated with severe developmental defects [23]. In contrast, trisomics of all chromosome types as well as more complex aneuploid types have been described in several plant species [23–27]. There is also considerable variation between plant species in the degree of lethality caused by dosage imbalance. For example, the progeny produced by triploids of different plant species vary in the extent and frequency of aneuploidy. During triploid meiosis, three sets of chromosomes must be allocated to two poles, producing mostly aneuploid gametes. The progeny produced by such gametes should consist of a swarm of aneuploid types ranging from near diploid to near tetraploid. Such a swarm is produced by triploids of certain species, such as A. thaliana [24], but triploids of other species fail to produce such a range of aneuploids generating instead mostly diploids and near diploids [3,23,28,29]. Finally, sensitivity to aneuploidy can differ between varieties of the same species. One such case was reported in tomato in which cherry tomato produced aneuploids with an average of one extra chromosome than those of a large-fruited tomato variety [30]. Similar observations were made in barley in which the aneuploids produced by a wild variety carried a higher number of extra chromosomes, were more vigorous, and exhibited higher fertility than the aneuploids produced by a triploid of a cultivated variety [31,32]. In A. thaliana, comparison of identical trisomics of the Columbia (Col-0) and Landsberg erecta ecotypes uncovered differences in fertility and transmission rate of the trisomic chromosome [5,33–35]. A detailed genetic characterization of natural variation for tolerance to aneuploidy and cloning of the responsible loci have so far not been possible. Recent technological advances in Arabidopsis that combine molecular karyotyping and quantitative genotyping now allow quantitative genetic analysis of aneuploid populations [36]. Here, we report the first step towards positional cloning of a locus affecting aneuploid survival in Arabidopsis.

We previously investigated the effect of genotype on the rate of aneuploidy production by comparing the karyotype swarms in the progeny of two triploids of A. thaliana. One genotype, the CCC triploid, was produced from a cross between diploid Col-0 and its synthetically derived tetraploid (4x-Col). The other, the CWW triploid, was produced from a cross between diploid Col-0 and the naturally occurring tetraploid Warschau (Wa-1). We demonstrated that both of these triploids were fertile and produced a swarm of aneuploid progeny [24]. Genotype influenced both fertility of the triploids and the composition and performance of their aneuploid swarms. Additionally, recombinant inbred lines (RILs) produced from the progeny of a CWW triploid [37] resolved into two cohorts of near-diploid and near-tetraploid genome contents (Figure 1). Genetic analysis of these RILs identified transmission distortion at genetic markers on Chromosome 1 in the near-tetraploid but not the near-diploid lines [24]. In the present report, we investigated the genetic mechanisms responsible for the ploidy-dependent selection of this locus, which we call SENSITIVE TO DOSAGE.
**Results**

Genetic analysis of the Col-0 by Wa-1 RILs identified a locus, SDI, linked to markers nga280 and MN1.2 on Chromosome 1. The high percentage of the Wa-1 allele at SDI in the near-tetraploid RILs could result from selection at different steps. For example, selection of the Wa-1 allele could occur via a role in tetraploid survival and stability. Indeed, previous analyses demonstrated that tetraploidy is unstable and that tetraploid individuals often produce aneuploids [36]. Alternatively, selection could occur in the early generations by modulating the sensitivity of plants to aneuploidy. Consistent with this alternative, a higher percentage of aneuploids were found in the near-tetraploid lines than in the near-diploid lines (Figure 1) [24]. A protracted aneuploid phase might result in selection for alleles that reduce the deleterious effects of aneuploidy. To test these two hypotheses and identify a mechanism for the ploidy-dependent selection at SDI, we analyzed the inheritance of markers linked to SDI in three other populations.

**The SDI Locus Is Not Selected in a Tetrploid Population**

We produced an F₂ family from tetraploid CCWW plants (Figure 2A). Ninety CCWW F₂ individuals were genotyped at 11 markers, including nga280 and the linked MN1.2. No markers exhibited transmission ratio distortion in this population (Figure 2B). Thus, the polymorphism at SDI is unlikely to be critical for the survival of tetraploids.

**The SDI Locus Is Selected in Aneuploid Individuals**

As expected from our previous studies of tetraploids [36], several aneuploid individuals were identified among this CCWW F₂ population (Figure 2A). To investigate whether the SDI allele from Wa-1 was selected in the aneuploid individuals in this F₂ population, the percentage of the Wa-1 allele in the aneuploid and tetraploid subpopulations were compared. Of the 11 markers tested, only MN1.2 (but not nga280) exhibited a significant difference between the two subpopulations. A higher percentage of the Wa-1 allele was present in the aneuploids than in the tetraploids (t-test p-value = 0.0396) (Figure 2C).

The previously characterized populations derived by selfing of the CWW triploid [24] were tested for selection at SDI. In both the CWW F₂ population and the near-diploid RILs, the percentage of Wa-1 allele at MN1.2 was, on average, higher in the aneuploid individuals than in the euploid individuals (Figure 3A, inset). This trend was weak and not significant (t-test p-value = 0.38 and 0.34, respectively). A shortcoming of grouping all CWW-produced aneuploids is that differences in the severity of aneuploidy and thus differences in selection for aneuploidy tolerance were not accounted for.

**Karyotype Frequency as a Proxy for Aneuploidy Lethality**

To investigate the relationship between selection of the Wa-1 allele and aneuploidy severity, a quantitative measure of karyotype-dependent selection was developed. As previously reported, the CWW F₂ is a complex swarm of aneuploids of various karyotypes [24]. This swarm does not match the predicted outcome of triploid meiosis, presumably due to lethality differentially affecting these karyotypes [24]. The expected frequency of each genome content class was calculated previously (see Figure 2C in [24]). For each genome content class, the ratio of expected-to-observed frequencies (Figure 3A) was used to calculate the aneuploidy selection index (ASI) (see Material and Methods for details) (Figure 3B). Negative and positive values for ASI indicated overrepresentation and underrepresentation of a class relative to the expected frequency, respectively.

To test the biological significance of the ASI, we examined the relationship between ASI and seed production in the CWW F₂ population. Both the percentage of plump seed (as an estimate of seed viability) and the total number of seed produced by each of the CWW F₂ individuals were recorded (Figure 4A). Their relationship with ASI was determined by regression analyses (Figure 4B). Both regressions were highly significant (p-value = 0.0002 for seed viability and <0.0001 for seed counts). This demonstrated that ASI is a biologically
between marker genotype and ASI was investigated by increased survival of aneuploid individuals, the relationship Genotype at SDI selection. excellent early indicator of karyotype-modulated viability selection in the next generation. Thus, ASI represents an upon aneuploid classes and predicts the strength of viability relevant measure correlated with the viability selection acting Genotype at SDI is Associated with the Strength of Selection in the Triploid Swarm

To test whether the Wa-1 allele at SDI could be linked to increased survival of aneuploid individuals, the relationship between marker genotype and ASI was investigated by regression analysis. The percentage of Wa-1 allele at three markers, all located at the bottom of Chromosome 1 were significantly associated with ASI (Table 1). The most significant association between genotype and ASI was found at MN1.2 (Figure 5). This association was consistent with a role for SDI in modulating the viability of aneuploids.

Unfortunately, because there is no diploid of Wa-1, it was not possible to perform a similar analysis on the progeny of a CCW triploid, which could have controlled for potential effects of preferential pairing or segregation. It is possible that an unidentified meiotic mechanism affected chromosome segregation such that it would be responsible for the effect observed at SDI. We have observed that the percentage Wa-1 allele at SDI increases with genome content (regression $p$-value $= 0.0006$, $r^2 = 0.12$). Yet, when the effects of genome content and ASI were tested simultaneously, only the effect of ASI ($p$-value $= 0.0096$) remained significant while that of genome content ($p$-value $= 0.41$) did not. This suggested that the apparent increase of percentage Wa-1 allele with genome content was due to the correlation between ASI and genome content (regression $p$-value $< 0.0001$, $r^2 = 0.44$) and not due to an overall increased percentage Wa-1 allele in disomic gametes.

The percentage Wa-1 allele at MN1.2 was lower than expected in the diploid individuals and aneuploids of low genome content (Figure 5). This observation was unexpected but appears to fit within a genome-wide phenomenon. In both the CCW $F_2$ and RIL populations, the percentage Wa-1 allele was on average lower than the expected 66% throughout the genome [37]. Although we do not have an explanation for this observation, marker MN1.2 is not unusual in this respect.

The association between genotype at MN1.2 and karyotype was also analyzed using the progeny of pseudo-backcrosses (pBC) involving the CCW triploid. In these populations, one of the two parents is a CCW triploid while the other parent is either diploid Col-0 or its tetraploid derivative (4x-Col). ASI values for each chromosome content class were calculated separately for each of the four pBC populations: CCW × Col-0; CCW × 4x-Col; Col-0 × CCW; 4x-Col × CCW. An association between ASI and marker genotype was again tested by regression. As observed in the CCW $F_2$ aneuploid swarm, the percentage of Wa-1 allele at MN1.2 increased with the ASI but the regression was not significant for any of the four populations studied ($p$-values between 0.23 and 0.92). Thus, selection for SDI in the progeny of a triploid was only visible in the context of a selfed triploid, where zygotes can be more severely imbalanced and where aneuploidy can have both maternal and paternal origin.

**Discussion**

Previously, ploidy-dependent transmission distortion was found at the SDI locus in a recombinant inbred population derived from a triploid produced by crossing Col-0 and the naturally collected tetraploid ecotype Wa-1 [24]. To investigate the mechanisms behind the selection of the Wa-1 allele at SDI in these RILs, we determined when this selection could have occurred during the derivation of the RILs (Figure 1). First, we demonstrated that allele frequencies at SDI were not distorted in tetraploid populations segregating for the Col-0 and Wa-1 alleles, suggesting that the Wa-1 allele of SDI was
not required for tetraploid maintenance (Figure 2). Markers linked to SDI were, however, selected in the aneuploid individuals produced by the CCWW tetraploids (Figure 2C) suggestive of a role for SDI in aneuploid viability. To further test this hypothesis, selection at SDI was investigated in the aneuploid swarm produced by the selfed CWW triploid. In this population, selection at SDI was associated with a quantitative measure of aneuploid survival (Figure 5). Specifically, the Wa-1 allele was strongly selected in those aneuploid genome content classes that exhibited the strongest viability selection (Figures 3 and 5). Selection against aneuploidy results from dosage imbalances affecting cellular functions required either in the gametes, at fertilization or in the fertilization products. We therefore named this locus SENSITIVE TO DOSAGE IMBALANCE or SDI.

Previously, we identified several other differences in the response of the Col-0 and Wa-1 genomes to triploidy and aneuploidy. Genotype influenced both fertility of the triploid and the composition and performance of the aneuploid swarm produced in the immediate filial generation following triploidy [24]. The CWW triploid produced a higher percentage of live seed and more triploid and aneuploid progeny than the CCC triploid [24]. We also previously observed that the natural tetraploid Wa-1 produced more viable aneuploid individuals than the synthetic tetraploid Col-0 [36]. These results are consistent with our interpretation that the Wa-1 allele at SDI improves aneuploid survival.

Could the Wa-1 Allele at SDI Rescue Extreme Aneuploids?

The percentage of Wa-1 allele at SDI increased with our measure of aneuploidy selection in the CWW F2 population. Thus, selection at SDI in the CWW F2 was karyotype-dependent. Yet, karyotype-dependent selection at SDI was not significant in the progeny of the pBC. Comparing the theoretical population of aneuploids produced by a selfed triploid to those produced in the pBC suggests possible explanations for this observation (Figure 6). The pBC populations are exclusively composed of moderate aneuploid (dosage deviation of no more than one chromosome, light blue) and euploid (red) individuals. These individuals are also present in the triploid F2 distribution, where they only represent a minor proportion (Figure 6A versus 6C). More extreme aneuploid individuals (in green in Figure 6), containing two copies of some chromosome types and four copies of others can be formed from a selfed triploid when aneuploid gametes carrying extra copies of the same

### Table 1. Analysis of the Relationship between Marker Genotype and ASI in the CWW F2 Population

| Marker | Chromosome | Position (Bps) | Distance to Next Marker on the Same Chromosome (cM) | Regression p-Value |
|--------|------------|----------------|-----------------------------------------------|-------------------|
| MN1.5  | 1          | 898,627        | 49.4                                          | 0.2510            |
| MN1.7  | 1          | 13,634,035     | 24.6                                          | 0.8750            |
| nga280 | 1          | 20,877,364     | 5.7                                           | 0.0006*           |
| MN1.2  | 1          | 21,949,869     | 11.7                                          | <0.0001*          |
| F5I14  | 1          | 24,374,008     | NA                                            | 0.0054*           |
| nga1145| 2          | 683,000        | 42.9                                          | 0.396             |
| nga1126| 2          | ~9,000,000     | NA                                            | 0.789             |
| MSAT5.19| 5         | 25,911,521     | NA                                            | 0.222             |

*Significant regression p-values. Test significance was set at a p-value < 0.05, equivalent to a Bonferroni correction for a p < 0.05 and five independent tests on three chromosome types.

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Consistent with the association of SDI selection with aneuploidy severity in the CWW F_2, the highest percentages of Wa-1 allele at SDI are associated with the genome content classes that are predicted to contain the highest percentage of severe aneuploids (genome content classes 2.8, 3.0, and 3.2).

As argued above, selection against severe aneuploids may be an important determinant of genome content distribution in triploid progeny. Our data suggest that other processes also contribute to it. If selection against severe aneuploids was the only driving force, one would expect a completely symmetrical bimodal distribution (encompassing the red, orange, and light blue individuals in Figure 6). The observed distribution (Figure 3A) is bimodal but not symmetrical. It has been hypothesized that carrying an excess of several types of chromosomes (such as in genome content classes 2.6 or 2.8) is more deleterious than carrying only one chromosome in excess (genome content classes 2.2 or 3.2). This hypothesis is based on the observation that most regulatory interactions are negative [10]. Therefore, increasing the number of copies at more loci (or stated differently, having a minority of loci in relative deficiency) should increase the probability of negatively affecting loci involved in crucial cellular or developmental processes and that are not located on the additional chromosome copies [10]. Our results agree with this hypothesis: in each half of the distribution, individuals with lower genome contents are more common than individuals with higher genome content (Figure 3A).

**What Are the Evolutionary Consequences of Surviving Dosage Imbalance?**

We have shown that the naturally collected tetraploid ecotype Wa-1 produced aneuploid individuals more often than Col-0 [36]. In addition, an allele from Wa-1 is associated with the survival of severe aneuploid individuals (Figures 2 and 5). Considering the obvious negative consequences of aneuploidy, is there a counterbalancing advantage that could justify the persistence of such a trait? Persistent aneuploidy has been reported in several specific situations where the aneuploid phenotype confers a selective advantage relative to the diploid phenotype. For example, segmental aneuploidy is frequent in yeast deletion mutants [39] and can confer a growth advantage to the aneuploid cells compared to the euploid ones [39]. In humans, aneuploid cells have recently been found to be an integral part of the functional pool of neurons and in placental trophoblasts, consistent with a functional role for aneuploidy in these contexts [12–15]. In plants, it is believed that aneuploidy can play a role in speciation and phenotype evolution [40]. Additionally, aneuploidy is very frequent in polyploid populations [2,40] as well as in the process of polyploid formation through the triploid bridge in species for which triploids are readily produced and are fertile [24,41]. In neopolyploids, aneuploidy may contribute to phenotypic variability [40] and become fixed through selection for advantageous karyotypes. An allele associated with increased tolerance to dosage imbalance would therefore increase the probability that advantageous karyotypes arise and reproduce successfully. In addition, it would increase the fertility of triploids produced from unreduced gametes or interploidy hybridization. This would enhance gene flow between diploid and polyploid subpopulations via a triploid bridge and aneuploid swarms and allow the sharing of alleles arising in either
population. Such recurrent triploid formation between diploid and polyploid populations and repeated formation of polyploid derivatives would therefore hinder polyploid speciation and may explain why multiple karyotypes are often cataloged as a single species based on shared ecological and morphological traits.

What Mechanism(s) Underlie the Action of SDI?

The fact that selection acting on SDI is associated with the presence of extreme aneuploids suggests that SDI acts to buffer the effects of dosage imbalance. This buffering could enhance the survival of aneuploid gametes or that of the fertilization products, either the zygote itself or the endosperm and could be mediated through a number of mechanisms.

Selection at SDI could stem directly from specific dosage effects on one or a few dosage-sensitive genes. Aneuploidy affects the regulation of genes located both on the varied chromosome and on the rest of the genome [17,42–45]. The intensity of these effects varies from gene to gene and can be positive or negative, illustrating the complexity of the regulatory networks [10,17,44,46]. Observations in maize and Drosophila have led to the idea of a “dosage-regulatory hierarchy,” in which the expression of a given gene might be regulated by several dosage-dependent regulators, which in turn are involved in the regulation of several target genes [10]. This hypothesis would be consistent with the possibility that selection at SDI stems from the misregulation of a specific dosage-sensitive gene linked to SDI.

Karyotype-dependent selection at SDI could also originate from a genome-wide effect mediated by SDI. Changes in chromosome number affect overall genome maintenance, function, and regulation [47,48]. For example, polyploidy results in variation in epigenetic regulation, as demonstrated by ploidy-sensitive gene silencing and paramutation in Arabidopsis [48–50]. Epigenetic silencing in polyploids and aneuploids may result directly from dosage imbalance. This idea is supported by our understanding of the mechanisms underlying dosage compensation in flies, mammals, and worms, which all rely on chromatin remodeling [51]. In plants, trisomy dependent epigenetic instability has been reported for a transgenic locus in tobacco [52,53]. Similarly, cancerous cells are associated with both aneuploidy and epigenetic modifications [17,54]. In addition, meiotic silencing of unpaired DNA has been demonstrated in Neurospora crassa [55], in X-chromosome imprinting in C. elegans [56], and in sex chromosome inactivation in mammals [57]. It is possible that the presence of unpaired chromosomes during triploid or aneuploid meiosis has similar consequences. Thus, it is possible that the SDI locus encodes a regulator mediating a genome-wide epigenetic response to dosage imbalance. The possible involvement of SDI in epigenetic modifications of the genome or in its regulation is an attractive hypothesis that the recent publication of the complete methylome of A. thaliana [58,59], natural variation in A. thaliana methylation level [60,61], and our ability to detect and karyotype aneuploid individuals [36] will help address.

In conclusion, we have established a quantitative measure
for aneuploid survival. Using this trait, we have demonstrated the feasibility of genetic mapping in aneuploids and associated a locus to the variation in aneuploid survival observed in Arabidopsis. Characterization of the gene(s) responsible for SDI should facilitate a better understanding of the mechanisms governing the sensitivity to dosage imbalance and aneuploid syndromes.

Materials and Methods

Plant growth conditions, lines, and crosses. All plants were grown on soil (Sunshine Professional Peat-Lite mix 4, SunGro Horticulture, http://www.sungro.com) in a growth room lit by fluorescent lamps (Model TL50; Philips, http://www.lighting.philips.com) at 22 ± 3 °C with a 16:8 h light:dark photoperiod or in a greenhouse at similar temperatures and light regimes, with supplemental light provided by sodium lamp illumination as required.

Tetraploid lines were described previously [24]. Col-0 represents the diploid ecotype Columbia, 4x-Col represents tetraploidized Col-0, and Wa-1 represents the naturally occurring tetraploid ecotype Warschau-1 (CS6885). C and W refer to basic genomes or alleles of Col-0 and Wa-1, respectively. The CWW F1 population (n = 90) was obtained by crossing 4x-Col as the seed parent to Wa-1 and allowing three F1 individuals to self-pollinate. The Col-0 × Wa-1 RILs described by Schiff and coworkers [37] were a kind gift from Shauna Somerville (Carnegie Institution, Stanford University). The CWW F1 population was generated as described [24]. In order to reduce the complexity of the aneuploid swarm produced by a tetraploid, pBC populations were generated by crossing CWW triploids to either diploid Col-0 or tetraploid 4x-Col, in both directions [36]. There were four types of pBC populations and the number of individuals analyzed in the context of this report were as follows: Col-0 × CWW (n = 80), CWW × Col-0 (n = 102), 4x-Col × CWW (n = 33), and CWW × 4x-Col (n = 47).

Phenotypic analysis of seed production. Single siliques were harvested into individual tubes, and all the seeds from each fruit were counted using a dissecting microscope. To estimate seed viability, seeds were characterized as “plump” if they contained a visible embryo structure at least 20% the size of wild-type seed or “shriveled” if they did not. On average, five individual siliques were counted for each individual. Mean values for each group of plants were compared pair-wise using Student’s t-test and p-values < 0.05 were considered significant.

Flow cytometric determination of genome content. All individuals in the pBC, the CWW F2, and the RIL populations were analyzed for genome content as previously described [24,36]. Briefly, control A. thaliana and known embryo genome content were run before, between, and after experimental samples and used to create a standard curve, allowing us to determine the genome content of our experimental samples. Individuals were categorized by genome content values expressed as a multiple of the haploid genome content of Col-0. On this scale, a 2.0 corresponds to a diploid individual, and a 4.0 corresponds to a tetraploid individual. The number of categories was chosen based on how many chromosome number classes were expected (four classes between diploid and tetraploid as well as four classes between triploidy and tetraploidy). We have previously shown that this method is accurate and precise for A. thaliana aneuploids by comparing our flow results to complete karyotypes (r² = 0.985 in [36]).

Quantitative genotyping and karyotyping. Quantitative genotyping was performed as previously described [36]. Different populations were genotyped at different markers. The progeny of the pBCs were genotyped at all 12 markers previously described [36], and the data were used to infer the complete karyotype of each individual [36]. The CWW F2 individuals were genotyped at eight of those 12 markers located on four of the five chromosome types, namely MN1.5, MN1.6, MN1.7, MN1.2, nga1126, nga1145, MN4.2, and MSAT5.19 as well as at the three additional markers nga280, F5I14, and nga692. The presence of additional or missing chromosomal copies were used to compare quantitative genotypes for at least one chromosome type indicated the presence of additional or missing chromosomal copies were classified as aneuploid (n = 18).

Statistical genetic analysis of pBCs. For each population, the numbers of Col-0 and Wa-1 alleles were counted. For example, a CWW genotype contributed three Wa-1 alleles and one Col-0 allele. The ratio of C to W within the population was compared to the expected 1:1 ratio using chi-squared tests. Transmission ratio distortion in the CWW F2 population was compared to the expected 1:1 ratio tested by chi-square analysis. Test significance was set at a p-value < 0.0083, equivalent to a Bonferroni correction for a p < 0.05 and six independent tests on four chromosome types. Only the euploid individuals were used for this analysis (n = 72), to eliminate any possible effect of aneuploidy on genotype.

In order to statistically test the effect of marker genotypes on traits, genome-content was expected (four classes between diploid and triploid as well as four classes between triploidy and tetraploidy). We have previously shown that this method is accurate and precise for A. thaliana aneuploids by comparing our flow results to complete karyotypes (r² = 0.985 in [36]).

Quantitative genotyping and karyotyping. Quantitative genotyping was performed as previously described [36]. Different populations were genotyped at different markers. The progeny of the pBCs were genotyped at all 12 markers previously described [36], and the data were used to infer the complete karyotype of each individual [36]. The CWW F2 individuals were genotyped at eight of those 12 markers located on four of the five chromosome types, namely MN1.5, MN1.6, MN1.7, MN1.2, nga1126, nga1145, MN4.2, and MSAT5.19 as well as at the three additional markers nga280, F5I14, and nga692 all located on Chromosome 1. The CWW F2 individuals were genotyped at MN1.5, MN1.7, MN1.2, nga280, F5I14, nga1126, nga1145, and MSAT5.19 located on three of the five chromosome types. Finally one marker, MN1.2, located approximately 5.7 cM distal to the centromere from nga280, was added to the Col-0 × Wa-1 RILs genotyping set (see Table 1). Selection at MN1.2 in the near-tetraploid RILs was evaluated according to previous protocols [24] and was identical to that of nga280. In the near-diploid population, the percentage of Wa-1 allele at MN1.2 was slightly higher than at nga280. As a result, comparisons between diploid and tetraploid RILs were not significant after a Bonferroni correction for 11 independent tests but a strong trend was evident (Fisher Exact test p-value = 0.0099, MN1.2 span 2 deletions polymorphism between Col-0 and Wa-1, while nga280 amplifies a polymorphic microsatellite repeat. Because of the technical advantages of using an indel polymorphism for quantitative genotyping [36], and because of the close linkage to nga280, we employed MN1.2 for the quantitative genetic analyses of the pBCs.

MN markers were designed by identifying short insertions or deletions between Col-0 and Wa-1 present in the sequence database provided by the Magnus Nordborg laboratory (http://walnut.usc.edu/apache2-default) [62]. The sequence and modification of these primers was summarized previously [36]. Forward primers for markers nga280, F5I14, and nga692 [24,63] were labeled with FAM, NED, and ROX respectively.

For the pBC populations, chromosome doses were inferred from quantitative fluorescent PCR as previously described [36]. Individuals were categorized depending on their chromosome number. Individuals with 10, 15, or 20 chromosomes were classified as euploids, while all other individuals were classified as aneuploid. Individuals from the CWW F2 population were only partially karyotyped as data were only available for four of the five chromosome types. Individuals for which all quantitative genotypes were consistent with tetraploidy (n = 72) were classified as euploid, while individuals for which the quantitative genotypes for at least one chromosome type indicated the presence of additional or missing chromosomal copies were classified as aneuploid (n = 18).

ASI. A value for ASI was calculated for each genome content class of the CWW F2 population. The expected frequency of each genome-content class was calculated assuming random assortment of three sets of chromosomes and no selection for or against karyotypes. For each genome content class, the observed frequency was calculated by dividing the number of individuals observed in that genome content class by the total number of individuals. The values for ASI for each genome content class were obtained using the following formula: ASI = log 2 (expected frequency/observed frequency). Using this formula, chromosome number classes that were overrepresented relative to their expected frequencies were assigned a negative ASI value, while positive ASI values indicated selection against a chromosome number class. Some of the genome content classes included few individuals. We tested an alternative version of ASI in which these classes were excluded from the analysis. Although the p-values obtained were higher, the percentage of Wa-1 alleles was significantly affected by ASI at the same markers as presented in Table 1.

A similar approach was applied to the pBC populations with the exception that genome content classes were replaced by chromosome number classes since each of the pBC individuals had been molecularly karyotyped [36]. ASI values were calculated separately for each pBC population.

Supporting Information

Accession Numbers

The Arabidopsis Information Resource (TAIR) (http://www.arabidopsis.org) accession number for Wa-1 is CS6885.
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References

1. Blakeslee A (1922) Variation in *Datura* due to changes in chromosome number. Am Nat 56: 16–31.
2. Khush G (1973) Cytogenetics of aneuploids. New York: Academic Press. 301 p.
3. McClintock B (1929) A cytological and genetical study of triploid maize. Genetics 14: 180–222.
4. Rick CM, Baron DW (1955) Cytological and genetical identification of the primary trisomics of the tomato. Genetics 39: 649–666.
5. Steinitz-Sears L (1962) Chromosome studies in *Arabidopsis thaliana*. Genetics 48: 493–499.
6. Papp B, Pal C, Hurst LD (2005) Dosage sensitivity and the evolution of gene families in yeast. Nature 429: 194–197.
7. Veitia RA (2005) Gene dosage balance: Deletions, duplications and dominance. Trends Genet 21: 35–36.
8. Fortin R, Smits R (2002) Cancer biology. A matter of dosage. Science 298: 761–763.
9. Giaever G, Shoemaker DD, Jones TW, Liang H, Winzeo EA, et al. (1999) Genomic profiling of drug sensitivities via induced haploinsufficiency. Nat Genet 21: 278–283.
10. Bircher JA, Bhadra U, Bhadra MP, Auger DL (2001) Dosage-dependent gene regulation in multicellular eukaryotes: Implications for dosage compensation, aneuploid syndromes, and quantitative traits. Dev Biol 234: 275–292.
11. Lindles DL, Sandler L, Baker BS, Carpenter AT, Denell RE, et al. (1972) Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics 71: 157–184.
12. Kingery MA, Friedman B, McConnell MJ, Rehen SK, Yang AH, et al. (2005) Aneuploid neurons are functionally active and integrated into brain circuitry. Proc Natl Acad Sci U S A 102: 6143–6147.
13. Weier JF, Feillette C, Baumgartner A, Jung CJ, Nguyen HN, et al. (2006) Molecular cytogenetic studies towards the full karyotype analysis of human blastocysts and cytotrophoblasts. Cytogenet Genome Res 114: 302–311.
14. Weier JF, Weier HU, Jung CJ, Gornley M, Zhou Y, et al. (2005) Human cytotrophoblasts acquire aneuploidy as they differentiate to an invasive phenotype. Dev Biol 290: 420–432.
15. Yang AH, Kaushal D, Rehen SK, Kriedt K, Kingery MA, et al. (2003) Chromosome segregation defects contribute to aneuploidy in normal neural progenitor cells. J Neurosci 23: 10454–10462.
16. Gagos S, Irminger-Finger I (2005) Chromosome instability in neoplasia: Chaotic roots to continuous growth. Int J Biochem Cell Biol 37: 1014–1033.
17. Matzke MA, Mette MF, Kanno T, Matzke AJ (1997) The location of linkage groups in *Arabidopsis thaliana*. Can J Genet Cytol 9: 381–384.
18. Steinitz-Sears L, Lee-Chen S (1970) Cytogenetic studies in *Arabidopsis thaliana*. Can J Genet Cytol 12: 217–223.
19. Henry IM, Dilkas BP, Lee-Chen S (2006) Molecular karyotyping and aneuploidy detection in *Arabidopsis thaliana* using quantitative fluorescent polymerase chain reaction. Plant J 48: 307–319.
20. Schiff CL, Wilson IW, Somerville SC (2001) Polygenic powdery mildew disease resistance in *Arabidopsis thaliana*. Quantitative trait analysis of the accession Warschau-1. Plant Pathol 50: 690–701.
21. Bircher JA (1995) Dosage analysis of maize endosperm development. Annu Rev Genet 27: 181–204.
22. Hughes TR, Roberts CJ, Dai H, Jones AR, Meyer MR, et al. (2000) Widespread aneuploidy revealed by DNA microarray expression profiling. Nat Genet 25: 333–337.
23. Ramsey J, Schemske DW (2002) Neopolyploidy in flowering plants. Annu Rev Ecol Syst 33: 589–609.
24. Ramsey J, Schemske DW (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annu Rev Ecol Syst 29: 467–501.
25. Fitz-Patrick D (2005) Transcriptional consequences of autosomal trisomy: Primary gene dosage with complex downstream effects. Trends Genet 21: 249–253.
26. Fitz-Patrick D, Ramsay J, McGill N, Shade M, Carothers A, et al. (2002) Transcriptome analysis of human autosomal trisomy. Hum Mol Genet 11: 399–409.
27. Guo M, Bircher J (1994) Trans-acting dosage effects on the expression of model gene systems in maize aneuploids. Science 266: 1999–2002.
28. Mao R, Zielke C, Zielke H, Pevsner J (2003) Global up-regulation of *Arabidopsis* epialleles and their paramutation-like interaction in tetraploid *Arabidopsis thaliana*. PLoS Genet 3: e700601.
29. McCaffery JA, Bell G, Hubschle R, Bakker G, Christie B, et al. (2003) Developmental impact on trans-acting dosage effects in maize aneuploids. Genesis 31: 64–71.
30. Matzke M, Scheid O, Matzke A (1999) Rapid structural and epigenetic changes in polyploid and aneuploid genomes. Bioessays 21: 761–767.
31. Pikaard C (2001) Genomic change and gene silencing in polyploids. Trends Genet 17: 675–677.
32. Mittelsten Scheid O, Afars K, Paszkowski J (2003) Formation of stable epialleles and their paramutation-like interaction in tetraploid *Arabidopsis thaliana*. Nat Genet 34: 450–454.
33. Mittelsten Scheid O, Jakovleva L, Afsar K, Maluszynska J, Oaszkowski J (2007) Inheritance and expression of a transgene insert in an aneuploid tobacco line. Mol Gen Genet 274: 489–497.
34. Henry IM, Dilkes BP, Young K, Watson B, Wu H, et al. (2005) Aneuploidy and genetic variation in the *Arabidopsis thaliana* triploid response. Genetics 170: 1979–1988.
35. Epstein C (1986) The consequences of chromosomal imbalance. In: Barlow PL, editors. Cambridge: Cambridge University Press. 1535–1655.
36. Johnsson H (1945) The triploid progeny of the cross diploid x tetraploid *Papulus tremula*. Hereditas 31: 411.
37. Lengyel (1942) The effect of chromosomal variation in sugar beets. Hereditas 26: 345–399.
38. Punyasinh K (1947) Chromosome numbers in crosses of diploid, triploid and tetraploid maize. Genetics 32: 541–554.

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57. Lee JT (2005) Sex chromosome inactivation: The importance of pairing. Curr Biol 15: R249–R252.
58. Schob H, Grossniklaus U (2006) The first high-resolution DNA “methylome”. Cell 126: 1025–1028.
59. Zhang X, Yazaki J, Sundaresan A, Cokus S, Chan SW, et al. (2006) Genome-wide high-resolution mapping and functional analysis of DNA methylation in Arabidopsis. Cell 126: 1189–1201.
60. Cervera MT, Ruiz-Garcia L, Martinez-Zapater JM (2002) Analysis of DNA methylation in Arabidopsis thaliana based on methylation-sensitive AFLP markers. Mol Genet Genomics 268: 543–552.
61. Riddle NC, Richards EJ (2002) The control of natural variation in cytosine methylation in Arabidopsis. Genetics 162: 555–565.
62. Nordborg M, Hu TT, Ishino Y, Jhaveri J, Toomajian C, et al. (2005) The pattern of polymorphism in Arabidopsis thaliana. PLoS Biol 3: e196. doi:10.1371/journal.pbio.0030196
63. Bell CJ, Ecker JR (1994) Assignment of 30 microsatellite loci to the linkage map of Arabidopsis. Genomics 19: 137–144.