Tetrad Analysis in Higher Plants. A Budding Technology

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Tetrad analysis, the ability to manipulate and individually study the four products of a single meiotic event, has been critical to understanding the mechanisms of heredity. The Arabidopsis quartet (*qrt*) mutation, which causes the four products of male meiosis to remain attached, enables plant biologists to apply this powerful tool to investigations of gamete development, cell division, chromosome dynamics, and recombination. Here we highlight several examples of how *qrt* has been used to perform tetrad analysis and suggest additional applications including a genetic screen for gametophytic mutants and methods for investigating gene interactions by synthetic lethal analysis.

In 1883 Van Beneden made an amazing observation: In newly fertilized *Ascaris megalocephala* eggs, the sperm and the egg nuclei each contained two chromosomes whereas the somatic cells contained four. Building on this observation, Weismann (1887) proposed that there must be a reductive cell division during the sexual life cycle to compensate for the fusion of gametes at fertilization. Farmer and Moore (1905) coined the term meiosis to describe this division. The cellular processes surrounding meiosis and the rules governing genetic inheritance have been the subjects of intense scientific scrutiny in the century since these early observations. During meiosis the cell reorganizes cytoplasmic components, initiates transcriptional programs, and activates specialized biosynthetic pathways. Equally dramatic events impact the genome: Each DNA strand is replicated, chromosomal homologs pair and recombine, and two cell divisions are executed to produce four haploid cells. Geneticists have employed several techniques to unravel the mechanisms of meiosis. Chief among these techniques is tetrad analysis, a method for investigating genetic mechanisms based upon the analysis of all four products of meiosis.

Tetrad analysis is particularly useful for examining meiotic recombination, and it has the flexibility to provide insight into many aspects of inheritance. Tetrad analysis can be used to detect chromosomal translocations, prove synthetic lethality in double mutants, and distinguish nuclear from organellar segregation. Similar to other methods for measuring recombination frequencies, tetrad analysis establishes linkage relationships that enable the construction of genetic maps (Mather and Beale, 1942). The most remarkable aspects of tetrad analysis are that it uniquely allows monitoring of every genetic exchange in an individual meiosis, unequivocal detection of gene conversion events, establishment of chromatid interference, and high precision genetic mapping of centromeres (Whitehouse, 1942; Mitchell, 1955; Fogel and Hurst, 1967). Because tetrad analysis requires the recovery of all four products of a meiosis, the analysis of complete tetrads has been historically restricted to fungal organisms and single-cell algae (Pascher, 1918). In contrast, the four meiotic products of higher eukaryotes either separate (male meiosis) or undergo selective cell death (female meiosis). The discovery of the *quartet* (*qrt*) mutant of Arabidopsis, a mutation that causes pollen grains to remain attached after cytokinesis (Fig. 1), allowed the extension of tetrad analysis to a multicellular genetic model system (Preuss et al., 1994). While an understanding of the theory and practice of tetrad analysis is essential for anyone exploring genetic mechanisms, these techniques can be extended into many other areas, including development and cell biology. Here we describe the use of tetrad analysis in a higher plant, review recent examples from the literature, and suggest additional opportunities.

CALCULATING LINKAGE WITH TETRAD ANALYSIS

The four meiotic products produced by *qrt* mutants, like those of *Saccharomyces cerevisiae*, are unordered, forming symmetrical tetrads with a geometry that does not reflect spindle orientation or the order of chromosome assortment. Marker pairs in these unordered tetrads assort in three possible patterns (Fig. 2). In parental ditype (PD) tetrads, each meiotic product contains the same pair of alleles as one or the other parent. In non-parental ditype (NPD) tetrads, each meiotic product is recombinant, with novel allelic combinations. In tetratype tetrads (TT), each of the four meiotic products has a different genotype: two parental and two recombinant. These patterns of allelic segregation reveal the linkage relationships between genetic loci, including centromeres.

When two loci are linked, PD tetrads are more abundant than NPD tetrads; if all of the tetrads are PD, the loci are completely linked. Single crossover events between linked loci yield TT tetrads, whereas double crossover events yield PD, TT, or NPD tetrads

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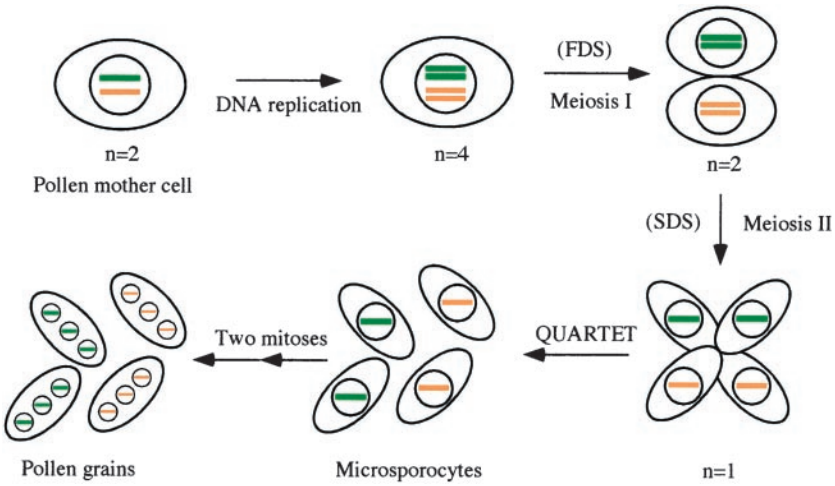

Figure 1. Arabidopsis pollen development. The diploid pollen mother cell undergoes a round of DNA replication resulting in a meiocyte in which $n = 4$. During meiosis I, first division segregation (FDS) separates homologous chromosome pairs generating two cells in which $n = 2$. During meiosis II, second division segregation (SDS) separates sister chromatids and gives rise to four haploid cells. In Arabidopsis, pectin components in the exine wall of the pollen grains are degraded resulting in separation of the pollen tetrad. In *qrt* mutants, failure to degrade the pectin components leaves the pollen tetrad intact.

depending on the number of chromatids involved. The frequencies of each of these classes of tetrads can be used to calculate distances between linked markers with the equation: centiMorgans (cM) = [(1/2TT + 3NPD) ÷ total no. of tetrads] × 100.

Unlinked loci alternatively yield an equal number of PD and NPD tetrads; in such cases, the percentage of TT tetrads can be used to calculate the linkage of each locus to its centromere. During meiosis I, homologous chromosomes are drawn to opposite poles via their connection to the spindle apparatus at the centromere. Thus centromeres and centromere-linked genetic markers always segregate to opposite poles; pairs of centromere-linked markers that reside on different chromosomes yield only PD and NPD patterns. In contrast, recombination frequently separates distal markers from their centromeres, yielding a TT pattern when compared to centromere-linked markers (Fig. 2). The distance between these markers and their centromeres is determined by the equation: cM = (1/2TT) ÷ total no. of tetrads.

Similar calculations can be made using half-tetrad analysis, a special case of tetrad analysis that is possible when only two of the four meiotic products can be analyzed. This method has been used in several plant and animal species, including fruitflies (*Drosophila melanogaster*), zebra fish (*Danio rerio*), humans, alfalfa (*Medicago sativa*), potatoes (*Solanum tuberosum*), and corn (*Zea mays*) (Anderson, 1925; Rhoades and Dempsey, 1966; Mendiburu and Peloquin, 1979; Johnson et al., 1995; Tavoletti, 1996). Although half-tetrads can be used to map centromeres, they are less helpful when analyzing genetic events that require knowledge of all four meiotic products (such as gene conversion or chromatid interference).

The genetic segregation data that result from tetrad analysis often require repetitive calculations that can easily be accommodated with a computer spreadsheet program. Each marker allele can be represented by a "1" or "0," making it possible to calculate PD, NPD, and TT frequencies quickly for any pair of markers (Fig. 2C). The frequencies of these classes can then be used with the mapping functions described above to determine genetic map distances. It is important to note that the frequency of TT tetrads for unlinked marker pairs can be used to calculate centromere positions.

RESOURCES FOR TETRAD ANALYSIS IN ARABIDOPSIS

Since the discovery of the Arabidopsis *qrt* mutation, tetrad analysis has become an efficient tool for plant biologists. Lesions in either the *QRT1* or *QRT2* genes of Arabidopsis lead to defects in pectin degradation following male meiosis, preventing the normal separation of developing pollen grains from one another (Rhee and Somerville, 1998). This absence of normal separation results in fusion of the pollen exine walls, but leaves *qrt* pollen viable and fertile in every other respect (Preuss et al., 1994; Copenhaver et al., 1998). Because the fusion of *qrt* pollen grains does not involve the inner intine wall, there is no mixing of cytoplasmic or nuclear components between the meiotic products. Although many other plants possess the capacity to package their meiotic products into pollen tetrads, including water lilies (Nymphaea), cattails (Typhaceae), heath (Ericaceae and Epacridceae), evening primroses (Onagraceae), sundews (Droseraceae), orchids (Orchidaceae), acacias (Mimosaceae), *Dysoxylum* spp. (Meliaceae), and petunias (Solanaceae) (Levan, 1942; Large and Mabberley, 1994; Preuss et al., 1994; Smyth, 1994), these organisms do not yet have the extensive genetic resources of Arabidopsis.

To determine the genotype of each member of a pollen tetrad, one could perform PCR analysis on individual grains (Matsunaga et al., 1999), a procedure that would require separation of the pollen, disruption of the exine layer, and efficient DNA amplification. The inherent technical difficulties, however, coupled with the limited number of loci that

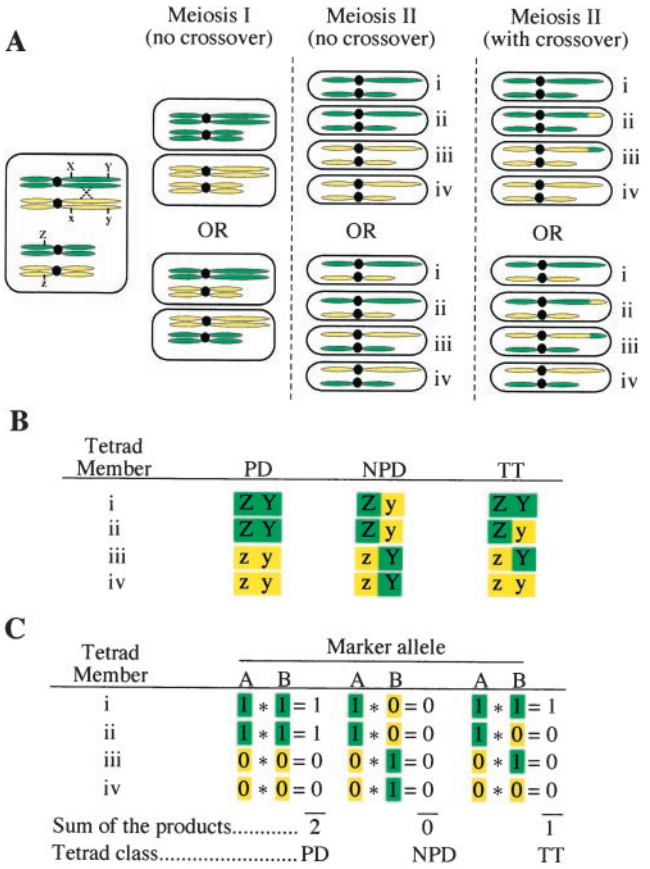

Figure 2. Segregation analysis in tetrads. A, Two pairs of chromosomes are diagrammed progressing through meiosis. Each chromosome pair is composed of four chromatids; two from each parent (green and yellow, respectively). Upon completion of meiosis each member of the tetrad (i–iv) inherits one chromatid. The segregation of markers (X–Z) with different alleles (upper and lowercase) depends on the alignment of chromosomes at meiosis I and the distribution of recombination events (dashed lines). B, Scoring marker pairs in each tetrad member reveals three possible segregation patterns: parental ditype (PD), non-parental ditype (NPD), or tetratype (TT). Recombination can result in TT patterns. With markers on different chromosomes (Z and Y), crossovers between one of the markers and its centromere yields a TT; similarly, with linked markers (X and Y), a single crossover between them results in TT (not shown). C, Assigning each parental allele a "1" or a "0" value allows PD, NPD, and TT tetrad patterns to be converted to numerical data (2, 0, and 1, respectively).

could be analyzed in each grain, make it preferable instead to obtain pollen tetrads that are segregating alleles of interest and to cross them to appropriate females and analyze the resulting progeny. For example, crossing two *qrt* plants from different ecotypes yields an F_1 plant that is heterozygous for multiple polymorphisms (Fig. 3). These polymorphic markers segregate in the expected 2:2 ratio in the pollen tetrads produced by the F_1 plant (Copenhaver et al., 1998). Pollen is collected from mature anthers by tapping them on a glass slide. A hair is attached to the end of a small wooden dowel and is subsequently used to lift a single pollen tetrad onto a stigma of an appropriate female. To avoid contamination from self-pollination, it is convenient to use stigmas from a male-sterile strain, such as *ms1* (van der Veen and Wirtz, 1967). Crosses with individual pollen tetrads yield three or four seeds approximately 40% of the time. The tissue produced by these progeny yields sufficient DNA for thousands of PCR reactions, and their seeds provide a permanent resource for genetic analysis. The segregation of any type of genetic marker can be followed in the four progeny plants. Codominant PCR-based molecular markers such as simple sequence length polymorphisms, cleaved-amplified polymorphic sequences, and single nucleotide polymorphisms SNPs are reliable, easy to score even in large numbers, and require only a small amount of purified DNA (Konieczny and Ausubel, 1993; Bell and Ecker, 1994; Cho et al., 1999). RFLPs can also be used, but these markers require larger DNA preparations. Morphological markers have the advantage of rapid analysis if multiply marked parental lines are used to create the F_1.

USING TETRAD ANALYSIS TO STUDY MEIOTIC MECHANISMS AND PLANT DEVELOPMENT

In addition to investigating the mechanisms of meiosis and recombination, tetrad analysis can also be used to identify key genes required for pollen development. Moreover, the ability to monitor the expres-

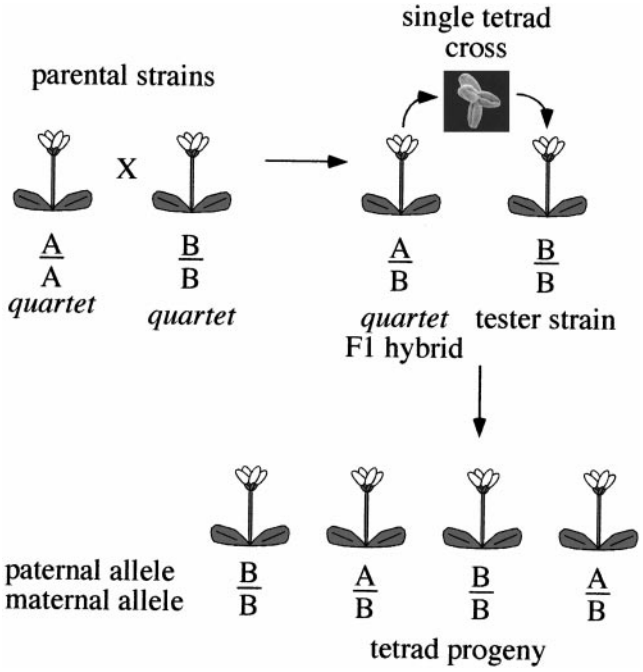

Figure 3. Performing tetrad crosses in plants. Two *qrt* parental strains (A and B) are crossed to produce a *qrt* F_1 plant that is heterozygous for all the polymorphisms between the two parents. Individual pollen tetrads from the F_1 plant are placed onto the stigmas of a receptor plant of known genotype (B). Each pollen grain in the tetrad fertilizes a different ovule resulting in four tetrad progeny.

sion of genes within pollen grains provides practical tools that could enhance the efficiency of plant genetic screens. Below, we summarize recent studies that employed *qrt* for tetrad analysis and suggest future applications for this technology.

Analyzing Genes Required for Pollen Development

Although some gene products contained within pollen grains are derived from the sporophytic (diploid) parent, including the pre-meiotic pollen mother cell and the surrounding tapetal tissues, a large fraction of the pollen contents are expressed during the gametophytic (haploid) phase that follows meiosis. In some species, as many as 60% of the genes expressed during vegetative development are also expressed in haploid pollen, and approximately 10% of all genes in these species are pollen specific (Stinson et al., 1987). The *qrt* mutation tremendously facilitates investigation of these haploid-specific genes. In heterozygotes, gametophytic mutant phenotypes segregate 2:2 in pollen tetrads; in contrast, genes under sporophytic control segregate in a 4:0 or 0:4 pattern for dominant or recessive mutations, respectively.

Tetrad analysis was used to prove the gametophytic function of two genes required for normal cell division in Arabidopsis pollen development: *SIDECAR POLLEN* (*SCP*) and *GEMINI POLLEN1* (*GEM1*) (Chen and McCormick, 1996; Park et al., 1998). In the Nossen-0 and Columbia-0 ecotypes the *scp* mutation causes a mixture of wild-type, aborted, and extracell pollen, but in the Landsberg *erecta* ecotype it causes pollen lethality. By crossing *scp* to *qrt*, Chen and McCormick generated *+/scp*; *qrt/qrt* plants in a Nossen-0/Landsberg *erecta* mixed background. These plants produced pollen tetrads with two wild-type grains and two aborted grains, indicating that *scp* was acting as a gametophytic pollen lethal (Fig. 4). A similar strategy was used to examine *gem1* mutants that produce twin-cell pollen grains due to an extra mitotic division during pollen development. Pollen produced by *+/gem1*; *qrt/qrt* plants never contained more than two aberrant grains but often contained fewer, indicating that *gem1* is an incompletely penetrant gametophytic mutation. The *qrt* mutant was further utilized to examine the geometry of the extra mitotic divisions in *gem1* pollen; in the aberrant pollen grains, these divisions were aligned on the normal division axis.

The *qrt* mutation can also be used to examine the uniformity of developmental events associated with individual meioses. Mutants in the Arabidopsis *MEI1* gene undergo an aberrant meiosis, resulting in more than four pollen grains that vary in size and DNA content (He et al., 1996). To discern exactly how many cells are produced by individual meioses in *MEI1* plants, He and Mascarenhas (1998) constructed *MEI1-qrt* double mutants, making it possible to isolate and count the meiotically related pollen clusters. The authors found significant variation in the number and size of cells within individual clusters and concluded that *MEI1* could function in several stages of meiosis.

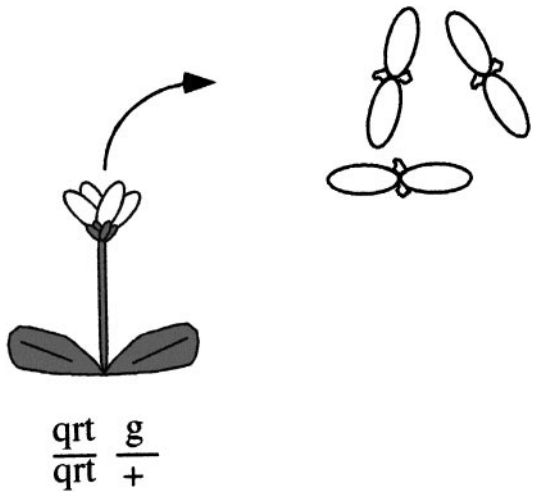

Figure 4. Gametophytic segregation. Gametophytic genes are expressed in the haploid products of meiosis. Plants that are heterozygous for a male-specific gametophytic allele (g) will yield pollen grains that segregate the phenotype in a 2:2 ratio, which can be readily verified with *qrt*. Tetrads containing two aborted and two viable pollen grains occur with gametophytic lethal allele.

These studies demonstrate that *qrt* is useful for analyzing a variety of defects in pollen development. In fact, *qrt* can be used as the basis for a genetic screen designed to detect lesions in any gametophytically important gene. We have expressed a visible marker, green fluorescent protein, under the control of a pollen-specific promoter (G.P. Copenhaver, J. MacGurn, and D. Preuss, unpublished data). Following *Agrobacterium tumefaciens*-mediated transformation we found that insertions into gametophytic genes required for pollen development resulted in pollen tetrads with green fluorescent protein-marked, inviable pollen grains. Of 143 primary transformants surveyed, eight show a clear 2:2 aborted: viable phenotype in the pollen tetrads.

Constructing Genetic Maps

As diagrammed in Figure 2, marker assortment in tetrads can be used to construct genetic maps. With this approach, fewer recombinant individuals are required to obtain map distances, gene order can be readily defined by examining all four chromatids, and the distance at which linkage can be detected expands. We have used tetrad analysis to analyze recombination across the entire Arabidopsis genome, scoring all of the crossovers that occurred in individual meioses in Arabidopsis (Copenhaver et al., 1998). The number and distribution of crossover events in 57 meioses were measured by analyzing the segregation of 52 PCR-based markers spaced at approxi-

mately 10-cM intervals. This study revealed that the number of crossover events in each meiosis ranged from five to 13 with an average of 8.9 ± 1.8 (SD). Almost every chromosome experienced at least one crossover, suggesting that recombination is required for proper chromosome disjunction in Arabidopsis.

Crossover interference, a bias in the expected frequency of double crossovers, can also be measured with these techniques (Whitehouse, 1942). Chromosomal interference is detected when the expected frequency of double crossovers in adjacent genetic intervals differs significantly from the observed frequency of single crossovers in the individual intervals (Fig. 5A). In contrast, chromatid interference results in a non-random distribution of double crossovers on DNA strands, producing a deviation from the expected 1:2:1 ratio of two:three:four-strand double crossovers (Fig. 5B). Although chromosomal interference can be measured with other methods, chromatid interference requires knowledge of the crossover status of all four DNA strands at meiosis I and thus can be determined only with tetrad analysis. In our previous study of genomic recombination in Arabidopsis, significant chromosomal interference was observed (33 double crossovers observed versus 93 predicted), but chromatid interference was not detected on any chromosome (Copenhaver et al., 1998).

Detecting Gene Conversion

The physical replacement of one allele with another is known as gene conversion, an event that can result from mismatched repair of heteroduplex DNA during recombination (Mitchell, 1955; Meselson and Radding, 1975; Paques and Haber, 1999). Meiotic gene conversion events can be formally proven only with tetrad analysis; in contrast, when genetic analysis is performed with random gametes, closely spaced double crossovers are assumed to reflect gene conversion. Examination of all four chromatids, however, can discriminate between actual gene conversion events and other possibilities such as local negative interference. With tetrad analysis, an allele that undergoes gene conversion segregates in a 3:1 pattern (Fig. 6), whereas flanking alleles segregate 2:2. It is surprising that in our work with Arabidopsis, we have yet to detect a gene conversion event. This observation may stem from insufficient marker density or may reflect an unexpectedly low frequency of gene conversion events.

Identifying Regions That Provide Centromere Function

A number of methods have been used to map centromeres in higher eukaryotes, including plants. Chromosome breakage experiments localize centromeres by identifying chromosome fragments capable of autonomous segregation (Sears and Lee-Chen, 1970; Koornneef et al., 1983; Tyler-Smith et al., 1993; Murphy and Karpen, 1995; Sacchi et al., 1996). This method can be limited by the difficulty of obtaining desired breakpoints and by the activation of cryptic centromeres on acentric DNA fragments. Cytological methods alternatively reveal heterochromatic regions of the chromosome or localize proteins implicated in centromere function (Rattner, 1991; Sunkel and Coelho, 1995; Fransz et al., 1998). Such techniques can have limited resolution and cannot precisely identify the DNA sequences critical for centromere function. Several classes of repetitive DNA are known to colocalize with cytologically defined centromeres, but it is still unclear if these classes of DNA are required for centromere activity (Round et al., 1997).

As detailed above (Fig. 2), tetrad analysis can uniquely define the region of each chromosome that segregates to the cell pole in meiosis I. We took advantage of this property to map, with high precision, all five centromeres in Arabidopsis (Copenhaver et al., 1998, 1999). The same set of 57 tetrads used for the genome-wide scan of recombination pro-

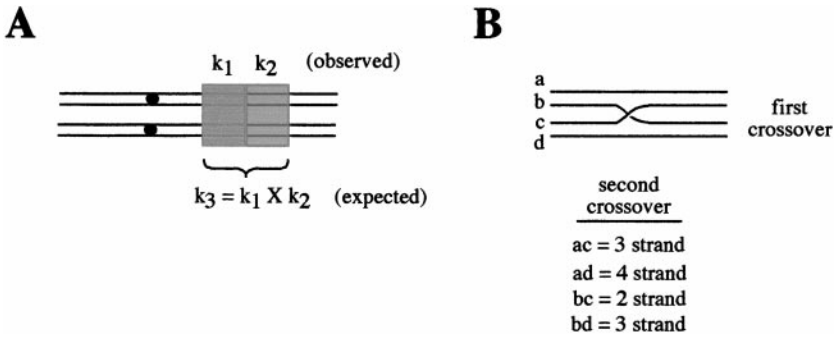

Figure 5. Crossover interference. A, If crossovers are independent of one another the product of the frequencies (k) of single crossovers within adjacent intervals (k_1 and k_2) equals the frequency of double crossovers (k_3) in the combined interval (bracket). If the observed number of double crossovers within this region is less than the expected frequency then the interval is experiencing positive interference; in contrast, negative interference will yield more crossovers than expected. B, If crossovers are distributed randomly among the four chromatids (a–d), double crossovers should occur in a 1:2:1 ratio of two-strand:three-strand:four-strand events.

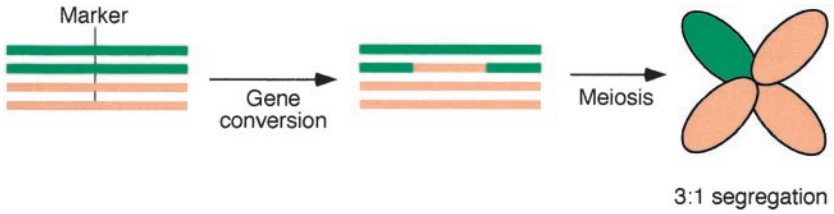

Figure 6. Detecting gene conversion in tetrads. Gene conversion occurs when genetic information is non-reciprocally transferred from one chromatid to another (orange and green bars) resulting in a non-Mendelian (3:1) segregation pattern. These events can be definitively detected with tetrad analysis because all four products of meiosis are available for inspection.

vided an initial centromere position for each chromosome. By developing additional PCR-based markers, assembling contigs of bacteria artificial chromosome (BAC) clones, and scoring over 1,000 tetrads, we refined these initial centromere positions. This study revealed that the recombinationally suppressed centromeric regions of Arabidopsis encompass an array of repetitive elements and are flanked by regions rich in mobile DNA elements. Despite their repetitive nature, the Arabidopsis centromeres contain many genes. We are currently extending these studies by using tetrad analysis to assess the assortment of chromosome fragments, aberrant chromosomes containing two centromeres, and synthetic minichromosomes (K.C. Keith and D. Preuss, unpublished data).

Detecting Chromosome Rearrangements

Since the construction of the earliest fruitfly mapping strains, balancer chromosomes that contain translocations or inversions have been recognized as important genetic tools (Casso et al., 2000). In plants, these rearrangements can often occur inadvertently as a consequence of *Agrobacterium tumefaciens*-mediated plant transformation (Castle et al., 1993; Nacry et al., 1998). Tetrad analysis is a useful method for rapidly detecting and analyzing these aberrations.

A plant that is heterozygous for a balanced translocation can undergo two types of meiotic segregation: adjacent or alternate (Fig. 7). In the latter case, all four meiotic products contain a balanced set of chromosomes, but in the former case, all four meiotic products have duplications and deficiencies that are usually lethal. Because the frequencies of adjacent and alternate segregation patterns are approximately equal, a *qrt* plant heterozygous for a translocation will yield equal numbers of tetrads containing all aborted or all wild-type pollen grains. Ray et al. (1997) took advantage of this property to confirm that they had found a desired reciprocal translocation, TL-1, caused by T-DNA mutagenesis. In their strains, *qrt* plants showed an aborted pollen phenotype that segregated in a 4:0 and 0:4 pattern with equal frequency. This line also produced some pollen tetrads segregating aborted pollen in 3:1, 2:2, and 1:3 patterns, suggesting that recombination events capable of restoring a balanced chromosome set were occurring. Using these strains, Ray et al. (1997) subsequently showed that, as expected, one-half of the female meioses were also aberrant and that the resulting defective female gametophytes were incapable of attracting pollen tubes.

Non-Mendelian Inheritance

Whereas tetrad segregation patterns of 2:2 indicate that a particular phenotype is under the control of the nuclear genome, consistent 0:4 or 4:0 patterns suggest that the phenotype is determined by an organelle, such as the mitochondrial or chloroplast genome, or is a cytoplasmic component inherited from the precursor diploid cell. When plants with two different organelle genotypes are crossed, the resulting F_1 will

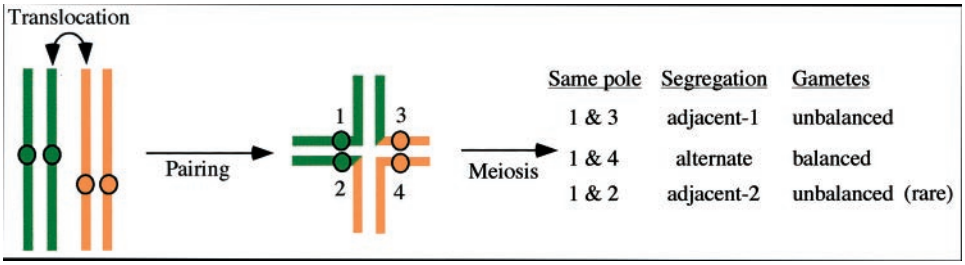

Figure 7. Chromosome segregation in translocation heterozygotes. In individuals heterozygous for a reciprocal translocation, the affected chromosomes form a tetravalent structure upon pairing. During meiosis I homologous centromeres (1–4) disjoin and migrate to the cell poles. Segregation in a tetravalent can occur in two ways: either adjacent chromosomes (1 and 3) or alternate chromosomes (1 and 4) can migrate to the same pole. In rare cases, homologous centromeres fail to disjoin and a second form of adjacent segregation (adjacent-2) can occur.

typically have the composition of the maternal parent, since most plants show maternal inheritance of organelles (Birky, 1978). The pollen tetrads from the F_1 will consequently segregate in a 4:0 pattern, reflecting the maternal allele. A similar effect is seen in yeast; although both parent cells contribute organelles to the F_1 zygote, subsequent mixing and distribution of organelles results in uniform 4:0 inheritance in the spores (Wolf et al., 1978).

During our effort to map the centromeres in Arabidopsis, the ability to distinguish between nuclear and organelle inheritance with tetrad analysis was critically important. As DNA clones were identified for sequencing on chromosome II, a BAC clone that contained nDNA fused to DNA that was highly similar to the sequence of the Arabidopsis mitochondrial genome was characterized (Unseld et al., 1997; Lin et al., 1999). The identification of a second BAC clone with a different mitochondrial-nuclear junction raised the possibility that these clones corresponded to a large insertion of mitochondrial DNA into the nuclear genome rather than chimeric constructs formed during construction of the libraries. To test whether there was indeed a large mitochondrial insertion into the chromosome, we designed PCR primers that detected polymorphisms at the junction of the nuclear and mitochrondrial DNA. Scoring these markers in the tetrads used to map the centromeres showed, in every case, a 2:2 pattern, confirming a large (270 kb) insert of the mitochondrial genome into chromosome II.

FUTURE OPPORTUNITIES: INVESTIGATING GENETIC REDUNDANCY

The enormous number of duplicated genes within the Arabidopsis genome will require creative approaches aimed at discerning gene function. It is imperative to investigate mutations in combinations that can reveal genetic interactions, including those interactions that result in synthetic lethality. The latter is a particularly useful phenomenon that has been important for discerning the functions of numerous genes in yeast and other organisms (Huffaker et al., 1987). Two mutations are described as having a synthetically lethal phenotype when their combination results in a non-viable double mutant. Such lethality raises the possibility that the genes contribute to the same biological process. Tetrad analysis provides essential proof that the desired double mutant is indeed lethal. By analyzing only a few NPD tetrads one can conclude that the mutations are synthetically lethal if the two surviving individuals always have a wild-type genotype. In contrast, providing such proof with a randomly segregating population requires large numbers of progeny and relies on statistical analysis.

We recommend the following methods when incorporating this approach in plants. Two *qrt* parental strains each homozygous for a different mutation should be crossed to each other to generate an F_1. In the pollen tetrads from this F_1, the mutant and wild-type alleles will segregate into PD, NPD, and TT patterns. If the genes play a gametophytic role in pollen development, then synthetic lethality will result in pollen tetrads that segregate viable:aborted pollen grains in 4:0, 3:1, and 2:2 patterns. Those tetrads exhibiting a 2:2 segregation pattern should then be crossed to an appropriate female to ensure that the two surviving pollen grains contain only wild-type gametes.

CONCLUSIONS

Recent years have brought an explosion of genomic information in several model organisms, including *S. cerevisiae*, *Caenorhabiditis elegans*, fruitflies, and Arabidopsis, each with their own collection of sophisticated genetic tools. Among these organisms, Arabidopsis stands alone in its ability to address complex questions of genetic inheritance, developmental biology, and cell biology using tetrad analysis. With the approaches described above, it is possible to identify the complete set of genes required for pollen development and function. Moreover, all of the genetic exchanges that occur in an entire genome can be surveyed routinely, yielding important insight into gene conversion and interference mechanisms. As the Arabidopsis community extends its genetic resources to the construction of strains with translocations, inversions, and deletions, tetrad analysis will likely play a key role in rapidly characterizing each of those rearrangements. In addition, tetrad analysis will provide a powerful tool for investigating functional interactions and redundancy among gametophytic genes. The confluence of rich genomic information resources and powerful genetic tools make Arabidopsis one of the most attractive model systems for studying these questions.

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