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Arabidopsis Species Hybrids—Emerging Model Systems for the Analysis of Species Differences

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ABSTRACT

Plant interspecific hybrids have been used to introgress desirable traits for crop improvement, but they have not been widely used in the analysis of plant development and genome evolution. Interspecific hybrids generated by crossing diploid species in the genus *Arabidopsis* are described. It is proposed that these *Arabidopsis* species hybrids represent a unique resource for the functional analysis of vascular plant genomes. The interspecific hybrid lineages are expected to expand the range of biologic phenomena that could be studied with the impressive arsenal of molecular tools available for the model plant *A. thaliana.*

Key words: *Arabidopsis*; *Arabis*; *A. thaliana*; *A. lyrata*; Interspecific hybridization

It is estimated that about a quarter million species of flowering plants exist. The large number of species has fascinated biologists ever since Linnaeus helped define the field of taxonomy in the eighteenth century, but it is the study of the origin and diversity of species that continues to interest and tantalize biologists. Today, as in the 1700s, the diversification that led to the evolution of multitude of species remains an important and fundamental question in biology.

G. Ledyard Stebbins is credited with applying modern evolutionary thinking to the study of plant speciation. His studies on species hybrids culminated in his influential book published in 1950 *Variation and Evolution in Plants* (Stebbins 1950). Stebbins contributed to the intellectual watershed known as the "evolutionary synthesis," the significance of which is ranked by Stephen Jay Gould among the major scientific achievements in this century. However, the study of plant speciation and interspecific variation has not seen the level of intense activity that it deserves, and in recent years there appears even to have been a decline in interest in plant interspecific hybrids, except for their use as a source of disease-resistance genes or genes for increasing yield in plant breeding programs. Plant biology in the twenty-first century will be well served by the development of model systems that exploit the enormous store of natural variation that is manifest in interspecies differences and by the study of the molecular basis of these differences. These models will no doubt enable plant scientists to discover some of the factors that have contributed to the production of the complex mosaic of biologic forms on this planet.

Traditionally, plant growth and development have been studied by generating relevant mutations or by analyzing naturally occurring variants within a species. In only a few cases has the tremendous interspecies variation that was generated over the millions of years of evolution been used. In recent

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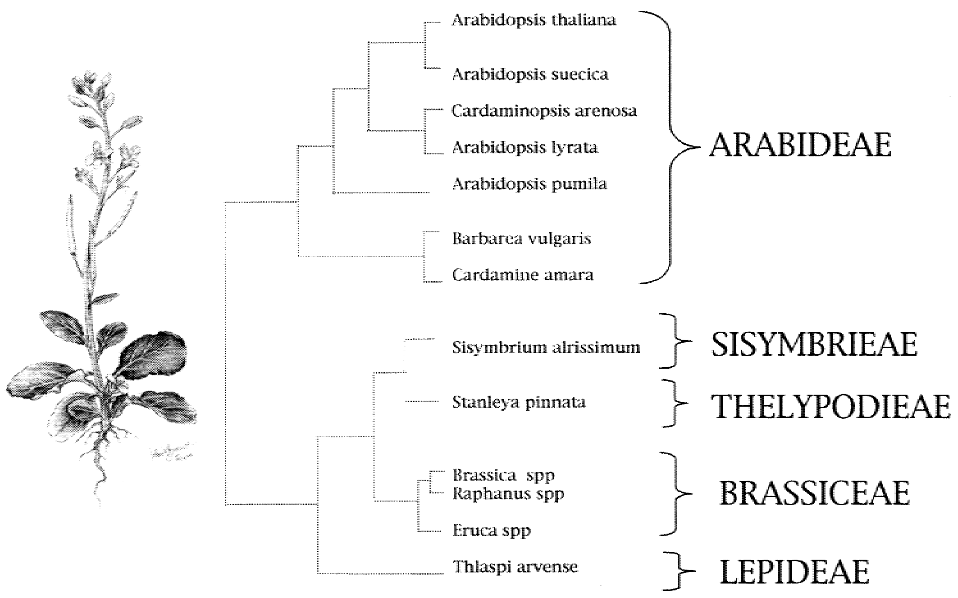

Figure 1. Phylogenetic relationships of *A. thaliana, A. lyrata,* and some other members of the Brassicaceae as determined from molecular data (adapted from Price and others 1994 and Koch and others 1999). Representative species from 5 of the 19 tribes of the Brassicaceae are shown. The tribes to which the species have been assigned are indicated to the right. A sketch of a crucifer plant is shown at left.

years, more people have recognized that natural variability is a major but untapped resource that could complement traditional approaches. For example, in the model plant *Arabidopsis thaliana,* considerable intraspecific genetic variation occurs among different geographical isolates, and this variation, which is largely quantitative in nature, is being studied by using methods developed for the analysis of quantitative trait loci (QTL) in crop plants (reviewed in Alonso-Blanco and Koornneef 2000). However, the enormous store of natural variation that is manifest in interspecies differences has been largely ignored. I suggest that the generation and analysis of interspecific hybrids between *A. thaliana* and related species would provide an additional and unique resource for the functional analysis of the *Arabidopsis* genome.

WHAT PLANT SPECIES AND SPECIES HYBRIDS WOULD MAKE USEFUL MODEL SYSTEMS?

Wide crosses and interspecific hybridizations have been used to investigate the genetic basis of complex traits that differentiate varieties within a species and related species in several plant families (Bernacchi and Tanksley 1997; Bernatzky and others 1995; Bradshaw and others 1995; Doebley and others 1990; Eubanks 1997; Lin and Ritland 1997). But it is in the crucifer family that the development of an interspecific hybrid model would be the most useful, for reasons that I hope are obvious. Taxa in this family are highly diverse and include crop species belonging to *Brassica* and *Raphanus,* as well as the wild crucifer *A. thaliana* (Figure 1), which is now one of the favorite model systems for the analysis of the molecular basis of plant development and physiology. That alone is attractive enough, but it is the availability of the *Arabidopsis* genome sequence in public databases that affords biologists unique opportunities to reexamine concepts of speciation and to understand in molecular detail some of the factors associated with species diversification.

The feasibility of generating interspecific hybrids of *Arabidopsis* and closely related species is suggested by the occurrence of *A. suecica,* an allotetraploid thought to be derived from *A. thaliana* and *Cardaminopsis arenosa* (Hylander 1957; Mummenhoff and Hurka 1995; O'Kane and others 1995) that occurs naturally and can be synthesized in the laboratory (Chen and others 1998; Comai and others 2000) by crossing autotetraploid *A. thaliana* (generated by colchicine treatment) and tetraploid *C. arenosa* (see Figure 1 for the phylogenetic relationships of these species). In the 1950s to 1970s (Berger 1966; Laibach 1958; Redei 1972, 1974), interspecific hybridizations were performed in an attempt to clarify the taxonomic relationships of *A. thaliana* to related species. Laibach (1958) performed crosses between *A. thaliana* and the allotetraploid *Cardaminopsis* (now *Arabidopsis*) *suecica* and produced, after embryo rescue, sterile F1 hybrids. Subsequently, Berger (1966) succeeded at producing seed by crossing *A. thaliana* and polyploid *A. pumila* (2n = 32), and Redei (1972, 1974) obtained viable seed and fairly fertile F1 hybrids by crossing *A. thaliana* with tetraploid *C. arenosa* (2n = 32). Hybridizations of *A. thaliana* with related diploid species were rarely performed, although both Mesicek (1967; quoted in Redei [1972]) and Redei (1974), crossed *A. thaliana* with *C. petraea* (2n = 16), each raising sterile hybrid plants (2n = 13) that were not characterized further. However, to our knowledge, crosses between diploid species in the genus *Arabidopsis* have not been used either to uncover naturally occurring variation or to construct stocks for genetic analysis of traits that differentiate species within the genus.

In our laboratory, we found (Nasrallah and others 2000) that *A. thaliana* can be crossed with *A. lyrata* (formerly *Arabis lyrata*), a species that has been recently incorporated into the genus *Arabidopsis* on the basis of molecular data (O'Kane and Al-Shehbaz 1997). The phylogenetic relationships of *A. lyrata* to *A. thaliana* and to the cultivated *Brassica* species are shown in Figure 1. Some of the advantageous attributes of the *A. thaliana–A. lyrata* species pair and their hybrids for the study of interspecific variation may be summarized as follows:

1. Molecular systematic analysis indicates that the two species are closely related and started to diverge one from the other approximately 5 million years ago (Koch and others 1999).

2. The genes of the two species share a high degree of sequence similarity, allowing facile transfer of molecular markers and other data generated by the *A. thaliana* genome project to *A. lyrata* (van Treuren and others 1997).

3. Differences in chromosome numbers between the two species (*A. thaliana* is 2n = 10 and *A. lyrata* is 2n = 16) indicate that genetic divergence over a 5-million-year period has significantly altered the basic genetic apparatus of the two species.

4. *A. lyrata* is self-incompatible and therefore it is by and large an outbreeding species in contrast to *A. thaliana,* which is a self-fertilizing species that rarely outbreeds.

5. From a developmental standpoint, the two species differ in a variety of morphologic traits. These include quantitative differences such as larger mass of floral organs, fruit, and seed in *A. lyrata* relative to *A. thaliana,* as well as qualitative differences such as an annual and ephemeral existence in *A. thaliana* and a perennial growth habit in *A. lyrata.*

Thus, although *A. thaliana* and *A. lyrata* are phylogenetically related, the differences in their mating system (inbreeding vs outbreeding) and growth habit (annual vs perennial) present us with sufficiently divergent gene pools to epitomize the life histories of most angiosperm species. The genetic differences that underlie these important and complex traits are amenable to analysis in *A. thaliana–A. lyrata* hybrids. It should also be noted that species within the immediate taxonomic vicinity of *Arabidopsis* (Price and others 1994) show a range of interesting traits (such as apomixis in *Arabis holboellii* [Bocher 1951; Roy 1995]). Therefore, it is conceivable that the range of traits that may be investigated by an interspecific hybridization approach will be further expanded in the future, should it prove possible to hybridize *A. thaliana* with these other related species.

GENERATION AND ANALYSIS OF *ARABIDOPSIS* INTERSPECIFIC HYBRIDS

To generate interspecific hybrids, we used plants from accessions (collected in Michigan and kindly provided to us by Charles Langley of University of California-Davis) of *A. lyrata* subspecies *lyrata*, whose range in North America extends from Minnesota and Wisconsin south into Missouri, east into Geogia, north into Vermont, and west into Ontario (O'Kane and A1-Shebbaz 1997) and *A. thaliana* ecotype Columbia. We found that sexual hybridization between these species results in the production of viable hybrid offspring and viable backcross progeny, despite the fact that the two species may have diverged about 5 million years ago and that their chromosome numbers differ.

Microscopic analysis revealed that the *A. thaliana* stigma epidermis supports efficient adhesion, hydration, tube emergence, and growth of *A. lyrata* pollen, and these interspecific pollinations resulted in the development of viable seed from which mature plants could be generated by ovule rescue (Nasrallah and others 2000). Several *A. thaliana X A. lyrata* crosses were made by emasculating *A. thaliana* flowers and manually pollinating their stigmas with *A. lyrata* pollen. The hybrid status of the progeny plants derived from these crosses was evident from a variety of morphologic characteristics, such as petal size (Figure 2). That these progenies were true interspecific hybrids was confirmed by cytologic analysis showing the presence of 13 chromosomes (which is the sum of the basic chromosome number of *A. thaliana* [$n = 5$] and that of *A. lyrata* [$n = 8$]), and by DNA gel blot analysis demonstrating the inheritance of restriction fragments from both parental species (Nasrallah and others 2000). Interestingly, and as often observed in interspecific hybrids (Rieseberg and others 2000), the *A. thaliana–lyrata* progenies exhibited a very high degree of hybrid vigor with more luxuriant vegetative growth and flower production than either of the parental species (Figure 3).

Figure 2. Flowers of *A. thaliana* (left), *A. lyrata* (right), and their interspecific hybrid (middle).

The *A. thaliana–lyrata* hybrids were pollen sterile and could not be selfed, as expected for the progeny of wide crosses between species that differ in chromosome number (Eubanks 1997). However, backcrosses of the hybrids to either parent have been successful and should allow the establishment of advanced backcross populations. Backcross 1 plants exhibited a range of phenotypes. They were intermediate in appearance for some traits, more similar to one of the parental species for other traits, and exhibited strong transgressive variation with novel phenotypes not observed in either parental species for still other traits. These observations indicate that genome and/or chromosome recombination is taking place between the diverged *A. thaliana* and *A. lyrata* genomes. Application of QTL mapping methods (Bernacchi and Tanksley 1997; Bernatzky and others 1995) to the analysis of more advanced backcross populations may therefore provide the opportunity for investigating dominance or epistatic genetic relationships underlying traits for which *A. thaliana* and *A. lyrata* differ. The availability of the complete genomic sequence and molecular resources in *A. thaliana* would then facilitate the identification of specific chromosome blocks that affect these traits and the subsequent cloning of the genes responsible for these species differences.

STRENGTHS OF *A. THALIANA-A. LYRATA* HYBRIDS: BIOLIGICAL QUESTIONS THAT CAN BE ADDRESSED IN THESE INTERSPECIFIC HYBRIDS

By increasing the genetic variability available for study, the *A. thaliana–lyrata* hybrid populations we have generated should be useful in the analysis of a number of different plant processes. Some examples are discussed in the following.

Master Control Genes in the Evolution of Mating Systems and Growth Habit

As stated earlier, the major differences between *A. thaliana* and *A. lyrata* are that *A. lyrata* is an obligate outbreeder because of the operation of a self-incompatibility system and exhibits a perennial growth habit, whereas *A. thaliana* is a predominantly selfing species with an annual growth habit. Thus, *A. thaliana–lyrata* interspecific hybrids and their backcross progenies should allow a direct analysis of the genetic differences that distinguish self-fertilizing and outcrossing species and those that distinguish annual and perennial species. Because the genetic basis of these traits is likely to be complex, a QTL mapping study would be the approach to use for identifying associations between each phenotype and particular markers. It will be possible to determine the number of loci that differentiate the *A. thaliana* and *A. lyrata* mating systems and growth habits, the magnitute of the effect of each, and which genes might exhibit modification in function or expression in the two species. With respect to mating system differentiation, these studies should reveal commonalities and differences between intraspecific and interspecific mating-system transitions and increase our understanding of the molecular mechanism(s) of pollen rejection and acceptance at the stigma surface. With respect to growth habit, these studies are likely to uncover differences in the behavior of meristems, which are ephemeral in annuals but long-lived and prolific in perennials.

Genome Evolution

Genome differentiation in closely related species. Backcross populations of *A. thaliana–lyrata* hybrids may be used to investigate the extent of chromosome differentiation between *A. thaliana* and *A. lyrata* and the degree to which it might interfere with chromosome pairing and gene flow between the two species (Rieseberg and others 1996, 1999, 2000; Ungerer and others 1998). It will be important to determine whether the backcross plants incur differential inheritance of different chromosome blocks. These studies should lead to the identification of loci that contribute to genetic isolation between the two species, because such loci are expected to be introgressed at a slower rate than neutral loci or loci that are positively selected (Rieseberg and others 1999).

The identification of positively selected chromosomal segments that increase the fitness of backcross

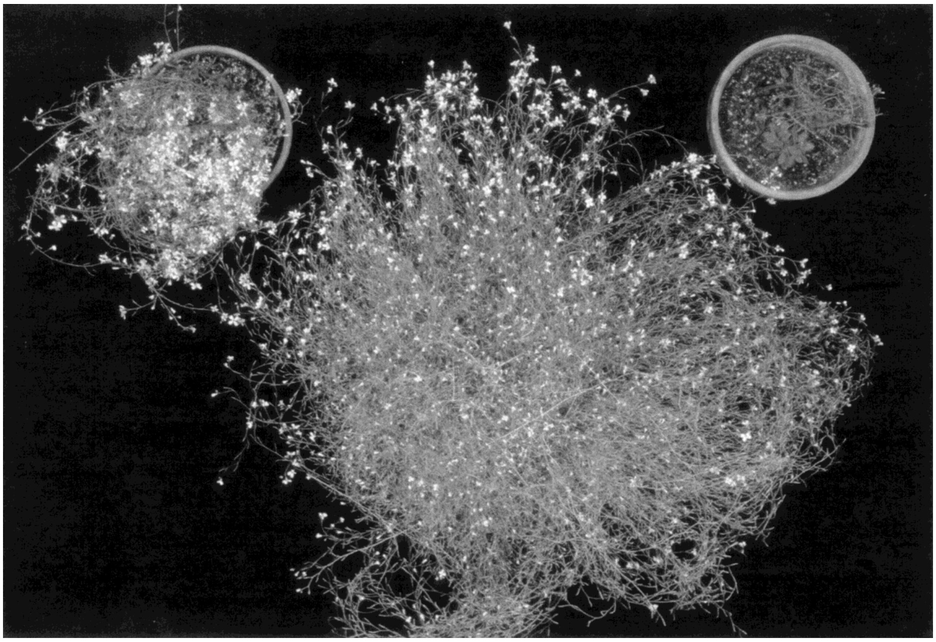

Figure 3. Luxuriant growth of the *A. thaliana–lyrata* interspecific hybrids. The interspecific hybrid (middle) exhibits heterosis for vegetative growth and flower production relative to its *A. lyrata* (left) and *A. thaliana* (right) parents. The three plants shown were grown in 6-inch pots.

progeny (Rieseberg and others 1999) would provide a basis for the study of heterosis. The phenomenon of heterosis or hybrid vigor is poorly understood despite its perceived importance in evolution and its practical significance in breeding programs that aim to increase yield of crop plants. Several models have been proposed to explain the genetic basis of heterosis, including the masking of deleterious alleles (dominance), complementation of allelic variants (overdominance), and interactions of different dominant genes (epistasis) (Rieseberg and others 2000). However, the few molecular marker studies that have addressed this issue have led to conflicting conclusions regarding the primary cause of heterosis (Mitchell-Olds 1995; Monforte and Tanksley 2000; Rieseberg and others 2000; Xiao and others 1995), possibly because a combination of causes might produce heterotic effects. Clearly, explanation of the genetic basis of heterosis will benefit from genome-wide high resolution analyses, for which the *A. thaliana–lyrata* hybrid lineages are well suited.

Origin and stabilization of amphiploid species. Biologists agree that polyploidy is not only common in plants but that it has played a major role in higher plant evolution (Clausen and others 1945; Leitch and Bennett 1997; Liu and others 1998; Soltis and Soltis 1999; Song and others 1995; Wendel 2000). In particular, amphiploidy, which involves the merger of two or more differentiated genomes has significant potential for species diversification. Fertile amphiploids arise by chromosome doubling in a sterile interspecific hybrid, which restores bivalent pairing and regular meiosis (Srb and others 1965). This chromosome doubling must occur spontaneously in nature but can be induced in the laboratory by colchicine treatment to generate artificial amphiploids (for example, Song and others 1995). Recent molecular studies have shown that amphiploidy is not as rare as previously thought and that it can occur repeatedly, with individual amphiploid species having originated independently and multiple times from the same diploid species (Soltis and Soltis 1999). However, the spontaneous genesis of fertile amphiploid neospecies from sterile species hybrids has been observed in only a few instances, all reported early in the twentieth century. A few fertile amphiploids were obtained in crosses between the two morphologically and cytologically distinguishable species *Nicotiana glutinosa* and *Nicotiana tabacum* ($n = 12$ and $n = 24$, respectively) (Clausen and Goodspeed 1925). The fertile neospecies *Primula kewensis* arose as a fertile shoot at Kew, England, on a sterile hybrid of *P. floribunda* (a species from Afghanistan) and *P. verticillata* (a species from Arabia) (Digby 1912; Pellew and Durham 1916). And the distinct taxanomic entity known as *Raphanobrassica* (Karpechenko 1927; Srb and others 1965) arose from a sterile hybrid derived from an intergeneric cross between *Brassica oleracea* and *Rhaphanus sativus* (Figure 4).

It has been suggested that a major factor favoring the generation and establishment of new amphiploids from two differentiated species that can hybridize with each other is "long perennial growth habit to increase the chances of somatic doubling or, as a partial compensation in short-lived annuals, an au-

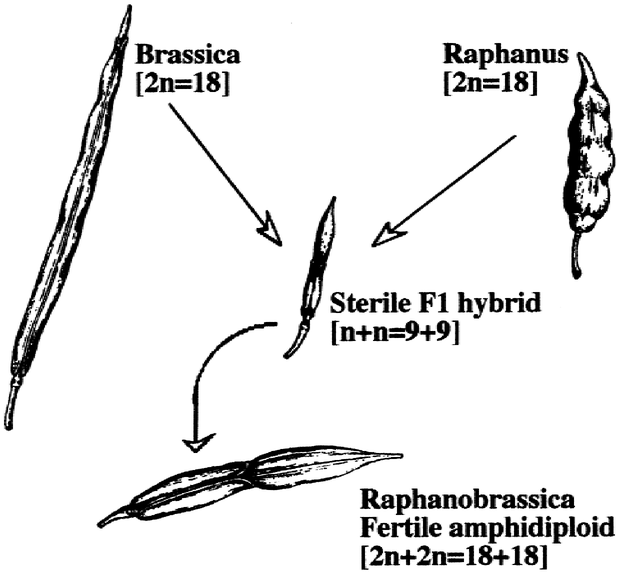

Figure 4. Origin of a classical crucifer amphidiploid: *Raphanobrassica* is shown as having arisen by chromosome doubling in a sterile *Brassica-Raphanus* hybrid (after Srb and others 1965).

togamous breeding system to increase the chances of union of unreduced gametes" (Grant 1981). The *A. thaliana–lyrata* hybrids we generated are long-lived and thus provide a test for the hypothesis that fertile amphiploid neospecies are generated from interspecific hybrids by somatic chromosome doubling.

In any event, the availability of *A. thaliana–lyrata* amphiploids, whether they occur spontaneously or are induced by colchicine treatment, will allow us to address several issues regarding genome evolution in polyploids at a level of detail not possible in other species because of the availability of the *A. thaliana* genome sequence. Furthermore, because these amphiploids would originate from diploid parental species, their analysis should be more straightforward than that of *A. suecica* amphiploids, which are derived from tetraploid parents (Chen and others 1998). Among the questions that might be addressed are: Does the genome of these amphidiploids undergo rapid karyotypic and genomic changes? Is there evidence for subsequent diploidization (Soltis and Soltis 1993)? Are chromosomes, chromosome segments, or specific sequences eliminated? Does the genome become rearranged? Do processes such as nucleolar dominance and epigenetic changes (such as differential DNA methylation, gene-dosage compensation, gene silencing) occur? And do these changes occur in a random fashion or in a nonrandom (and thus predictable) fashion in different amphidiploid plants?

In summary, the *A. thaliana–lyrata* hybrids and their backcross populations that we have generated provide a unique resource that should complement ongoing studies of the model plant *A. thaliana.* The strengths of this interspecific hybrid system relate to its potential to facilitate progress in several important research areas:

- The analysis of biological processes that do not exist and therefore cannot be studied in *A. thaliana,* such as self-incompatibility and perennial growth habit
- The understanding of genome evolution, specifically as a complement to the commonly used strategy of comparative mapping of the differentiated genomes of related species
- The genetic basis of heterosis
- Explanation of biologic issues that cannot be addressed by the analysis of one species alone, namely the evolution of mating systems and the origin and stabilization of polyploid species, two processes that have played major roles in plant evolution.

It should be noted, however, that interspecific hybrid systems have several limitations:

- Interspecific pollination barriers, although not a major issue in the *Arabidopsis* interspecific hybrid system described here, may not allow the production of hybrids in other taxa.
- The production of hybrids is labor intensive because of the large number of pollinations and embryo rescue strategies that are typically required to overcome reproductive barriers between species.
- The establishment of backcross populations is a long process that requires several generations.
- Interspecific hybrids are typically sterile; therefore, F_2 populations are not available for genetic investigations.
- Interspecific hybrids can exhibit nuclear/cytoplasmic incongruity and other barriers to gene flow that might prevent the transfer of certain traits.

Ultimately, the success of the *Arabidopsis* species hybrids, as well as any interspecific hybrid system, will depend on the establishment of advanced backcross populations in which the segregation of interesting traits may be analyzed.

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