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Hybridization, introgression, and linkage evolution

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Abstract

Genetic mapping methods provide a unique opportunity to study the interactions of differentiated genes and genomes in a hybrid genetic background. After a brief discussion of theoretical and analytical concerns, we review the application of these methods to a wide range of evolutionary issues. Map-based studies of experimental hybrids indicate that most postzygotic reproductive barriers in plants are polygenic and that the expression of extreme or novel traits in segregating hybrids (transgressive segregation) results from the complementary action of divergent parental alleles. However, genetic studies of hybrid vigor do not concur in their interpretations of the relative roles of dominance, overdominance, and epistasis. Map-based studies of natural hybrids are much rarer, but the few existing studies confirm the polygenic basis of postzygotic barriers and demonstrate the utility of genetic linkage for detecting cryptic introgression. In addition, studies of experimental and natural hybrid lineages provide compelling evidence that homoploid hybrid speciation has occurred in nature, and that it represents a rapid and repeatable mode of speciation. Data further indicate that this mode is facilitated by strong fertility selection and high chromosomal mutation rates. We recommend that future studies of hybrid genomes focus on natural hybrids, not only because of the paucity of data in this area, but also because of the availability of highly recombinant hybrid genotypes in hybrid zones. Of particular value will be studies of long-lived or difficult-to-propagate organisms, which previously have not been amenable to genetic study.

Introduction

Hybridization can be viewed as a reunion between differentiated genetic material. Until recently, the results of these reunions could only be studied in a fairly indirect manner. One method was to analyze the phenotype of hybrids, such as the symmetry of morphological characters or the viability of pollen or seed. Alternatively, meiosis in hybrids could be studied by light microscopy and the degree of differentiation between hybridizing taxa estimated by analyses of chromosome pairing behavior and meiotic abnormalities. Although both of these approaches have been extremely valuable, they can only provide glimpses into the complex interactions of alien genes and genomes following genetic reunions.

This decade has seen two technological advances that have revolutionized our ability to study hybrid genomes: molecular-marker-based genetic linkage mapping [145, 146] and *in situ* hybridization (ISH) of genomic probes to cytological preparations [10, 61, 66, 74, 88, 111]. These approaches are advantageous relative to traditional methods because they allow the dynamics of parental species chromosomal segments to be monitored in a hybrid genetic background.

With the ISH approach, hybrid or introgressive karyotypes are 'painted' using genome-specific DNA probes [69]. Probes typically are generated by fluorescence labeling of entire genomes or repetitive sequences [43, 124]. The extent of introgression across the entire genome can then be visualized in a single hybridization experiment. However, the method is limited by the requirement of substantial genomic divergence between the taxa studied and by the difficulty of detecting introgression of small chromosomal segments. In addition, the data generated are not conducive to analysis with quantitative genetic theory

because the method does not provide recombination frequency-based estimates of chromosomal fragment sizes.

The use of genetically mapped markers may be less efficient than ISH, but perhaps more versatile since hybridization between genomes of almost any degree of divergence can be analyzed and even very small introgressed segments can be detected. In addition, small or large parts of the genome may be monitored by varying the number and location of markers assayed. Most important, however, is the fact that the sizes of chromosomal segments are based on recombination frequencies, which make the segment size data amenable to theoretical analyses.

This chapter will focus on the use of genetically mapped markers to study the genomic, evolutionary, and ecological consequences of hybridization and introgression. We first review the theoretical and analytical tools required for hybrid genome analyses. This is followed by a detailed discussion of the application of these tools to the study of experimental hybrids. Our focus will then shift to natural hybrids, emphasizing studies of hybrid zones and hybrid species. The review will be concluded by a brief discussion of promising areas for future research and of possible approaches that may facilitate studies in this area. Our focus will be on homoploid hybrids rather than allopolyploids, although our understanding of polyploid evolution also has been enhanced by genetic mapping tools [58, 70, 116].

Readers will notice that the latter portion of this review is biased toward our research on hybridization and introgression in wild sunflowers. This emphasis results from the fact that genetic mapping technology has not yet been applied to natural hybrids in other plant groups, although we know of a number of labs that are beginning to explore this area. Nonetheless, many labs have contributed to the study of experimental hybrids, and this material is discussed in considerable detail.

Theoretical and analytical considerations

Genetic map construction

Genetic maps are based on the principle that the degree of gene linkage, as measured by recombination frequencies, is correlated with differences in the physical distance separating genes on chromosomes [72, 126]. Recombination frequencies between genes or molecular markers can be determined by monitoring meiotic segregation in crosses between individuals that are divergent at these loci. Genetic map distances are based on the frequency of crossing-over between markers, where one map unit or centimorgan (cM) is equal to 1% recombination. The average ratio of genetic to physical distance varies widely across chromosomal regions within species, as well as across taxonomic groups. For example, 1 cM corresponds to 2.5–3.0 kb in yeast [79], ca. 140 kb in *Arabidopsis* [18], and 350 kb in *Eucalyptus* [15].

Over the past two decades, advances in molecular biology have made available a wide variety of molecular markers that can be used for mapping. These include isozymes, restriction fragment length polymorphisms (RFLPs), random-amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and microsatellites. Descriptions of these markers and their advantages and limitations for genetic mapping studies are available elsewhere [95]. The important point for this review is that the almost unlimited number of molecular markers provided by these methods have made it possible to rapidly generate maps of the nuclear genome for almost any species [72, 126]. As a result, genetic maps now exist for most crop plants, as well as many wild plant species [51, 95].

Once genetic maps have been generated for a particular group of species, hybrid individuals can be assayed with mapped molecular markers to estimate hybrid genomic composition. Data resulting from these marker-assisted studies of early-generation hybrids may be analyzed visually using graphical methods developed by Young and Tanksley [145] or analytically using the method of junctions first suggested by Fisher [32] and explored in more detail by Baird [5].

Graphical genotypes

Multilocus genotypes generated by molecular marker assays of hybrid individuals can be difficult to interpret or comprehend in alphabetic or numerical form. To convert these multilocus genotypes into more useful graphical images, Young and Tanksley [145] proposed the concept of ‘graphical genotypes’. Graphical genotypes are similar to cytological karyotypes in describing the entire genome in a single image, but differ in that the genomic constitution and parental derivation for all points on the genome are depicted in a graphical genotype (Figure 1). The graphical genotype approach assumes that the parental derivation of link-

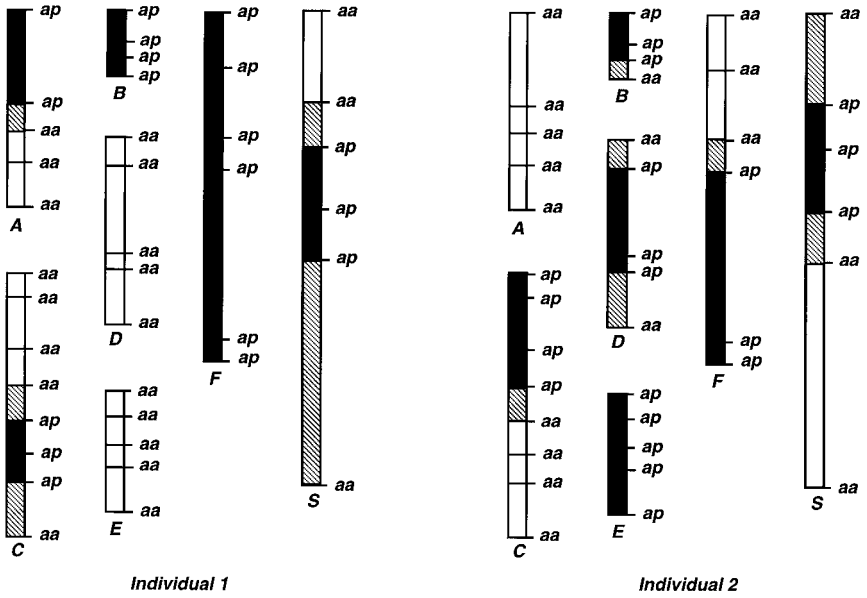

Figure 1. Graphical genotype for two individuals from a BC_1 population of sunflower in which *Helianthus petiolaris* was the donor parent, and *H. annuus* was the recipient parent. Only the seven linkage groups that are collinear between the two species are shown. White blocks indicate regions derived exclusively from *H. annuus*, filled blocks indicate regions derived from *H. petiolaris*, and hashed blocks indicate the presence of a crossover event. Numerical genotypes are also shown for each locus: *aa*, homozygous for *H. annuus*; *ap*, heterozygous.

age blocks may be inferred from flanking markers of known parental origin. Thus, if two adjacent markers are shown to be derived from the same parent, then the intervening block is also assumed to be derived from that parent (Figure 1). Conversely, if adjacent markers differ in parental origin, then the intervening block is assumed to contain a crossover event. A graphical genotype is then constructed by minimizing the total number of crossover events required to explain a given multilocus genotype.

It is possible, of course, that more than one crossover event could have occurred within an interval during its crossing history, falsifying the inference of parental derivation. The probability of multiple crossovers is low for early-generation hybrids or when the interval between adjacent markers is small. However, multiple crossovers may represent a substantial problem for later-generation hybrids or for large linkage blocks.

Although graphical genotypes for first- or second-generation hybrids can easily be constructed by hand, the analysis of more complex pedigrees may require computer assistance. Two computer programs are available to aid in graphical genotype construction: HyperGene [144] and SuperGene [12]. Both require a Macintosh operating system for use.

Junction theory

Junction theory can be viewed as the analytical counterpart of graphical genotypes, and actually predates the graphical genotype method. Junction theory was developed by R.A. Fisher as a theoretical device for tracking parental chromosomal blocks in inbred populations [33, 34]. Because it is not computationally feasible to track all points in the genome, Fisher suggested that recombination breakpoints or 'junctions' between genetic material of different ancestry be monitored instead. Junctions can be gained and lost over multiple generations just like point mutations, and the size and location of parental chromosomal blocks can be inferred from the distribution of junctions.

Fisher used junction theory to predict changes in chromosome block sizes in selfing and sib-mating breeding populations over time. Shortly after Fisher's initial publication, the method was extended to plants with tetrasomic inheritance [9] or progenies with more complex mating designs [53, 54]. Junction theory was independently rediscovered by Robertson [108] as a method for analyzing multilocus evolution under truncation selection and by Barton [6] in a study of migration in multilocus hybrid zones.

Most recently, Baird [5] has suggested that the accumulation of junctions in hybrids can be used as a

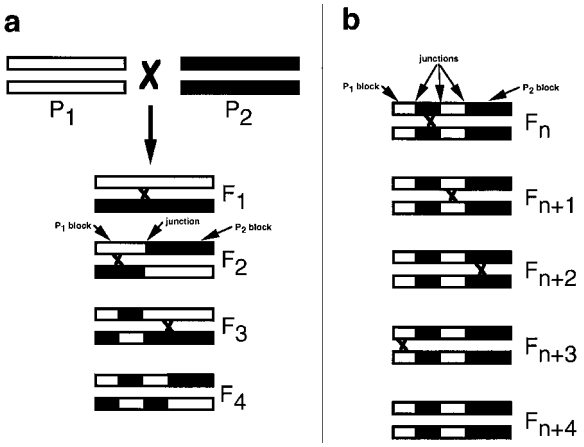

Figure 2. Illustration of the reduction in size and fixation of parental chromosomal blocks (black versus white) over successive generations of hybridization. Chiasmata, and hence junction origin, are designated by an 'X' between intra-generational chromosomes. a. Hypothetical scenario demonstrating how parental species' chromosomal block size decreases and junction number increases over successive generations of hybridization. b. When genomic composition becomes fixed or stabilized, junctions cease to be produced and no further reduction in block size can occur, despite continued recombination in successive generations. Modified from Ungerer *et al.* [125].

kind of clock to estimate the ages of hybrid zones (Figure 2a). The junctions clock can be viewed as analogous to a molecular clock, except that the production of junctions may slow down over time. In fact, in the case of hybrid speciation, the junctions clock will stop once the hybrid genome becomes stabilized (Figure 2b); recombination only occurs between genomic blocks derived from the same parental species [132]. In this situation, the distribution of junctions provides an estimate of the speed or tempo of hybrid speciation rather than the age of the hybrid species.

In addition to providing a method for dating hybrid zones or estimating the tempo of hybrid speciation, junction theory provides an analytical framework for exploring the effects of various selection models, migration rates, and population sizes on the lengths and distribution of chromosomal blocks in simulated populations [5, 6, 108, 132]. A thorough understanding of these parameters and their potential effects is necessary to interpret empirical data sets correctly.

Experimental hybrids

To date, most map-based studies of hybrids have focused on experimental hybrids. There appear to be two reasons for this. First, it is much easier to study experimental than natural hybrids since the markers employed need not distinguish the taxa. Rather, they only need to differentiate the parental individuals used in the cross of interest. Moreover, if inbred lines are used to generate the hybrids, analytical difficulties associated with parental heterozygosity or intraspecific polymorphisms are eliminated. Second, marker-assisted introgression has proven to be a powerful tool for moving traits of agronomic interest from wild plants into domesticated germplasm. Marker-assisted introgression has been the focus of most map-based studies of experimental hybrids, although a few labs have used experimental hybrids to examine evolutionary questions such as the genetic architecture of postzygotic reproductive barriers, the genetic basis of hybrid vigor and transgressive segregation, and the genetics of hybrid speciation.

Marker-assisted introgression

Marker-assisted introgression in agricultural systems may not seem to be immediately relevant to evolutionary biology, but we think that this area has significant future promise for evolutionary studies for two reasons. First, the movement of complex traits from wild into domesticated species may provide insights on how this process might occur in nature. Second, marker-assisted introgression provides a means for reconstructing genotypes that may represent critical steps in adaptation or speciation. The fitness of these genotypes can then be tested under natural conditions, allowing the sequence of genetic changes responsible for the origin of complex adaptations or new species to be elucidated. In our discussion of marker-assisted introgression, the term 'donor parent' will be used to refer to the parent that is only involved in generating first-generation hybrids and thus supplies chromosomal blocks. The 'recurrent' or 'recipient parent' refers to the parent that is crossed with the first generation hybrids to generate backcross plants and thus receives chromosomal blocks from the donor parent.

The potential utility of molecular markers in breeding studies was first recognized by Tanksley and Rick [127], who suggested that markers could be used to test for the presence of a desired gene when direct phenotyping was not possible. They also remarked on the possibility of whole genome selection, in which desired quantitative trait loci (QTL) could be retained and unwanted DNA eliminated. Later in the 1980s, the conceptualization of graphical genotypes [145] enhanced the utility of whole genome selection, and the

method was demonstrated by the rapid reduction of linkage drag (retention of unwanted donor segments of DNA) around the *Tm-2* locus in tomato during backcross breeding [146]. Marker-assisted genomic selection is now fairly common, and many variants of this basic approach are employed [73, 125, 130]. In some instances, only the markers flanking QTL of interest are assayed because the proportion of unlinked donor DNA declines rapidly across backcross generations [146]. In other cases, single-copy or low-copy markers with defined map positions (e.g. RFLPs) are used as flanking markers to ensure the introgression of desired traits, whereas markers with higher information content per reaction (e.g. AFLPs or minisatellites) are used to select for individuals with the maximum proportion of the recurrent parent genome [130].

This empirical work has been accompanied by a fairly substantial body of theoretical studies [37–39, 64, 71, 138] that have focused on the efficiency of marker-assisted breeding relative to phenotypic selection. In general, marker-assisted selection can provide a large increase in efficiency over phenotypic selection if population sizes are large and heritabilities of phenotypes are low. Because of the rapid breakdown of linkage disequilibrium between flanking markers and QTL, marker-assisted selection will be most useful in early hybrid generations. Surprisingly, the number of markers assayed had little effect on the efficiency of selection unless there were fewer than three markers per chromosome [37, 63]. These results generally apply to both additive and non-additive traits [38].

The above studies have focused on the selective response of the hybrid population rather than on the rate at which the genome of the recurrent parent is recovered. However, this latter question may be of the most direct concern to evolutionists trying to generate genotypes that only differ by a particular set of QTL. Computer simulations of whole genome selection suggest a gain of about two backcross generations relative to that expected without selection [63]. That is, genome content of the recurrent parent after three backcross generations with marker-assisted selection will be equivalent to that found in fifth-generation backcrosses lacking selection. A much greater gain in efficiency can be expected, however, if the primary goal is to reduce linkage drag around a single region. For example, in a traditional selection program, ca. 100 generations of selection and backcrossing to the recurrent parent would be required to reduce an introgressed segment to ca. 2 cM, whereas by selection on flanking markers, the same result could be achieved by screening 555 individuals over just two generations [81].

Hybrid sterility, inviability, or breakdown

Genic factors

Genetic mapping studies provide an opportunity to address a fundamental question in evolutionary biology: what is the mechanistic basis of sterility or inviability in hybrids? This is a critical issue because mode and tempo of speciation can be strongly affected by the number and magnitude of genetic changes required for the evolution of reproductive isolation.

The most widely accepted model for the evolution of postzygotic isolating barriers was first proposed by Dobzhansky [27]. In this ‘standard model’, a gene from one species interacts negatively with a gene from the other species, causing some degree of inviability or sterility. Wu and Palopoli [142] argue that the most plausible interpretation of this model is that the hybrid sterility/inviability gene acts like a mutation whose deleterious effects are suppressed by another gene in the source species. However, when placed in the genetic background of another species, the deleterious effects of the sterility/inviability gene are expressed.

A somewhat different model for the evolution of hybrid inviability/sterility is that a much larger number of diverging loci interact negatively in a hybrid genetic background, and these weak interactions act cumulatively to cause inviability or sterility [142].

Classical Mendelian analyses of segregating hybrid populations involving many different pairs of plant species tend to support the standard model in which one or two genes appear to have major effects on hybrid sterility or inviability. Examples include barley [139], cowpea [110], *Crepis* [62], cotton [42], iris [14], *Melilotus* [109], *Mimulus* [20, 82], rice [90], and wheat [59]. However, these observations do not rule out the possibility that additional genes may affect inviability and sterility in these species.

In crosses between subspecies of rice, for example, marker-based QTL analyses of hybrid sterility and hybrid breakdown (reduced fertility or viability of segregating hybrids) suggest that several mechanisms are involved [76, 134]. These include a cytoplasmic gene that causes both male and female sterility and interactions between at least two pairs of complementary genes that lead to greatly reduced fertility. Both of these mechanisms fit the standard model. In addition, recombination between differentiated ‘supergenes’ appears to represent a major source of

sterility [76]. Map comparisons suggest that these regions may contain inversion polymorphisms and that sterility may be due to crossing over between cryptic structural rearrangements (cytologically undetectable chromosomal aberrations) [119].

Hybrid breakdown in rice also fits the polygenic model and appears to result from the uncoupling of coadapted subspecific gene complexes by recombination [76]. In later-generation hybrids, semisterility appears to be due largely to incompatibility interactions among many loci, and hybrid weakness appears to result from the break-up of coadapted gene complexes that affect fitness traits such as heading age and floret number per panicle. The presence of these coadapted gene complexes in rice has long been suspected because of observations that intersubspecific hybrids tend to revert quickly back to one of the parental types in successive hybrid generations. The complex genetic basis of postmating reproductive isolation in rice accords well with studies of *Drosophila* [131, 141], which indicate that sterility and breakdown in fly hybrids involve many genes and higher-order epistatic interactions.

The number and location of factors affecting hybrid fertility or viability may also be inferred from observations of segregation distortion in segregating hybrid populations or from patterns of introgression. In both instances, chromosomal segments that occur less frequently than expected are assumed to harbor genes or chromosomal rearrangements that contribute to reproductive isolation. For example, 10 chromosomal segments were shown to exhibit segregation distortion in crosses between the same subspecies of rice described above [56]. Likewise, segregation distortion was reported at 54% of loci from interspecific crosses involving lentil, pepper and tomato, compared to only 13% in intraspecific crosses [148]. In *Helianthus*, segregation distortion has been observed at 7–13% of loci in intraspecific mapping populations [11, 40, 98] compared to 23–90% of loci in interspecific crosses [92, 96, 102]. Not only are distorted ratios prevalent, they can also be extreme. For example, segregation ratios that were skewed 12:1 in favor of 'wild' alleles have been reported in crosses between cultivated pearl millet (*Pennisetum glaucum*) and one of its wild relatives (*P. violaceum*) [80]. Thus, the hybrid progeny may receive more alleles from one parent than would be expected under Mendelian rules of segregation and thus resemble that parent more closely than Mendelian rules would predict.

Multigenerational introgression experiments may be a more sensitive tool for detecting chromosomal segments that affect hybrid fitness because they represent a cumulative estimate of fitness effects of chromosomal segments over several generations of introgression. For example, Rieseberg *et al.* [96] identified 14 chromosomal segments that introgressed at significantly lower than expected frequencies in three independently generated hybrid lineages between the sunflower species *Helianthus annuus* and *H. petiolaris* (Figure 3). Because these lines were shaped by natural selection for pollen viability, the 14 negatively selected segments were assumed to carry genes that reduced pollen viability in a hybrid genetic background. Similar results have been reported from a study of introgression lines between the domesticated tomato (*Lycopersicon esculentum*) and a related wild species (*L. peruvianum*) [35]. By the BC_3 generation, nine wild segments had already been completely eliminated and an additional nine occurred at significantly lower than expected frequencies. The loss of some of these segments is likely due to sterility problems in the BC_1 [35]. However, due to the small population size (13 plants), only one marker could be detected that had a significant effect on fertility. This marker was located near the self-incompatibility gene, a region which is expected to accumulate deleterious alleles due to low recombination rates. In addition, due to low fruit set in the BC_1 (only 5 plants set fruit), some of the deviations in marker frequencies probably result from drift rather than from selection. Several other investigations have documented significant reductions in the frequencies of one or more alien chromosomal segments in introgressed populations [13, 36, 68, 86, 135, 140], but data from these studies have not been directed to questions of genetic architecture.

Although blocks from the donor genome are typically reduced in frequency relative to neutral expectations, there have been several exceptions to this general rule. For example, the white lint gene of the donor genome was favored over brown lint alleles of the recipient parent in backcrosses from *Gossypium barbadense* into *G. hirsutum* [120]. Likewise, assays of mapped markers in interspecific sunflower [103] and tomato [35] backcross hybrids revealed several chromosomal blocks that introgressed at significantly higher than predicted rates in both experiments. In addition, Wang *et al.* [135] noted that the same *Gossypium hirsutum* chromosome fragments were maintained in independently generated *G. barbadense* introgression lines. It is not clear whether

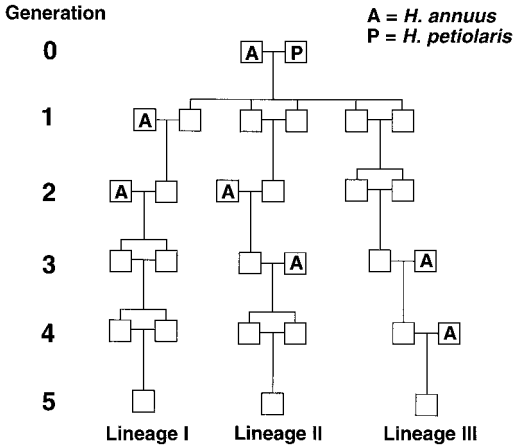

Figure 3. Pedigrees of three hybrid lineages between *Helianthus annuus* and *H. petiolaris.* A minimum of 20 plants was used for each generation of crossing. Crosses were performed by applying pooled pollen from all plants from a given generation to stigmas of the same individuals. All achenes from each generation were pooled, and 30 achenes were arbitrarily chosen to start the next generation.

these loci or chromosomal fragments are selectively favored in the recurrent genome or whether they represent examples of selfish genes – genes that enhance the success of gametes they inhabit even if they pose a significant fitness cost during the diploid phase of the life cycle [52].

Chromosomal rearrangements

In crosses between chromosomally divergent species, hybrid sterility is often attributed to the effects of chromosomal rearrangements on meiotic pairing. However, this assumption has been questioned recently because individuals heterozygous for chromosomal rearrangements often show little meiotic impairment or loss of fertility [24, 113]. These authors have suggested that genic factors may explain much of the loss of fertility typically attributed to chromosomal rearrangements. Unfortunately, it has been difficult until recently to distinguish between chromosomal and genic effects.

Two approaches have been successfully employed to separate the effects of chromosomal rearrangements and genic factors on sterility in interspecific crosses. One approach involves genetic mapping of quantitative trait loci (QTL) for sterility. An early example involves hybrids between two lentil species, *Lens culinaris* and *L. ervoides* [123], which differ by a single translocation. Mapping studies identified four allozymes that were associated with the translocation end-points. Plants heterozygous for these markers, and therefore for the translocation, were more sterile (pollen viability $<65\%$) than plants homozygous for the translocation (pollen viability $>85\%$), suggesting that the chromosomal translocation represents the primary postzygotic reproductive barrier between these two species. However, the possibility that genes tightly linked to the translocation breakpoints cause sterility cannot be ruled out.

In a similar study, the segregation of 48 genetic markers was monitored in a BC_1 progeny of an interspecific hybrid between *Helianthus argophyllus* and the common sunflower, *H. annuus* [92]. A wide range of variability in pollen viability was observed in the BC_1 mapping family (27% to 93%). Over 80% of this variation was explained by three genetic intervals located on linkage groups 1, 2, and 3, respectively. Analyses of meiosis in the backcross hybrids revealed that meiotic abnormalities also were tightly correlated with these intervals, indicating that chromosomal rearrangements are most likely responsible for reduced hybrid fertility. This finding accorded well with earlier cytogenetic work, which indicated that the species differ by one or two reciprocal translocations [17].

A second approach that has been employed successfully to distinguish between chromosomal and genic effects involves analysis of introgression patterns across the sterility barrier. If the chromosomal rearrangements contribute to reduced hybrid fitness, then linkage blocks carrying these rearrangements will be selected against in introgressed progeny. An example of this approach comes from analysis of the hybrid lineages between *Helianthus annuus* and *H. petiolaris* discussed previously [96, 102]. Comparative genetic mapping studies have identified ten chromosomes that differ in gene order between the two species. The remaining seven chromosomes appear to be collinear. Analysis of the distribution of interspecific genetic material in the hybrid lineages revealed that introgression across collinear linkages was 4 to 12 times greater than across rearranged linkages. Thus, as predicted by the QTL analysis [92], chromosomal rearrangements do represent significant barriers to gene exchange.

Hybrid vigor

Although interspecific hybrid combinations vary widely in fertility and vigor, one general rule is that first-generation hybrids, particularly between geographic races or closely related species, tend to exceed their parents in vegetative vigor or robustness [49]. This phenomenon is referred to as hybrid vigor or het-

erosis and often is used to maximize yields in crop plants. Heterosis has major implications for evolutionary biology and at least partly explains the success of allopolyploid species and many clonal hybrid lineages [51, 65]. It also may contribute to the successful establishment of introgressive hybrid races or hybrid species, but this argument is less convincing since hybrid vigor is more difficult to maintain in segregating hybrid generations.

Although heterosis is a likely contributor to the evolutionary success of hybrids or hybrid lineages, its genetic basis is still poorly understood. Possible explanations of heterosis include [87]: (1) dominance (the masking of deleterious recessives), (2) overdominance (single-locus heterosis), or (3) epistasis (enhanced performance of traits derived from different lineages due to non-additive interactions of QTL). It is difficult to distinguish among these models by Mendelian analysis because the effects of individual loci cannot be distinguished. The models have been tested by a handful of marker-based quantitative genetic studies (see below), but as will be seen the data are too few to permit generalizations.

The first detailed study of this type was conducted in maize [121]. Of nine QTL affecting yield, eight showed significant overdominance. However, these data conflict with the evidence from maize breeding experiments in which maize inbred lines have been developed that have higher yields than would be expected if overdominance was important [25, 107]. In addition, a reanalysis of the maize data set by Cockerham and Zeng [22] suggests that the apparent overdominance observed may represent an example of pseudo-overdominance due to the presence of several linked QTL. Cockerham and Zeng argue that the data are most consistent with the model of dominance of favorable genes, but admit that epistasis also could play an important role and that overdominance could not be ruled out. However, one of eight major QTL exhibiting overdominance in the original study has recently been dissected into two smaller QTL in repulsion phase linkage [45]. Both QTL act in a dominant manner as predicted by the dominance theory of heterosis.

Two studies of rice hybrids also provide conflicting data regarding the genetic basis of heterosis: one supports the dominance hypothesis [143], whereas the other implicates epistasis as the primary source of heterosis [147]. Neither provides strong evidence for overdominance. It is difficult to account for the different outcome of the two studies since they involve mapping populations of similar size. The highly recombinant inbred lines employed in the former study may have enhanced the ability to discriminate between the dominance and overdominance hypotheses, but does not explain differing observations with respect to epistasis.

There is convincing evidence for the overdominance model in *Arabidopsis*, in which Mitchell-Olds [87] identified a QTL that resulted in a 50% increase in viability in heterozygotes relative to homozygotes. Mitchell-Olds argues that overdominance will be most important in partially inbred species because major deleterious recessives are likely to be rare and recessives with minor effects are likely to be purged from the population by inbreeding.

Transgressive segregation

Many studies of segregating plant hybrids have reported the presence of phenotypes that exceed the parental phenotypic values [100]. The generation of these extreme phenotypes is referred to as transgressive segregation, and this is the primary mechanism by which the extreme or novel adaptations observed in new hybrid ecotypes or species are thought to arise. Note that transgressive segregation is a phenomenon specific to segregating hybrid generations and refers to that fraction of individuals that exceed parental phenotype values in either a negative or positive fashion. This differs from hybrid vigor, which is most pronounced in first-generation hybrids, and is implicated when the mean trait value of the hybrids exceeds (in a positive fashion only) the phenotypic value of both parental species. As will be shown below, the genetic basis of transgressive segregation appears to be largely distinct from that underlying heterosis.

Traits exhibiting transgressive segregation range from disease resistance [67] to various ecological adaptations [1, 16]. For example, F_2 offspring between two subspecies of *Potentilla glandulosa* have wider ecological tolerances than either parent [21]. Similarly, introgressed populations of tetraphid flies are better able to adapt to higher-temperature regimes than either parental species [75].

Several explanations have been offered to account for the expression of transgressive characters in hybrids. These include: (1) an increased mutation rate in hybrids [93]; (2) reduced developmental stability [49, 133]; (3) the complementary action of new combinations of normal alleles [93]; (4) epistasis [44]; (5) the unmasking of recessive alleles normally heterozygous in the parents [93]; and (6) overdominance [26].

Some of these seem unlikely from the outset. Novel mutations (explanation 1) are known to occur in *Drosophila* inter-strain crosses due to the activation of previously quiescent transposable elements, but they are unlikely to account for the almost universal occurrence of transgression in interspecies crosses. Likewise, selection experiments demonstrate that transgressive phenotypes are highly heritable, indicating that transgression cannot be due to developmental instability alone (explanation 2) [75, 112].

Marker-based QTL studies point to the complementary action of genes from the two parental species (explanation 3) as the major cause of transgression. That is, transgression appears to occur when parental lines are fixed for sets of alleles with opposing effects (Table 1). This is illustrated by a QTL analysis of 11 quantitative traits in an interspecific tomato cross [26]. Transgressive segregation was observed for 8 of the 11 traits, and alleles at 36% of the QTL detected had effects that were in the opposite direction of the species differences for those traits. That is, alleles reducing a trait were sometimes derived from the species that had the highest value for that trait and vice versa. The number of significant pairwise digenic interactions did not exceed that expected by chance, indicating that epistasis (explanation 4) was unlikely to be a major cause of transgression. However, overdominance (explanation 6) was implicated as a secondary cause of transgression. Consistent with this explanation, the more similar the phenotype of the parentals, the more likely trangressive segregation was to be observed for that trait [26]. Other reports of transgressive segregation are largely consistent with these results [4, 69, 83, 84, 91, 136, 137]. These observations do not rule out the other mechanisms listed above, but evidence for these alternatives is weak.

Although these studies involve artificial hybrids, they do suggest that differentiated populations or species are likely to possess complementary alleles for most if not all quantitative traits [114]. New combinations of complementary alleles can generate phenotypes that greatly exceed the parental values. These observations lend credence to the view that hybridization can provide the raw material for rapid adaptation [1, 2, 75, 117]. However, it is not yet clear whether interspecific transgression has contributed to adaptive evolution or hybrid speciation in nature [26].

Experimental studies of homoploid hybrid speciation

Numerous authors have suggested that under favorable evolutionary conditions interspecific hybridization may lead to the establishment of stable hybrid derivatives that are fertile *inter se* and partially reproductively isolated from both parental species [46, 89, 117, 128]. This hypothesis was explored experimentally in several plant groups earlier this century [47, 48, 105, 118], but due to technical limitations, it was not feasible to monitor the genomic changes that accompanied the stabilization of synthetic hybrid lineages or to compare the genomes of the synthetic hybrids with those derived via natural processes.

In a recent experiment, however, genetic mapping tools allowed the genomic changes accompanying the birth of a new hybrid species to be followed precisely [103]. The experiments were conducted using three sunflower species, *Helianthus annuus*, *H. petiolaris*, and *H. anomalus*, which represent two parental species and their putative hybrid derivative, respectively. In an attempt to reconstruct the sequence of genetic changes that resulted in the origin of *H. anomalus*, three independent hybrid lineages were synthesized between *H. annuus* and *H. petiolaris* [103]. The crossing scheme used to generate the three hybrid lineages (Figure 3) was designed to mimic the probable sequence of matings that gave rise to *H. anomalus.* In particular, backcrosses toward *H. annuus* were considered likely during the formation of *H. anomalus* since backcrosses in this direction are more fertile and easily produced than any other genotypic class of hybrids. The existence of several large blocks in the genome of *H. anomalus* derived solely from *H. annuus* also suggest backcrosses in this direction. Thus, the sequence of matings used to generate the hybrid lineages included backcrosses toward *H. annuus* as well as random matings among individuals from each generation. In addition, natural selection for fertility was allowed in order to replicate as far as possible the selection regime in natural hybrid populations. However, no attempts were made to replicate the sand dune ecology of *H. anomalus* in the greenhouse.

The most striking result of this experiment was the rapid convergence of the three lineages toward the same set of marker combinations even though they were generated independently and from a different sequence of matings (Figure 3). Moreover, this set of gene combinations was remarkably similar to that found in *H. anomalus*, which probably originated more than 100 000 years ago. This congruence in ge-

Table 1. Hypothetical example of transgressive segregation due to the complementary action of genes with additive effects. Letters in parenthesis after values indicate the species origin of a given QTL in the F_2s.

QTL	Phenotypic values			
	species A	species B	transgressive F_2	transgressive F_2
1	+1	−1	+1 (A)	−1 (B)
2	+1	−1	+1 (A)	−1 (B)
3	+1	−1	+1 (A)	−1 (B)
4	−1	+1	+1 (B)	−1 (A)
5	−1	+1	+1 (B)	−1 (A)
Total	+1	−1	+5	−5

nomic composition attests not only to the repeatability of this mode of speciation [23], but also to the importance of fertility selection (pollen viability and pollen competition) in shaping the genome of *H. anomalus.* Although *H. anomalus* is similar in genomic composition to the synthetic hybrid lineages, substantial differences are evident as well. Possibly, these correspond to regions that are selectively neutral in a hybrid genetic background or are under ecological rather than fertility selection.

Rieseberg *et al.* [103] also observed that unlinked markers derived from the same parental species tended to show stronger associations than would be expected by chance (linkage disequilibrium). These associations were attributed to epistatic selection, but the possibility that they could be generated by assortative mating and/or genetic drift was not discussed in the original study. Genetic drift seems unlikely to be responsible for all of the associations observed, since many were detected in all three hybrid lineages. However, associations restricted to individual lineages might be due to drift. Assortative mating seems less likely to account for the observed patterns since pollen from all plants was pooled and mixed before applying it to styles. On the other hand, assortative fertilization must have occurred, and this is the mechanism through which the marker associations are likely to have arisen. Presumably, gene combinations that contributed to enhanced gametic viability or competitiveness would be retained at higher frequencies than those that did not, generating most of the epistatic associations observed.

Although this is the only experimental study of homoploid hybrid speciation that has employed mapping approaches, it would be worthwhile to explore other aspects of this poorly understood mode of speciation. Problems to be addressed include the effects of different mating designs, selection regimes, the degree of parental genome divergence, and parental chromosome numbers on the genomic composition and stability of new hybrid lineages. It also would be of interest to explore these same parameters with respect to their influence on the number and distribution of junctions in the hybrid neospecies. This would allow for more confident use of these data for interpreting the tempo of hybrid speciation (below).

Natural hybrids

Documentation of hybridization and introgression

One of the major difficulties associated with estimating the evolutionary significance of hybridization and introgression has been the difficulty of unambiguous documentation, particularly for later generations or ancient hybrid derivatives [105]. The difficulty of hybrid identification stems from the fact that there are multiple explanations for morphological intermediacy, molecular additivity (the presence of molecular markers from two different species in the same individual), or phylogenetic incongruence (when phylogenetic trees based on different data sets suggest different organismal histories), which are the three most commonly employed methods for documenting hybrids. Morphological intermediacy, for example, can arise through convergent evolution or the retention of ancestral character states. Molecular markers reduce the problem of convergent evolution, but provide little improvement relative to morphological characters for discriminating between patterns resulting from hybridization and those due to the retention of ancestral polymorphisms. Gene tree data are subject to the

same problem – the sorting of gene lineages following speciation in a polymorphic ancestor. The analysis of linked nuclear markers provides a robust solution to problems of hybrid identification since one would expect significant associations between linked markers under a scenario of introgression but not from convergence or common descent. Linkage disequilibrium is not predicted under a lineage sorting scenario because the unit of sorting is small for chromosomal blocks, often less than a single gene [129]. However, weak linkage disequilibria could be generated by strong epistatic selection. In addition, linkage disequilibria may be difficult to detect in ancient hybrids, unless loci are very tightly linked.

As far as we are aware, the first application of this approach was in maize, in which an indvidual of *Zea diploperennis*, a wild relative of maize, was shown to possess two allozymes that had not previously been observed for the species, but which are common in maize [28]. The allozymes were known to be tightly linked, suggesting introgression of the chromosomal segment carrying them.

More recently, mapped RAPD markers were used to examine levels of introgression between cultivated and wild *Helianthus annuus* [70]. Markers specific to the cultivated sunflower occurred at high frequencies in sympatric wild populations (32–38%), but were absent in allopatric populations – a pattern strongly suggestive of introgression. In addition, significant linkage disequilibrium was observed among all pairs or triplets of linked cultivar markers in the wild populations, providing even more compelling evidence of introgression. Similar evidence has been compiled for introgression between the cultivated sunflower and wild populations of *H. petiolaris* [101].

Linkage disequilibrium also provides unambiguous confirmation of the hybrid origin of *H. anomalus.* Unbroken sequences of parental species markers (i.e., chromosomal blocks) are much longer than would be expected if *H. anomalus* was ancestral or sister to its two parents. No significant associations among parental species markers are predicted in the latter two scenarios.

It also seems feasible that linkage data could be used to differentiate between clines resulting from ecological divergence *in situ* and those resulting from secondary contact and hybridization. In the former, linkage disequilibria should be fairly uniform across both linked and unlinked loci, whereas in the latter, the strength of linkage disequilibrium should be correlated with map distances. Phylogenetic data may represent a more straightforward solution to this problem, however, since primary zones of contact are only likely between sister species [100].

Although mapped markers can provide unambiguous 'footprints' of introgression, they cannot prove that the introgression observed was in any way adaptive. An example of this problem is illustrated by studies of a proposed example of adaptive trait introgression [57, 97; S.-C. Kim, unpublished]. Heiser [57] presented morphological and cytological evidence suggesting that the common wild sunflower, *H. annuus*, was able to colonize Texas by acquiring advantageous alleles of *H. debilis*, a species already adapted to the area. Introgression between the taxa was confirmed by molecular marker assays [97]. However, presumably neutral molecular markers were assayed rather than genes encoding adaptively significant traits. Thus, Rieseberg *et al.* [97] were cautious in their interpretation of the data, noting that 'molecular evidence for introgression does not necessarily prove that the introgression of *H. debilis* into *H. annuus* was in any way adaptive'.

To determine whether adaptive traits actually have introgressed, we are currently conducting a QTL analysis of morphological and seed oil characters that differentiate *H. debilis* from allopatric populations of *H. annuus*, but which are found in the Texan form of *H. annuus* (jagged leaf serration; speckled stems; basal branching patterns; low ray number; small disks, phyllaries, and seeds). After *H. debilis* molecular markers flanking these traits have been identified, natural populations of Texan *H. annuus* will be assayed for the flanking *H. debilis* markers. Detection of pairs of such flanking markers in Texan *H. annuus* populations would provide strong evidence for the introgressive origin of the morphological traits linked to them.

Hybrid zones

Hybrid zones are broadly defined as areas in which genetically differentiated groups of individuals meet, mate, and produce at least some offspring of mixed ancestry [55]. Most of the hybrid zones that have been described in the literature are between species or subspecies, but hybrid zones are also common between locally differentiated races within species [60]. Hybrid zones are most commonly thought to be maintained by balance between the dispersal of parental individuals into hybrid zones and selection against hybrids (although see Arnold [2] for an alternative view). In

some instances, hybrid zones are maintained by intrinsic (genetic incompatibility) selection only. These zones are typically referred to as tension zones. In other cases both intrinsic and extrinsic (environmental) selection are important, producing mosaic hybrid zones. Regardless of the nature of selection, if hybrids are selected against, the hybrid zones can be used to study the genetic architecture of the reproductive barrier between the hybridizing species.

Unlike experimental hybridizations that necessarily are limited to one or a few generations of recombination, hybrid zones may contain a wide variety of genotypes that result from hundreds or even thousands of generations of recombination. Thus, hybrid zones have the potential to greatly increase the resolving power of quantitative genetic studies, as well as to facilitate genetic studies of long-lived taxa or taxa that are difficult to propagate in the lab or greenhouse.

Several different approaches may be used to estimate genetic architecture in natural hybrid zones. One of these is based on cline theory and uses estimates of the width of the region of reduced hybrid viability, dispersal rate, patterns of linkage disequilibria, and strength of selection against hybrids to determine the number of genes that contribute to reduced hybrid fitness [8]. Application of this approach to well-characterized hybrid zones in *Podisma* [7] and *Bombina* [122] yields gene number estimates of 50–500 in *Podisma* and 26–88 in *Bombina.* These estimates are consistent with the hypothesis that changes at many genes are required for speciation [32] but tell us little about the location, effects, and interactions of specific genes.

Another approach relies on differential patterns of introgression across hybrid zones and is based on the same logic employed in experimental introgression experiments [55, 102]. Introgression of loci (and linked markers) contributing to isolation is expected to be retarded, whereas neutral or positively selected chromosomal segments (and linked markers) should introgress at higher frequencies. If the markers have been genetically mapped, the observed patterns of introgression should also make it possible to locate chromosomal segments contributing to isolation. A third approach exploits the highly recombinant genotypes in hybrid zones as a QTL mapping population, in which modified QTL mapping procedures are employed to search for correlations between mapped markers and the trait of interest.

As far as we know, the only example of the latter two approaches comes from a recent study of three natural hybrid zones between *Helianthus annuus* and *H. petiolaris* [106]. The primary goals of this study were to estimate the number of genes contributing to the reproductive barrier between these two species and to determine whether there was intraspecific polymorphism for isolating factors (most genetic studies of speciation assume that intraspecific polymorphism is low for factors that affect hybrid viability or fertility).

A total of 88 mapped molecular markers representing all 17 linkage groups in sunflower were used to analyze the introgression of *H. petiolaris* chromosomal segments into *H. annuus* in the three hybrid zones. Comparison of rates of introgression in the seven collinear versus ten rearranged chromosomes revealed a 50% reduction in introgressed marker frequencies in the rearranged linkage groups – further evidence that chromosomal rearrangements represent a substantial impediment to interspecific gene flow.

Because the overall frequency of introgression in collinear linkages did not differ from neutral expectations in the three synthetic hybrid lineages described previously [96, 102, 103], average introgression across collinear linkages in the natural hybrid zones was used to provide a rough estimate of expected rates of introgression under neutral conditions. Patterns of introgression were surprisingly uniform across the three hybrid zones (Figure 4). Of the 88 markers, 65 (74%) introgressed at frequencies which deviated from expectations in the same direction in all three hybrid zones. The uniformity of these patterns indicates that most of the genome is under the same selective constraints in the three hybrid zones and that levels of intraspecific polymorphism for isolating factors are low in these species.

Pooling of the data from the three hybrid zones revealed that almost half of the markers (48%) introgressed at significantly lower than expected frequencies, compared to 9% that introgressed at significantly higher than expected frequencies. After consideration of linkage, a total of 26 independent chromosomal segments could be identified that were negatively selected.

To determine why these chromosomal segments were negatively selected, searches for correlations between the mapped markers and reduced pollen viability were performed. Of these segments 16 were significantly associated with pollen sterility, providing a straightforward explanation for their reduced frequency in the hybrid zones. Presumably, the remaining 10 segments contribute to isolating factors that were not analyzed in this study such as pollen competi-

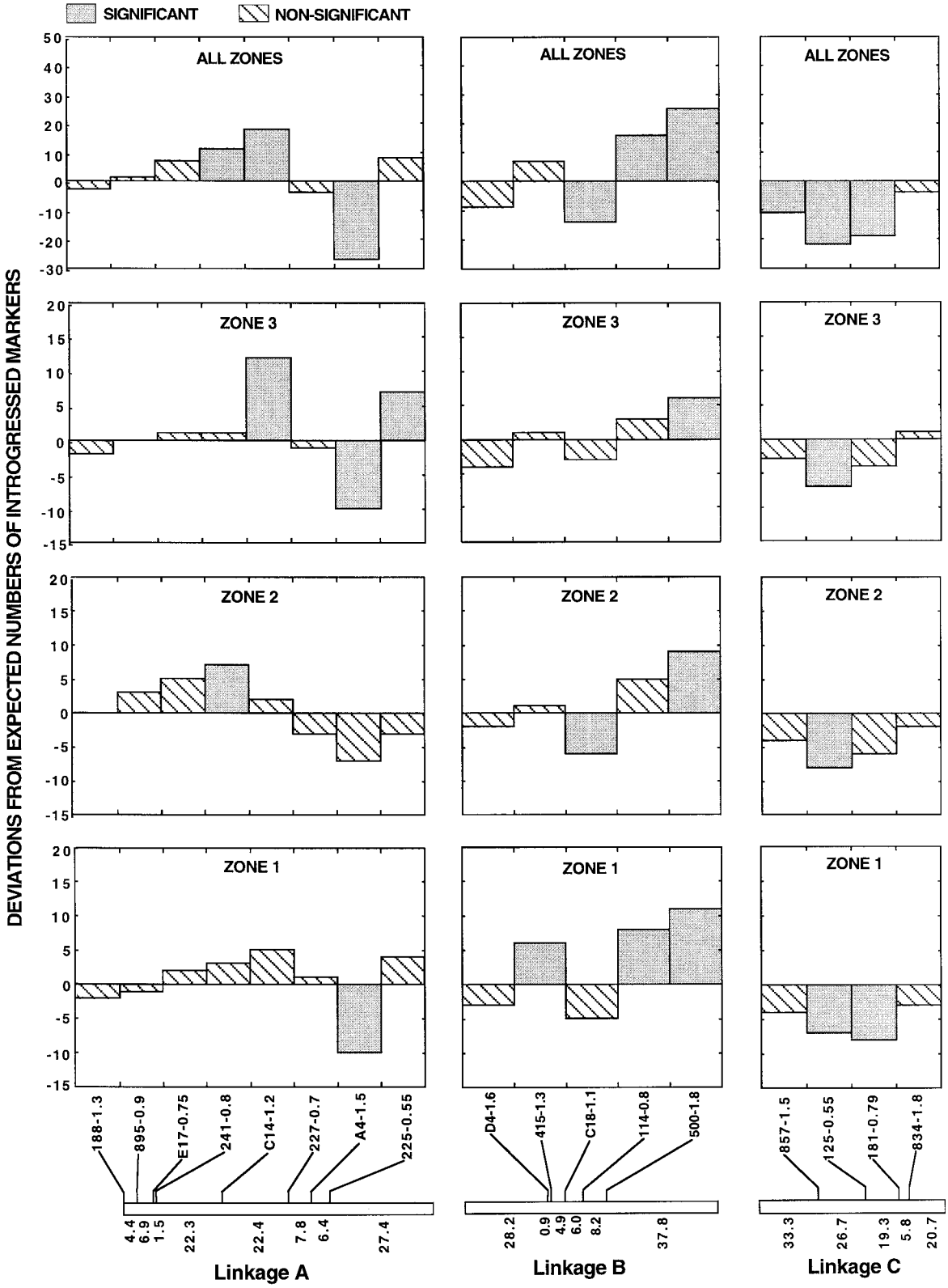

Figure 4. Deviations from the expected numbers of introgressed markers in each of three natural hybrid zones between *H. annuus* and *H. petiolaris* and in a pooled data set for linkages A, B and C. Map distances are given below each linkage group and represent averages across three genetic maps for wild sunflowers [99]. Markers are given above each linkage group and are shown in the same order as found in *H. annuus*. Marker nomenclature includes, from bottom to top, the primer designation [99] and the size in base pairs of the segregating fragment scored.

tion [99] or habitat differentiation (*H. petiolaris* is found in dry, sandy soils, whereas *H. annuus* tends to prefer wetter, clay-based soils). These results indicate that postzygotic reproductive barriers in plants, as in animals, appear to be an incidental byproduct of the numerous genetic changes that gradually accrue between geographically isolated populations.

This investigation also demonstrates the utility of hybrid zones for genetic map-based studies of species barriers. Hybrid zones offer increased resolution for genetic mapping studies due to the availability of highly recombinant hybrid genotypes, enable hybrid fitness to be tested under natural conditions, and allow genetic studies of long-lived or difficult-to-propagate organisms. Although we used preexisting genetic maps to ensure the validity of our results, our conclusions would have been essentially identical if we had generated a map using data from the hybrid zone itself. Thus, this general approach should be widely applicable to the many plant and animal species that hybridize in nature.

Hybrid species

Chromosomal evolution

Early genetic models of homoploid hybrid speciation suggested that the sorting of parental chromosomal rearrangements could lead to the formation of a hybrid lineage that was homozygous for a new combination of parental chromosomal rearrangements [41, 46, 89]. The partial isolation afforded by this new karyotype might allow the hybrid lineage to become established before it is swamped by gene flow with the parental species. More recently, Templeton [128] suggested that chromosomal breakage as a result of hybridization might be equally important in the establishment of a new homokaryotype.

Comparative genetic mapping studies of the natural hybrid species. *H. anomalus* and its two parents, *H. annuus* and *H. petiolaris*, confirm that both processes may operate [104]. The two parental species differ by a minimum of 10 chromosomal rearrangements. Sorting of these rearrangements in the hybrid speciation process placed two rearrangements from each parental species into the *H. anomalus* genome. However, considerable chromosomal rearrangement occurred during the speciation process as well: three chromosomal breakages, three fusions, and one duplication are required to derive the *H. anomalus* genome from its parents. If there is substantial intraspecific or temporal polymorphism for chromosomal rearrangements within the parental species, this may be an overestimate of the karyotypic change required.

Although hybrid speciation must be initiated in sympatry, computer simulation studies indicate that speciation is more likely if the hybrids are spatially isolated from the parental species (A. Buerkle, unpublished data). Thus, hybrid founder events may play a critical role in speciation via this mode [19, 94]. If most hybrid species actually arise in allopatry, the importance of karyotypic evolution or other postzygotic barriers in the speciation process might be questioned. However, once the hybrid species becomes established and expands its range, it seems likely to come back into contact with one or both parental species. Karyotypic divergence may enable the hybrid neospecies to survive the challenge of sympatry.

We suspect that hybrid speciation is fairly frequent, but that most hybrid species are weakly isolated from the parental species and merge back with one or the other parental species due to asymmetric levels of interspecific gene flow. Over time, this process may create a bias toward strongly isolated hybrid species, such as that observed in *Helianthus.*

Tempo of speciation

Estimating the speed with which new species are formed is one of the most difficult problems in evolutionary biology since the vast majority of species arose thousands or millions of years ago [3, 29, 30]. However homoploid hybrid speciation has a unique property that may allow tempo to be estimated fairly precisely. As discussed earlier, the recombinant nature of homoploid hybrids creates a junction clock, in which junctions accumulate in a predictable fashion following the initial hybridization event (Figure 2a). In the case of hybrid speciation, the junction clock stops once the hybrid genome becomes stabilized and parental species blocks become homozygous (Figure 2b). At this point, recombination takes place between blocks of the same parental species, and no new junctions are formed. Thus, the distribution of junctions provides an estimate of the speed or tempo of hybrid speciation rather than the age of the hybrid species.

To date, this approach has only been applied to *H. anomalus*, the homoploid hybrid species described earlier in this section [132]. The current map for *H. anomalus* is based on 701 AFLP, isozyme, and RAPD markers. Surveys of natural populations of both parental species revealed that close to half of these markers are species-specific and can be used to

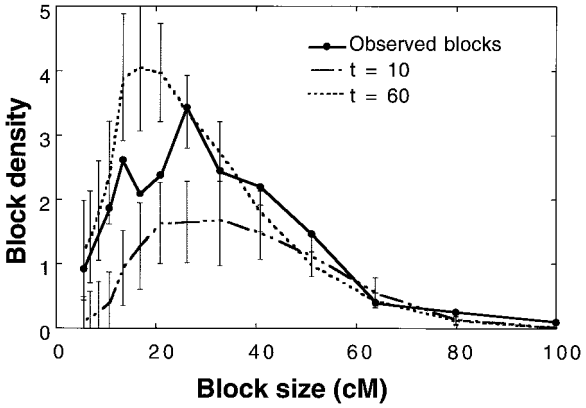

Figure 5. Comparison of the frequency spectra [29] of maximum possible parental species block sizes in the *H. anomalus* genome to those of simulation populations after 10 and 60 generations of hybridization. The frequency spectrum shows the number of blocks in a class (block density) scaled by the size of that class and is the standard way of representing the distribution of block sizes [5, 6]. The increase in the area under the curve over time indicates the increasing degree to which the genome is broken up by junctions. Error bars indicate 95% confidence intervals. Modified from Ungerer *et al.* [125].

estimate the distribution and sizes of parental chromosomal blocks in the *H. anomalus* genome (and, by inference, the distribution of junctions).

For comparative purposes, Ungerer *et al.* [132] analyzed junction production and block sizes in simulated hybrid populations that were assumed to be spatially isolated from the parental species due to hybrid founder events. Comparison of the simulated block distribution with that of *H. anomalus* indicated a rapid origin for *H. anomalus*, probably in fewer than sixty generations (Figure 5). A sensitivity analysis revealed that this result was robust to wide variation in the selection regime or population size. An analytical treatment of this data set is in progress (S. Baird, unpublished) and hopefully will increase the power of this result.

Several other factors in addition to the junction data suggest that this mode is likely to be rapid. First, experimental studies of hybrid speciation have shown that for most crosses between plant species, fertile, recombinant derivatives typically can be obtained in fewer than ten generations of hybridization and selection [47, 48, 115, 118]. For crosses between the parents of *H. anomalus*, for example, full fertility was achieved after only five generations of hybridization [103]. Second, simulation studies of homoploid hybrid speciation [85; Buerkle, unpublished] indicate that it is a punctuated process, in which long periods of hybrid zone stasis are followed by abrupt transitions to a new recombinant type. Third, the concordance between the genome of *H. anomalus* and those of the synthetic hybrid lineages derived from the same parental species also is suggestive of rapid speciation [103].

There are few little empirical data available that can be used to prove that a junction clock is accurate. Perhaps the best data come from a study of chromosome block sizes in three backcross generations between two species of tomato, *Lycopersicon peruvianum* and *L. esculentum* [35]. Mean block sizes in the BC_1, BC_2, and BC_3 generations were 47 cM, 31 cM, and 27 cM, respectively. Despite evidence that many of the chromosome blocks in this population are strongly negatively selected, observed block sizes correspond favorably to simulated block sizes in an unselected population: 50 cM in a BC_1, 34 cM in a BC_2, and 27 cM in a BC_3. These results confirm predictions generated by the sensitivity analysis of Ungerer *et al.* [132], which indicate that block lengths are not strongly affected by variation in the selection regime. Thus, we believe that junction analysis provides a general method of dating hybrid zones and estimating the tempo of hybrid speciation.

Conclusions and future directions

We have been perplexed by the apparent reluctance of both plant and animal evolutionists to apply genetic mapping methods to the study of natural hybridization phenomena. Perhaps this reluctance stems from the small size of many of the labs that focus on evolutionary questions and the perception that mapping is time-consuming and expensive. In addition, many evolutionists work with long-lived or difficult-to-propagate organisms, which are refractory to traditional genetic analyses.

We hope this review will at least partially dispel these fears. First, technological advances over the past decade in marker development and screening have greatly reduced the time and expense required for a typical mapping study. These studies still require a substantial investment of lab resources, but no longer represent the multi-year, resource-draining projects they once did. Second, our work demonstrates the potential utility of hybrid zones for studying the genetics of organismal groups that are difficult to investigate via experimental crossing programs. Not only can this approach save years of crossing effort, it can achieve significantly enhanced resolution due

to the availability of highly recombinant hybrid genotypes. Finally, the development of graphical genotypes and junction theory has provided powerful tools for analyzing the data resulting from map-based studies of hybrids and has allowed both old and new questions about the origins of hybrid zones and hybrid species to be addressed.

Although a general priority must be the application of map-based approaches to natural hybrid zones or hybrid species in a diverse array of organisms, there are some major questions that require particular attention. Below is our top ten list.

1. Does the genetic architecture of postzygotic barriers in plants vary according to life history (e.g. long versus short lifespan)?

2. Are interpretations of genetic architecture strongly affected by the methods we use to analyze it?

3. How does uneven selection across genomes affect the distribution of junctions in hybrid populations?

4. How old are plant hybrid zones? Have most of them recently been generated by disturbance or do they often represent ancient and stable species interfaces?

5. What is the tempo of hybrid speciation in groups other than sunflower? What type and magnitude of data will permit us to generalize that this is a very rapid mode of speciation?

6. Can we provide unambiguous evidence for the interspecific transfer of genetic adaptations, and does it occur frequently?

7. Has transgressive segregation contributed to the origin of the novel or extreme phenotypes in hybrid ecotypes or species, and is it a frequent phenomenon?

8. Are some interspecific gene combinations positively selected in hybrid zones, and if so, why? Is it due to heterosis or to transgressive segregation?

9. Is homoploid hybrid speciation repeatable in groups other than *Helianthus*, and if so, is there evidence that the same species has arisen on multiple independent occasions?

10. What kinds of karyotypic changes have occurred during the origin of hybrid species in other groups? Is rapid karyotypic evolution the rule rather than the exception?

In addition to the need for empirical data, it is clear that the appropriate analyses of the resulting data will require the continued exploration of junction theory and the development of useful analytical tools. K. Gardner and S. Baird are developing QTL and junction approaches for the analysis of mapping data in natural hybrids, and software resulting from these efforts will be made publicly available as this work proceeds.

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