CHROMOSOME STABILITY AND LABILITY IN PLANTS

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ABSTRACT: Theories of chromosome evolution have focused on factors promoting change. Stable chromosome systems have received little attention despite their importance in assessing factors responsible for patterns of chromosome lability and stability observed in plants. Numerous instances of chromosome stability maintained over great time spans under conditions strongly favoring change provide evidence of a previously unrecognized form of stabilizing selection. Recent studies offer evidence that this stabilizing selection stems from molecular level genetic position effects distributed throughout the genome. Each genomic arrangement apparently possesses a specific selective value that is often markedly influenced by major chromosome alterations. Thus, genomic arrangement is not typically a neutral trait, as assumed in most current models of chromosome evolution. In stable habitats most chromosome rearrangements are probably deleterious and are actively eliminated. In other instances, e.g., as plants change from a perennial to annual growth habit, some newly derived position effects resulting from chromosome repatterning are likely to be advantageous or even essential to survival during transition from one adaptive peak to the next. Therefore, natural selection may prevent or promote chromosome evolution depending on prevailing conditions. Several plant groups providing evidence supporting these unorthodox notions are discussed.

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INTRODUCTION

Chromosome alterations typically occur as unique mutational events and thus are highly subject to loss by chance. Moreover, because chromosome rearrangements arise in the heterozygous state, often with substantial fertility loss due to meiotic irregularities, the likelihood that novel chromosome rearrangements will be eliminated from populations is further increased. Despite these formidable obstacles, new chromosome arrangements do become established in populations and may even produce extensive genomic structural repatterning in certain taxa.

Two basic explanations have been proposed to account for establishing new chromosome arrangements in the face of these barriers. One explanation holds that natural selection is chiefly responsible (cf. John and Lewis, 1957, 1958, 1959; Lewis and John, 1957; Dobzhansky, 1970; Bengtsson and Bodmer, 1976; Bickman and Baker, 1979; Vosselman and Van Heemert, 1980; Atchley and Woodruff, 1981; Bush, 1981; Baker et al., 1983; Chesser and Baker, 1986). Those postulating an alternative explanation stress the paramount importance of stochastic factors and other elements that are largely independent of natural selection (cf. Lewis, 1973; Hedrick, 1981; Lande, 1979, 1984), including special population structure (cf. Wright, 1941; White, 1978*a*, 1978*b*, 1982; Hall, 1983; Hedrick and Levin, 1984; Larson et al., 1984).

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Hedrick (1981) mathematically analyzed the factors most theorists consider to be of key importance in chromosome evolution, i.e., genetic drift, meiotic drive, a selective advantage of the new homokaryotype, and inbreeding. He concluded that four situations involving these factors appear to be important for fixing new chromosome arrangements: meiotic drive alone, meiotic drive in combination with genetic drift, inbreeding in combination with a selective advantage of the new homokaryotype, and inbreeding in combination with genetic drift. Hedrick further concluded that a selective advantage of the new homokaryotype appears to have only a relatively small potential for aiding the fixation of new chromosomal types.

The widely accepted view that chance is the prime determinant of chromosome evolution recently has been reiterated by Stebbins (1989, pp. 114 and 115) as follows: "Morphological and ecological divergence can be promoted by diversifying natural selection in large populations, whereas hybrid sterility in plants, caused chiefly by chromosomal repatterning, results from the action of chance as populations pass repeatedly through bottlenecks of small size and undergo simultaneously strong but temporary divergent selection pressures. ... If colonization is rapid, populations do not remain small for periods long enough to permit chance fixation of adaptively neutral differences, such as the majority of chromosomal rearrangements."

Stebbins' latter statement alludes to a key assumption of stochastic models and theories of chromosome evolution; namely, that alternative homozygous chromosome arrangements are assumed to be adaptively neutral, and therefore interchangeable, with neither detriment nor advantage to the organism. If the assumption of genomic arrangement neutrality is incorrect then many predictions and conclusions based on stochastic theory would not reflect what actually occurs in nature.

General observations of the pattern of chromosome variation in plants (cf. Stebbins, 1950, 1958; Grant, 1981) and a critical review of several well documented biosystematic studies establish major paradoxes in these patterns that cannot be explained on the basis of stochastic factors. Moreover, there are now several lines of genetic evidence that argue against the notion of genomic arrangement neutrality. This evidence involves genetic position effects at the molecular-physiological level and reveals that alternative homokaryotypes frequently are not selectively equivalent and thus are not interchangeable with impunity. The paradoxes posed by the assumption of genomic neutrality become fully resolved in light of genetic position effects.

MOLECULAR EVIDENCE AGAINST GENOMIC ARRANGEMENT NEUTRALITY

Modern molecular genetics has established that a gene's position within the genome often has a substantial effect on that gene's performance. These effects are typically cryptic in terms of external phenotypic traits and hence are detectable only at the physiological/molecular level. Because they are cryptic, these influences would not ordinarily be classified as genetic position effects in the traditional sense. Despite their cryptic nature, these position-mediated effects very likely are subject to the action of natural selection.

One line of evidence for cryptic genetic position effects is revealed by the frequent association between oncogenesis and specific chromosome rearrangements in animal systems (e.g., Mitelman, 1984; Piccoli et al., 1984; Weil et al., 1988). Although these findings come from animal studies, the principles they reveal likely apply to organisms in general, including plants. One of the best documented examples of this phenomenon involves Burkitt lymphoma in humans (see Erikson et al., 1982; Dalla-Favera et al., 1982; Taub et al., 1982; and Klein, 1983). In this example, it was found that when the c-myc oncogene of chromosome 8 is relocated by a reciprocal translocation to the vicinity of immunoglobulin genes and their associated enhancers on chromosome 2, 14, or 22, the c-myc oncogene transcription level greatly increases and Burkitt lymphoma is induced.

These findings also established that the c-myc oncogene is unaltered when translocated and it continues to produce its regular product; the oncogene is simply deregulated as a result of proximity to antibody producing genes. It has also been shown that if oncogene c-myc remains in its normal position on chromosome 8 and the immunoglobulin genes and their enhancers are translocated to its vicinity, Burkitt lymphoma again results. These studies establish that the normal function of the c-myc oncogene, and even the survival of the organism in this case, depends on where the c-myc gene is located relative to other quite different and presumably unrelated genetic factors.

Molecular level position effects that induce oncogenesis, such as the Burkitt lymphoma example above, are particularly dramatic. However, they may be dismissed by some biologists as nothing more than interesting aberrations having little to do with normal mainstream functions. On the contrary, we suggest oncogenetic events elicited by genetic position effects disclose an important general phenomenon that is usually expressed more subtly. In other words, these lethal oncogenetic phenomena represent only the most conspicuous manifestation of a wide spectrum of molecular level position effects. These other position effects, although less spectacular, may have a very significant impact on an organism. For instance, many genes other than oncogenes, if deregulated as a result of position-mediated effects, might be induced to produce an inappropriate amount of their product. This could jeopardize the competitive performance of the organism. The potential for this sort of modification may well be common and when realized could be just as lethal as the initiation of a cancer.

In recent years a considerable number and variety of molecular level position effects have been described. Most of this research has been done on animal systems (e.g., Goldberg et al., 1983; Lacey et al., 1983; Scholnick et al., 1983; Spradling and Rubin, 1983; De Cicco and Spradling, 1984; Hazelrigg et al., 1984; Bourouis and Richards, 1985; Palmiter and Brinster, 1985; Stief et al., 1989; and Talbot et al., 1989), but similar evidence also has been obtained in plant systems (e.g., Jones et al., 1985; Nagy et al., 1985; Napoli et al., 1990; van der Krol et al., 1990). Most frequently, molecular level position effects have been detected in research where genes have been experimentally inserted at random into animal or plant nuclear genomes at a variety of locations by means of various vector systems. What is commonly seen following these gene insertions is that the amount of product made by the identical gene varies greatly at different genomic locations. At some locations the inserted gene may even become totally inactive and frequently the gene forms a smaller amount of product than it does when it is in its normal position within the nuclear genome. Experimental results of this sort are encountered so often, they appear to be the norm rather than the exception. Quantitative changes in gene transcription levels, that are induced by molecular level position effects, often involve a several fold difference, with 25 to 50 fold differences fairly common. Additionally, temporal changes in gene action and shifts in tissue specificity of gene expression are found, but much less often than quantitative changes. Little imagination is required to recognize these molecular level genetic position effects may be subject to natural selection and thus could be significant in determining the fitness of an organism. However, their detection among the myriad developmental and physiological processes of an organism would probably be quite difficult.

In addition to establishing the importance of a gene's location for its optimal function, recent evidence indicates that the entire nuclear genome is an extremely complex, highly integrated system. This view is reinforced in a recent review article by Manuelidis (1990) which states, for example, (p. 1538) "Some transgenic position effects probably derive from even larger neighborhoods of band or even coil size. Changes in gene position have also been shown to be accompanied by changes in both replication time and transcriptional activity, further indicating the influence of larger chromosomal domains." The article further states (p.1539) that "Genes are strategically positioned and hierarchically segregated in the interphase nucleus. Sequential compartmentalization of genes within domains of different size, ... and the nucleus as a whole, may additively influence phenotypic expression."

Considering the current evidence, it is difficult to sustain the view that major chromosome rearrangements could occur without also frequently inducing molecular level position effects. This is because several to many genetic loci should be in the vicinity of the two break points that are required to produce a reciprocal translocation or an inversion. Each of these genes when repositioned by chromosome rearrangement is likely to express some sort of position effect modification. Thus, the factors controlling chromosome evolution are apt to be far more complex than previously conceded and this possibility has very important implications for models and theories of chromosome evolution. The abundant evidence for molecular level position effects suggests that each genomic arrangement has an intrinsic selective value in addition to any linkage effects that may also exist.

The detection of an intrinsic selective value for any given chromosome arrangement in natural populations requires separation of the effects of random processes from those of natural selection. Among organisms that have experienced structural evolution of their chromosomes, it is virtually impossible to discriminate between any contributions made by stochastic factors as opposed to those made by natural selection. However, groups that have maintained extreme chromosome stability over vast time spans in the face of factors that have strongly favored chromosome evolution provide an opportunity to distinguish stochastic and selective factors. Under these circumstances only natural selection, not chance factors, can account for such rigid chromosome stability. The best examples of this sort are to be found in certain plant groups that are highly inbreeding, i.e., autogamous.

BIOSYSTEMATIC EVIDENCE AGAINST GENOMIC ARRANGEMENT NEUTRALITY

Chromosome stability in autogamous systems. — Small breeding populations offer the greatest opportunity for the establishment and fixation of new chromosome arrangements by chance. This is because inbreeding and genetic drift are greatly enhanced as effective population size is reduced to very small numbers (Wright, 1940, 1941; Hedrick, 1981; Lande, 1984). Theoretically, intense inbreeding and genetic drift, as well as meiotic drive, are capable of overcoming the semi-sterility disadvantage that often accompanies the initial, structurally heterozygous stages of chromosome evolution (Hedrick, 1981). Indeed, these factors may essentially eliminate or very greatly minimize this major barrier to chromosome change. Thus, it is apparent that a population composed of reproductively discrete individuals would be the most favorable situation for the stochastic fixation of new genomic arrangements. An examination of such populations would provide evidence to test the effectiveness of stochastic factors versus natural selection in controlling chromosome evolution. Additionally, such studies would yield evidence bearing on the pivotal question of genomic arrangement neutrality versus a possible intrinsic selective value for genomic arrangement that derives from numerous molecular level, genetic position effects throughout the nuclear genome.

Populations of reproductively discrete individuals needed for such a test are provided by plants that are highly autogamous, i.e., essentially self-pollinating. With such a high degree of selfing, breeding population size is consistently one, except for rare instances of outcrossing. This means that nearly every reproductive event undergoes the most extreme bottlenecking possible. Moreover, because of autogamy, when a heterokaryotype spontaneously arises, a quarter of its progeny is automatically fixed for the new homokaryotypic arrangement simply by normal meiotic processes. Additionally, the autogamous breeding system prevents any swamping effect that would otherwise be caused by the great excess of gametes bearing the original chromosome arrangement. Thus, once a new homokaryotype appears in a population, autogamy protects it from the genetic disruption of hybridization and genetic recombination and attendant loss of reproductive potential that would normally be experienced by an outcrossing plant possessing a novel chromosome arrangement. Autogamy, therefore, not only maximally promotes the fixation of new homokaryotypes, but also greatly enhances the likelihood they will persist in populations.

Consequently, the influence of stochastic factors within autogamous breeding systems seems likely to be strong enough to overwhelm all other evolutionary forces, if one assumes genomic arrangement neutrality.

If alternative chromosome arrangements are selectively neutral, then an examination of cytotypes within a long-established, widespread autogamous taxon should reveal numerous genomic arrangements fixed among its populations. This is because such a taxon very likely would have experienced numerous spontaneous chromosome alterations in its long history, and many of these alterations should have become fixed by the autogamous breeding system. This pattern is likely, even allowing for phenomena such as meiotic drive favoring the original chromosome arrangement, or lethal factors occasionally being associated with some of these spontaneous chromosome rearrangements. Indeed, numerous genomic arrangements have been fixed in certain autogamous taxa, e.g., *Erophila verna* (Winge, 1940) and *Elymus glaucus* Snyder, 1950, 1951). However, in the case of chromosomally variable autogamous taxa it is impossible to determine whether various cytotypes have been fixed by chance or natural selection, or by both of these factors acting together. Hence, such taxa provide only ambiguous information on the relative importance of natural selection as opposed to chance in chromosome evolution.

On the other hand, well-documented examples of long-established, widespread autogamous taxa that are essentially uniform chromosomally would constitute sound evidence that chromosome stability has been maintained by stabilizing natural selection. Among such selfing plants, stochastic and meiotic factors would so heavily favor the evolution of chromosomes, that a neutral chromosome arrangement would have virtually no probability of remaining stable. Moreover, within autogamous taxa, chromosome stability would not be maintained by natural selection favoring adaptive gene linkages (e.g., supergenes or coadapted gene complexes), because strict selfing effectively converts the entire genetic complement to a single linkage group. Hence, with autogamy, any chromosome arrangement should function just as well as any other for maintaining adaptive gene complexes. Seemingly, only molecular level position effects remain as a plausible explanation for any instances where chromosome stability persists within highly autogamous breeding systems over long time spans.

We conclude that determining the relative frequency of chromosomally variable and chromosomally stable autogamous plant groups would provide no useful evidence bearing on the underlying mechanisms of chromosome evolution. The crucial question is not quantitative, it is qualitative -- namely, whether any sizable, highly autogamous, chromosomally stable groups of species have existed at all over long time spans. In fact, there are several well-documented examples in the biosystematic literature. Perhaps the best example of this sort is the segment of *Epilobium* in the southern hemisphere that has been elegantly monographed by Raven and Raven, (1976).

i) The Australasian Epilobium *example*. — Some 45 species in New Guinea, Australia, and New Zealand comprise the Australasian *Epilobium* assemblage. Of these, 23 species share a single chromosome arrangement designated BB. This finding is based on extensive biosystematic research spanning more than 20 years and involves some 900 controlled crosses. The 23 species involved in the crossing program represent a comprehensive sample of the diversity present in the entire group of 45 species, hence it is considered unlikely that any of the 22 remaining Australasian species possesses a different chromosome arrangement (Raven and Raven, 1976). Long-term experimental studies have demonstrated that the great majority of these species are highly autogamous. It has also been observed that pollinators only rarely visit the flowers of these species in natural populations. Moreover, at least seven of these species are cleistogamous, with pollination and fertilization taking place in the unopened flower.

These Australasian epilobiums represent a striking example of a sizable, highly autogamous group that has rigidly maintained a single chromosome arrangement over the very long time period required to evolve 45 diverse species. The earliest fossils recorded for these Australasian epilobiums in New Zealand

are from the upper Pliocene, which places their age at 5 million years (Raven and Raven, 1976). Because the ancestors of these Australasian epilobiums originated in the northern hemisphere and subsequently migrated into New Guinea, then to Australia, and finally to New Zealand, these Australasian species probably have been evolving in the southern hemisphere somewhat longer. Thus, one could view this example as a natural experiment involving intense inbreeding that has been in progress for several million years. The remarkable result of this very long-term selfing experiment is that a single chromosome arrangement has remained essentially unchanged throughout this great time span. Only stabilizing selection could account for such long-term chromosome stability, especially in the face of factors that, due to autogamy, so heavily favor rapid chromosome diversification.

An important aspect of the biosystematic investigations of the Australasian epilobiums is the finding of a single individual in New Zealand with a homozygous chromosome arrangement differing from the standard BB arrangement by a single reciprocal translocation (Raven and Raven, 1976). However, five other individuals of the same species in the same general locality did have the expected BB chromosome arrangement. This different genomic arrangement, therefore, was apparently characteristic of only a very small segment of the population, or even a single individual, and likely originated *in situ*. This finding suggests that other individuals with novel chromosome arrangements exist within the extant populations of Australasian epilobiums, and that still others must also have existed during the long evolutionary history of this group. Despite this, the BB chromosome arrangement is the only one that has persisted and become widespread. It seems that novel chromosome arrangements in Australasian epilobiums exist only locally and temporarily, and have failed to establish any permanent new cytotypes occupying areas of any significant size.

It is also notable that within the South American species, *Epilobium hirtigerum* (of Australasian origin), an individual strain was discovered that differs from the BB arrangement by a single translocation. This novel chromosome arrangement apparently is restricted to a single population or perhaps even to a single individual (Seavey and Soloman, 1984). This example provides additional evidence that the raw material for chromosome evolution within these autogamous taxa is present, but apparently has failed to lead to the establishment of even moderately successful new cytotypes. The Australasian epilobiums are not the only chromosomally stable group of autogamous plants. A similar example is known in *Panicum*, as well as other taxa that are described below.

ii) *The* Panicum (Dichanthelium) *example*. — Spellenberg (1975) described experimental hybridization studies in a complex of perennial, highly autogamous species of *Panicum*. This research involved more than 1100 crosses and approximately 50 interspecific and interpopulational combinations. The results indicate the existence of essentially complete chromosome homology among the many populations tested throughout the western United States. In addition, three trans-continental hybridizations with species of the eastern United States were also included in this research and again no differentiation by major structural rearrangements of their nine chromosome pairs was evident. Abnormalities seen at meiosis involved occasional lagging chromosomes and univalents, but major structural differences such as interchanges and inversions were nearly absent. Fertility was typically depressed in all interpopulational and interspecific crosses, but this was attributed to genetic differences.

It is important to note that one local population of *P. thermale* differed by a single interchange from the widespread basic chromosome arrangement. A similar case was found in one local population of *P. occidentale*. Tests revealed that these two novel translocations were not identical, as they involved different chromosomes. This evidence demonstrates that novel cytotypes are available as a basis for chromosome evolution within this group but as in the previous example of the Australasian epilobiums, essentially only a single widespread chromosome arrangement has persisted and is shared by nearly all of these highly autogamous populations across North America.

iii) *The* Lycopersicon *example.* — As a result of a number of long-term studies of *Lycopersicon* by various researchers, particularly Charles Rick and his associates, a great deal of biosystematic information is available for this genus. The breeding systems of the nine species of *Lycopersicon* range from strong self-incompatibility in *L. chilense*, *L. hirsutum*, *L. peruvianum*, and *L. pennellii*, to various degrees of self-fertility in *L. chmielewskii*, *L. esculentum*, and *L. pimpinellifolium*, to almost complete autogamy in *L. cheesmanii* and *L. parviflorum* (Rick, 1979). *Lycopersicon pimpinellifolium* is interesting in that its populations in northwestern Peru have relatively high rates of outcrossing. Both to the north and south of this locality, populations of this species display progressively increasing levels of selfing, culminating in highly autogamous populations at both the northern and southern edge of range of the species (Rick et al., 1977). Thus, it appears likely that autogamy has evolved independently twice in this species.

It is especially noteworthy that the several species and biotypes in *Lycopersicon* that have evolved autogamous breeding systems and other diverse traits maintain a single chromosome arrangement that is shared by all members of the genus. As Rick (1979, page 673) pointed out, "speciation in *Lycopersicon* has occurred via gene substitution, not by chromosomal differentiation". Thus, the extensive and extremely well documented research in *Lycopersicon* provides another example of a single chromosome arrangement maintained against very strong influences for change fostered by intense inbreeding and genetic drift in derived, highly autogamous populations.

iv) *The* Clarkia *example.* — *Clarkia lassenensis* is a highly autogamous, annual species native to northern California and adjacent Oregon. Cytogenetic relationships within *C. lassenensis* have been extensively investigated by Snow and Imam (1964). This work revealed that a single standard chromosome arrangement occurs throughout the geographic range of this species. Only a few of the westernmost populations differ by a single interchange, but even in this area the standard arrangement is also present. In this study the detection of three chromosomally heterozygous *C. lassenensis* individuals in natural populations demonstrates that raw material for chromosome evolution is present, but the consequences of this heterozygosity have been extremely modest. This is especially noteworthy because *Clarkia* is exceptional as an annual group in lacking long-term seed storage (Lewis, 1962). Thus, in an autogamous species like *Clarkia lassenensis*, the stochastic influences promoting chromosome change should be at a maximum.

v) *The Madiinae example.* — Within the Compositae, Clausen (1951) described several instances in which autogamous members of subtribe Madiinae (Heliantheae) have remained genomically stable despite strict inbreeding and the strong influence this would exert to bring about chromosome repatterning. In one instance it was found that three 16-chromosome paired, highly autogamous species, *Madia sativa*, *M. capitata*, and *M. chilensis*, could easily be intercrossed, producing hybrids with entirely normal meiotic chromosome pairing. In this case it was particularly notable that *M. sativa* from California and *M. chilensis* from Chile produced hybrids with normal chromosome pairing even though the parents came from different continents.

Additionally, the hybrid between the obligate outcrosser, *Madia elegans* ssp. *wheeleri* and the highly autogamous *Madia citriodora* displayed normal meiotic chromosome pairing of 8 bivalents (Clausen, 1951), again demonstrating chromosome structural stability within a strongly inbreeding species. Clausen also reported a similar situation involving the highly autogamous *Layia hieracioides* and the obligately outcrossing, *L. gaillardioides*. This hybrid produced 8 normal chromosome pairs at meiosis and is fully fertile, indicating an absence of major chromosome repatterning within the highly inbreeding *L. hieracioides*.

The realization that chromosome arrangements within highly autogamous breeding systems can remain stable over vast time periods under the conditions most conducive for change, strongly suggests that one or more important evolutionary factors have been omitted from current, widely accepted theory. Such chromosome arrangement stability within highly autogamous breeding systems seemingly can only

be explained on the basis that particular nuclear genomic arrangements afford a significant long term selective advantage and hence are not neutral.

Chromosome stability in the tribe Aloineae. — The elegant biosystematic research of Brandham and coworkers on the liliaceous tribe Aloineae (e.g., Brandham, 1976, 1983; Brandham and Johnson, 1977) provides a quite different example of chromosome stability in a large and quite diverse group of higher plants where stability is unexpected. Some of Brandham's work (1976) reveals that individuals heterozygous for a large variety of interchanges and inversions are widespread in both cultivated and natural populations of Aloineae. It therefore is surprising to learn that the more than 1000 experimentally produced interspecific and intergeneric hybrids generated in this research demonstrate that every one of the large number of parental species tested in this tribe share essentially the same chromosome arrangement.

Brandham (1983) suggested that the absence of individuals possessing new homozygous interchange arrangements is due to a lack of positive selection for new chromosome arrangements coupled with the swamping effect of the numerous gametes carrying the original chromosome arrangement. However, it is extremely difficult to accept this explanation, because despite the ready availability of heterokaryotypes in many Aloineae populations, not even one new interchange homozygote has been detected. This is rather astonishing given the many millions of years of evolution that must have been required to produce the almost 500 species in this tribe. Most Aloineae are self-incompatible but even among the self-compatible species of this tribe, wherein new homokaryotypes could more readily result from heterokaryotype selfing, none are known. Thus, it seems likely that selection against new interchange homozygotes is the reason for their absence in the entire tribe, even though Brandham (1983) for some situations at least, concluded otherwise from his experiments. In these experiments a number of vigorous individuals homozygous for new interchanges were produced in cultivation. The frequencies of chromosome arrangements appearing within the experimental progeny were consistent with the hypothesis that no selection occurs against either the old or new interchange homozygotes. From these results it was concluded that in cultivation, and therefore probably in natural populations as well, there is no selection for or against new interchange homozygotes.

However, one cannot necessarily predict how plants will perform in natural environments based on observations made under the artificial conditions of cultivation. That a chromosome arrangement, gene complex, or single gene behaves in an apparently neutral fashion in the garden does not guarantee a similar behavior in the natural environment. This point is exemplified by studies in *Encelia* (Kyhos et al., 1981). In the *Encelia* research a large number of fruits were collected from putative first generation hybrids that were growing in a natural, undisturbed habitat. These fruits were then grown as a progeny test. It was found that the several hundred progeny consisted of interspecific recombinant and backcross individuals that were consistently vigorous and fully fertile. None of the hybrid derivative plants gave the slightest hint it would be at a selective disadvantage in the natural environment. However, in the natural, undisturbed population from which these plants came, interspecific backcross and recombinant individuals are absent, even though the progeny tests showed they are regularly produced in substantial numbers. These findings demonstrate that survival in natural habitats may be determined by subtle differences that are easily overlooked when plants are grown in cultivation.

As interesting and informative as the chromosome interchange situation in the Aloineae is, the chromosome inversion picture that emerges from this same study provides what may be one of the most convincing examples of the role of selection in chromosome evolution. Brandham (1976) reported that in natural populations of Aloineae a large variety of inversions commonly occurs in the heterozygous state. Despite the presence of these numerous inversion heterozygotes as a rich source of new inversion homozygotes, there is no instance in the entire tribe in which any species or individual is known to be homozygously fixed for an inversion relative to any other species or individual. It seems inconceivable

that this phenomenon is the result of the lack of selection for or against homozygotes coupled with the swamping effect from the original chromosome arrangement, as was suggested by Brandham (1983) for translocations. This argument is untenable because many inversion heterozygotes experience little or none of the depressed fertility that is commonly associated with interchange heterozygotes. Thus, the lack of detection of novel inversion homozygotes appears most likely to be due to their elimination by selection throughout the many millions of years the nearly 500 species of Aloineae have been evolving great diversity in many of their other features.

The Hawaiian natural experiment. — Another very different instance involving an unexpected stability of chromosome arrangement occurs within the Hawaiian archipelago where inbreeding and genetic drift should exert an exceptionally strong influence favoring chromosome change. The Hawaiian Islands, with their essentially perennial native flora, provide a unique opportunity to examine the effects of natural selection and chance-related factors on chromosome evolution. These islands are entirely volcanic and have some characteristics that make them a particularly suitable subject for a natural experiment. As the Pacific Plate moves toward the northwest over a nearly stationary "hot spot" beneath the ocean floor, new volcanic islands are progressively added to the southeast end of this archipelago. These geologic processes periodically produce entirely new land masses with a wide range of completely open habitats that are available for colonization. As these islands age, they experience substantial erosion which finally reduces them to mere reefs. Thus, as an island is formed and reaches its maximum size, its number of habitats becomes progressively greater and more varied. Subsequently, when volcanism ceases, an island will continually erode and its various habitats will progressively shift their locations and diminish in extent and variety, forcing their occupants to disperse and recolonize the same kind of habitat somewhere else, or evolve new adaptations, or become extinct.

The Hawaiian archipelago is isolated from any other significant land mass by more than two thousand miles of ocean. The ancestors of the plants that are native to these islands obviously must have originated elsewhere and became established in Hawaii only after being dispersed a very long distance (Baldwin et al., 1991). Under these circumstances, potential colonists are most likely to have arrived as single individuals (cf. Baker, 1955). Only if these colonizing plants were self-compatible or had an effective means of vegetative reproduction could they have become established. Indeed, this expectation is well supported, since all Hawaiian plants tested so far have proved to be self-compatible, with the exception of a single group (cf. Carr et al., 1986). Self-compatibility also would have greatly assisted plants as they dispersed and colonized additional suitable habitats on the same island or on new islands. This sort of breeding system permits new plant colonies to become established from single individuals, thereby strongly promoting inbreeding and genetic drift. It is also significant that Hawaiian plant populations have frequently been disrupted and fragmented by volcanic activity. As a result, the number of individuals in these populations often have been greatly reduced. Under these circumstances self-compatibility would allow such decimated populations to continue reproducing even when reduced to a single individual. This further increases the likelihood of inbreeding and genetic drift. Lastly, it is relevant that genetic drift and inbreeding is often favored even in mature Hawaiian plant communities, because populations of many species are typically scattered and consist of few individuals.

The "Hawaiian experiment" has been in progress for millions of years and the opportunity for inbreeding and genetic drift has been unusually great because of the maximized role of chance in small, isolated, self-compatible populations that have continually been forced to recolonize. Despite this almost unparalleled opportunity for chance to determine the course of chromosome evolution, nearly all native plant groups that have been critically studied within the Hawaiian ecosystem are apparently chromosomally stable. Well-documented examples include *Bidens* (Gillett and Lim, 1970; Ganders and Nagata, 1984), *Hibiscadelphus* (Carr, 1977a), *Lipochaeta* (Rabakonandrianina, 1980) and *Tetramolopium* (Lowrey and Crawford, 1985). Although less completely documented, chromosomal

stability is also indicated in Hawaiian species of *Scaevola* (Gillett, 1966). Only a single plant group, the Hawaiian Madiinae, is known to have undergone any chromosomal repatterning (Carr and Kyhos, 1981, 1986). Paradoxically, this latter group is the only one in Hawaii that is known to be primitively self-incompatible and predominantly remains so today. In stochastic terms this sort of breeding system should hamper rather than promote chromosome evolution (Carr et al., 1986).

The extreme chromosome stability characteristic of the Hawaiian flora stands in sharp contrast to the rather extensive and sometimes spectacular morphological, anatomical and ecophysiological evolution that has occurred in these very same Hawaiian plant groups. Thus, in the Hawaiian ecosystem, as in the previously discussed examples, it appears that natural selection has favored variation at the level of the gene, but has rigorously selected against variation at the level of the chromosome. The effects of stochastic factors on chromosome evolution in this oceanic flora are essentially nonexistent, despite current widely accepted stochastic theory that would predict otherwise.

Chromosome arrangement stability among perennial taxa. — It has been known for several decades that long-lived woody perennials in general, as well as many herbaceous perennials are highly stable chromosomally (see Stebbins, 1950, 1958; Grant, 1981); congeners among these perennial groups seldom display any chromosome diversification based on major structural alterations. Because such chromosome stability is particularly dramatic in the case of perennial vicariad taxa, a few specific examples are briefly discussed.

As a result of natural events, related populations have become separated for many millions of years with no opportunity to exchange genetic material. These naturally occurring situations afford the opportunity to gain insights that otherwise would not be possible. The often cited *Platanus occidentalis* from eastern North America and *P. orientalis* from the eastern Mediterranean is a prime example of extremely long-term chromosome stability within taxa that are very isolated geographically (Sax, 1933). These species have been separated on different continents for upwards of 25 million years (Stebbins, pers. comm.) and yet today, when artificially hybridized, they produce fully fertile hybrids with normal meiosis. This example demonstrates that populations can retain identical chromosome arrangements despite their complete separation for a vast time period, and despite their substantial genetic divergence. Similarly, both molecular and paleobotanical evidence indicate that *Liriodendron chinense* of mainland China and *L. tulipifera* of the eastern United States have been isolated from one another for between 10 to 16 million years (Parks and Wendel, 1990) and yet their hybrids display normal meiosis.

Similar examples of chromosome stability among widely separated taxa occur in *Catalpa* (Smith, 1941) and *Campsis* (Sax, 1933) of eastern North America and China, in *Rhododendron* of China, Japan and North America (Sax, 1930), and *Larix* of central Europe and Japan (Sax, 1932). Moreover, it is most likely that numerous comparable examples among other groups of long-lived plants exist but additional research of this sort is badly needed in order to determine the extent of such chromosome stability.

It may be argued that the chromosome stability of perennials is due simply to their slow population turnover rates, whereas a faster population turnover may explain the more rapid chromosome evolution among annuals. However, the divergent population turnover rates that distinguish perennial and annual plants are substantially offset by other factors. For example, while slow turnover rates in perennials should favor chromosome stability, the opposite effect should result from their frequent lack of long-lived seed banks. Furthermore, chromosome variability in perennials should also be favored because annually they produce large numbers of somatic cells which presumably are as likely to experience chromosome mutations as are cells produced by annual populations. The important difference is that in perennials, somatic cells carrying chromosome mutations can persist for an indefinitely long period as chimeras and ultimately may contribute to reproductive tissues. Over time this could result in the accumulation of numerous and varied chromosome mutations thereby providing multiple opportunities for the sexual

transmission of new chromosome arrangements to ensuing generations. In annuals, by contrast, novel chromosome alterations are subject to loss each year, which should be an impediment to chromosome change. Lastly, it is apparent that chromosome evolution among perennials is also favored because reduced fertility associated with chromosome heterozygosity should be far less detrimental to long-lived plants than to annuals. Hence, new heterokaryotypes should be much less subject to selective elimination in perennial populations than in annual populations. All factors considered, it appears that new chromosome arrangements should become established at least as easily, or perhaps more easily in perennials than in annuals. Despite these expectations, the empirical evidence reveals that perennial species characteristically display great chromosome stability, in contrast to annuals (Grant, 1981). These observations are not satisifactorily explained by stochastic principles but are easily reconcilable on the basis that genomic arrangement is not neutral and that natural selection is an important determinant of chromosome evolution.

EVIDENCE FOR THE ROLE OF NATURAL SELECTION IN CHROMOSOME LABILITY

Whereas woody congeneric perennial species characteristically share the same chromosome arrangement, the situation is generally reversed among congeneric annual species (cf. Stebbins, 1950; Grant, 1981). Among annual species and often even within a single species, chromosome evolution by major structural rearrangements is apparently often rapid and frequent; morphological evolution and ecological divergence of these same species is typically a slower process. Within a number of diploid annual species this phenomenon has been carried to an extreme, with many instances known in which distinct biological species are morphologically so similar that they were not detected until biosystematic research revealed their chromosomal differences and/or reproductive barriers (see e. g., Clausen, 1951; Jackson, 1962; Ornduff, 1966; Lewis, 1973; Smith, 1974; Carr, 1975; Kyhos et al., 1977; Palmer, 1982). Such patterns are largely absent among long-lived perennials.

These sharply contrasting patterns of evolution at the level of the gene and the chromosome in long-lived, versus annual plants present a formidable paradox. One aspect of this paradox is that by far the greatest amount of chromosome evolution occurs among annual species even though they can least tolerate the reduced fertility that commonly accompanies the structurally heterozygous initial phases of chromosome evolution. Normal fertility is obviously crucial for annuals because their continued survival depends ultimately on seed production. The other part of this paradox is that chromosome evolution by major structural rearrangements is comparatively rare among woody, and many herbaceous perennials, even though they can far more easily tolerate the reduced fertility associated with chromosome structural heterozygosity. These paradoxical variation patterns are difficult to explain in terms of stochastic processes. Even more difficult to explain in stochastic terms are the chromosome variation patterns among self-incompatible annual taxa.

The enigma of self-incompatible annuals. — Many self-incompatible annual species are characterized by a great deal of chromosome evolution involving major structural rearrangements. Numerous instances of this sort have been reported, for example, in the genera *Brassica* (Tsunoda et al., 1980), *Calycadenia* (Carr, 1975, 1977b, 1980; Carr and Carr, 1983), *Chaenactis* (Kyhos, 1965), *Coreopsis* (Smith, 1974), *Haplopappus* (Jackson, 1962), *Helianthus* (Heiser et al., 1962; Chandler et al., 1986), *Hemizonia* (Clausen, 1951; Tanowitz, 1977; Venkatesh, 1958), *Holocarpha* (Clausen, 1951; Palmer, 1982), *Lagophylla* (Thompson, 1983), *Lasthenia* (Ornduff, 1966), *Layia* (Clausen, 1951), *Lesquerella* (Rollins and Shaw, 1973), *Madia* (Clausen, 1951), and *Pentzia* (Mitsuoka and Ehrendorfer, 1972). The chromosome alterations observed in these groups, especially interchanges, substantially depress fertility when they initially arise as heterokaryotypes. Although highly disadvantageous even in self-compatible annuals, heterozygosity for such chromosome alterations is far more detrimental if the

annual is self-incompatible. While selfing affords a novel heterokaryotypic annual a reasonable chance of producing some progeny that are homozygous for the new chromosome arrangement before the end of its short life span, this option is not open to self-incompatible species. A novel self-incompatible annual chromosome heterozygote can only mate with individuals that are homozygous for the original chromosome arrangement. Consequently, half of its progeny consists of fertile individuals that are homozygous for the original chromosome arrangement and the other half are chromosome heterozygotes with the same reduced fertility as itself; no new homokaryotypes with restored fertility are produced during the initial year. Furthermore, the presence of long-lived seed banks typical of most annual species (Epling et al., 1960; Major and Pyott, 1966) means that the novel heterokaryotype is swamped in future years by gametes carrying the original homokaryotype.

The existence of long-lived seed banks also means that the wide variations of population numbers often observed in annual species are deceptive. Such numerical fluctuations in annual populations suggest that extinction-colonization events and the accompanying population bottlenecks explain rapid chromosome evolution in annuals. However, the enormous stability that long-lived seed banks normally confer on annual populations (*Clarkia* being a notable exception) render this explanation untenable.

Although chromosome repatterning is extremely common among annual species, it is by no means universal. One of the most interesting and instructive exceptions involves two self-incompatible species complexes within *Calycadenia* (Carr, 1975, 1977b; Carr and Carr, 1983). In all discernible features the members of these two species complexes are extremely similar and very closely related. They can all be intercrossed easily to produce hybrids with a high level of meiotic chromosome pairing and varying degrees of fertility that is determined by their chromosome arrangements. One of these groups, the *C. ciliosa* - *C. pauciflora* complex, includes many fixed cytotypes. The other group, the *C. multiglandulosa* - *C. hispida* complex, has only a single fixed chromosome arrangement. The uniformity of chromosome arrangement within the *C. multiglandulosa* - *C. hispida* species complex cannot be attributed to a lack of chromosome mutations. Populations of both of these species complexes include substantial numbers of "floating" interchange heterozygotes (Carr, 1977b, Carr and Carr, 1983; and unpubl.). Paradoxically, the available evidence indicates that the chromosomally stable *C. multiglandulosa* - *C. hispida* complex has a higher frequency of populations with interchange heterozygotes than the chromosomally diverse *C. ciliosa* - *C. pauciflora* complex. There are no apparent differences in the attributes of these two extremely closely related species complexes that offer a ready explanation for their diametrically opposite patterns of chromosome evolution. It seems most likely that natural selection has produced and is maintaining these contrasting patterns of chromosome variation.

Despite major obstacles to chromosome evolution among self-incompatible annuals, chromosome diversification in these plants is extremely common. Indeed, the available evidence indicates that self-incompatible annuals are probably the most active of all plant life forms in terms of chromosome structural rearrangements. This paradoxical pattern of chromosome evolution runs counter to expectations based on chance and is difficult to explain as a result of anything other than natural selection.

Evidence from genera with both annual and perennial taxa. — The same sharply contrasting variation pattern observed for higher plants in general, is also apparent when closely related annual and perennial species occur within the same genus. In these cases, it is again the annual members of a genus that display chromosome repatterning, with little or none seen among their congeneric perennial relatives. These instances, involving closely related annual and perennial species within a single genus, demonstrate that chromosomal repatterning is not dependent on some inherent tendency within a particular group of plants, but instead is closely correlated with the life form of the plants.

Grant (1981) cited examples of three such genera: *Helianthus* (Heiser et al., 1962), *Crepis* (Babcock, 1947), and *Knautia* (Ehrendorfer, 1962). To this list can be added the genus *Chaenactis* (Asteraceae). Within *Chaenactis* a number of annual species have been experimentally hybridized with several of the

perennial species to yield partially fertile hybrids, thus revealing the particularly close relationship between these annuals and perennials. Despite this close relationship, experimental crosses among the perennial *Chaenactis* species reveal a very different pattern from that of the annuals. Wide crosses between morphologically quite divergent perennial *Chaenactis* species from distant geographic regions have typically produced hybrids with only normal meiotic bivalent pairing. This strongly indicates a lack of major chromosomal repatterning among these perennial species in contrast to the annuals where no two species are known to share the same chromosome arrangement. Some annual species even have several genomic arrangements (Kyhos, 1965 and unpubl.). Thus, it appears that the paradox involving chromosomally stable perennials and chromosomally labile annuals even extends down to the individual, closely related members of a single genus. These contrasting patterns of chromosome evolution within *Chaenactis* cannot be related to differences in breeding system because all species are obligately self-incompatible.

GENETIC LINKAGE EFFECTS AND THE ORIGIN OF THE ANNUAL GROWTH HABIT

In addition to molecular level genetic position effects, genetic linkage effects probably play an important role in chromosome evolution. The rationale for this view is that the shift from the perennial to the annual habit potentially brings about a great increase in the amount of genetic recombination per unit of time that is experienced by the newly derived annual population. This effect may be extremely exaggerated if the initial perennial ancestor has an effective means of vegetative reproduction in addition to its ability to reproduce sexually. While vegetative reproduction permits indefinite maintenance and faithful reproduction of any highly adapted genotype and/or genomic arrangement by the perennial ancestor, this capability is lost with the shift to the annual habit. Hence, for the derived, sexually reproducing annual population, the ability to maintain adaptive gene combinations is drastically reduced relative to its perennial ancestor. Consequently, for the derived annual, the point of compromise between immediate fitness (genetic constancy) and long-term fitness (genetic variability) is markedly shifted away from the former toward the latter. Such a radical shift away from immediate fitness has the potential to reduce greatly the competitiveness of the annual population. This places a selective premium upon any mechanisms that could enhance the annual's ability to bring together and preserve adaptive gene combinations, in other words, restore the original balance between genetic constancy and variability.

The selective value of mechanisms capable of protecting adaptive gene combinations probably depends in large measure upon the richness of the gene pool of the derived, annual population. Any newly derived, annual population is likely to have originated from a small number of individuals, hence its genetic variability is correspondingly limited. An increased genetic recombination potential would have much less impact on such a genetically depauperate population than it would on one with a rich gene pool. Thus, it is reasonable to expect that in a newly derived population consisting of shorter-lived individuals, a potential increase in the rate of genetic recombination is likely to have a delayed effect that would become important only when the derived population has had time to experience an enrichment of its gene pool. This effect, however, could be more immediate if the derived population were in contact and hybridizing with a differently adapted population, such as, for example, its ancestral population. With the progressive enrichment of the annual's gene pool, its already elevated potential rate of genetic recombination would have an increasingly adverse effect on immediate fitness by rapidly disrupting adaptive gene combinations.

In this situation selfing may be favored in those annual populations that can tolerate intense inbreeding. Indeed, selfing is a trait that is common among annuals. On the other hand, in populations that are strongly or obligately outcrossing, selection may favor other mechanisms, e.g., reduced chiasma frequency, localization of chiasmata, and various chromosomal rearrangements, as a means of creating

and holding together adaptive gene combinations. These expectations appear to be realistic inasmuch as such mechanisms have long been known to occur commonly among annual taxa (see Stebbins, 1950, 1958), especially those that are self-incompatible.

SUMMARY AND CONCLUSIONS

Theories and models of chromosome evolution have focused primarily on the identification of factors that promote chromosome divergence, whereas the occurrence of chromosome stability in situations where potent factors favor chromosome repatterning has received comparatively little attention. This is unfortunate because chromosome evolution is very likely controlled by a balance between one set of factors that promotes change and another that promotes stability; hence, chromosomes will either change or remain stable depending on the relative strength of these opposing sets of factors. As a result, both stable and evolving systems offer opportunities to gain insights into the factors underlying chromosome evolution.

The Australasian epilobiums and the Aloineae described herein are prime examples of instances in which chromosomes have remained stable despite the influence of factors and processes strongly favoring chromosome evolution. The special significance of stable systems is that they cannot be explained on the basis of stochastic factors. Thus, paradoxically, much may be learned about chromosome evolution by studying chromosome systems that have remained stable for a very long time. Nevertheless, little attention has been given to such examples, perhaps because they afford so few opportunities for mathematical analysis.

The general neglect of the very important phenomenon of stasis in chromosome evolution and its underlying basis presents a striking parallel to the history of research on population bottlenecking and founder effect theory. In studies of bottlenecking phenomena, it had been widely assumed that genetically-based variance decreases after a population bottleneck event. However, the experiments of Bryant et al. (1986) demonstrated that this assumption is inaccurate, at least in some cases, and perhaps generally. This incorrect assumption was based on a large body of mathematical analysis and theory that was limited by necessity to a consideration of the genetics of simple additive variance. The fact that much genetic variance is nonadditive (e.g., overdominance and epistasis), and anything but simple, was apparently set aside and more or less forgotten, apparently owing to its mathematical intractability (Lewin, 1987). When Byrant and his coworkers showed that frequently as a result of population bottlenecking, instead of decreasing, genetically-based variance actually increased, in some instances dramatically, it became apparent that widely accepted theory and mathematical models had failed to include a fundamental factor, nonadditive genetic variance. This omission resulted in an incomplete and inaccurate view of biological reality.

Similarly, current theories and models of chromosome evolution also may have failed to include what appears to be a fundamental factor. This omitted factor, we suggest, is genetic position effects at the molecular level, which may be responsible for many otherwise inexplicable instances of very long-term chromosome stability, as well as equally baffling examples of extensive chromosome repatterning. Such molecular level position effects are to be expected during chromosome repatterning as a number of genes adjacent to sites of chromosome breakage and reunion are potentially removed from their original spheres of regulatory influence and placed in new ones. The sum of these position effects together with the selective value of all coadapted gene complexes determines the new selective value for the novel chromosome arrangement.

The evidence currently available, largely from molecular genetic and biotechnological research with transgenic organisms, suggests that essentially every gene in an organism's complement is potentially subject to molecular level position effects. It has been commonly found that the insertion of the same

gene into different locations within the nuclear chromosome complement results in substantially variable functional responses of that gene. Significantly, it has been shown that in these instances the gene has not been altered, but only brought into a different regulatory domain. We surmise that major chromosome rearrangements will similarly relocate genes into new or altered regulatory domains and thereby elicit modified functional responses of the affected genes.

Current research indicates that regulatory elements, such as enhancers, likely are dispersed widely throughout nuclear chromosome complements. Although the mechanism is not well understood, it is known that within the same chromosome, enhancers are capable of affecting genetic loci at relatively great distances. Thus, each enhancer effectively represents a field of regulatory influence. Collectively, all enhancers may create a complex of regulatory fields that envelop the entire genome (see Manuelidis, 1990), the disruption of which by major chromosome rearrangements, for example, would likely have important consequences for the organism. Hence, most large scale chromosome rearrangements occurring within a well adapted organism would probably be disadvantageous, and their elimination would be evidenced as stabilizing selection. In contrast, organisms caught in conditions of environmental flux should experience a reduced intensity of stabilizing selection, and thus should have increased opportunities for large scale chromosome repatterning that could move them via genetic position effects and new gene linkages from one adaptive plateau to another.

In the context discussed above, it seems that very small, local chromosome rearrangements that ordinarily would be categorized as cryptic are less likely to affect genetic-physiological function than rearrangements involving larger chromosome segments. Such cryptic chromosome rearrangements may even be generally neutral. Thus, the inability to detect very small chromosome alterations of the sort visible only in giant polytene chromosomes of Diptera, may not severely handicap attempts to establish relationships between molecular level position effects, functional modifications, and/or chromosome variation patterns in natural populations.

In the Australasian epilobiums, the importance of position effects is indicated by rigid chromosome stability that has persisted at least 5 million years, and which seems inexplicable in terms of stochastic theory. The autogamous breeding system of these epilobiums and other highly autogamous plant groups described herein provides the greatest opportunity for stochastic fixation of many genomic arrangements, and yet this has not happened. This argues that stabilizing selection is responsible for the rigid maintenance of a single genomic arrangement for vast time spans, despite intense inbreeding and the demonstrated availability of sporadic chromosome variants as a ready source of raw material for chromosome evolution.

Likewise, the total absence of any new inversion homozygotes in the nearly 500 species of Aloineae, despite an abundance of inversion heterozygotes, strongly implicates powerful stabilizing selection as the causal factor. Furthermore, it seems the absence of novel inversion homozygotes in Aloineae cannot be attributed to a disruption of coadapted genes as was proposed by Shaw and Coates (1983) for the Australian grasshopper *Caledia captiva*. In *C. captiva*, two chromosome races were shown by hybridization to differ by several inversions. Heterozygosity for these several inversions caused chiasmata to form in novel positions, apparently resulting in the break up of coadapted gene combinations which proved to be lethal early in the development of the next generation. These several inversions apparently are components of a coordinated system that serves as an isolating mechanism preserving coadapted genes that are vitally important and unique to each chromosomal race. The situation in Aloineae is quite different in that the varied and numerous inversions are "floating" structural heterozygotes and not part of any integrated system. Hence, there is no basis to suggest that the Aloineae inversions serve as isolating mechanisms preserving coadapted genes. Even if one did invoke this improbable explanation, it would be necessary to assume that each and every one of the numerous unique floating inversion heterozygotes would adversely affect some coadapted gene complex.

Whereas stochastic theory and models do not seem to account for long-term chromosome stasis in Aloineae, *Epilobium*, and other highly autogamous plant groups discussed herein, molecular level position effects do offer a plausible explanation. Indeed, many enigmatic phenomena in plants become explicable if one accepts the argument that gene sequences and regulatory factors in most long-lived perennial plant species are so optimally arranged in terms of position effects and adaptive gene linkages that very few alterations to their genomes would be superior. Thus, chromosome evolution is very limited in these perennial taxa. Among these chromosomally stable perennials, even fertility neutral chromosome rearrangements apparently are either quickly eliminated by selection, or typically exist only as heterokaryotypes, e.g., the Aloineae described herein. In terms of chromosome arrangements and their concomitant position effects, most perennials have apparently reached "adaptive peaks" from which they are not easily dislodged.

In contrast, annual species are generally acknowledged to be highly specialized derivatives of perennial ancestors. These ancestral perennials, by virtue of their longer period of existence and thus longer exposure to the selective forces that have shaped their evolution, should have genomes and attendant position effects that are highly perfected for their perennial way of life. Conversely, derived annuals consist of relatively recently evolved populations that have not yet experienced an extensive period of selective refinement for their new life style. Thus, they are far more likely to be in a highly active evolutionary phase, genetically and chromosomally.

Because the shift from a perennial to an annual growth habit is a very radical change, many of the genetic factors and regulatory elements that were required for the ancestral perennial to function optimally throughout its entire yearly cycle may become obsolete. For example, an annual grows for only a part of the year and in extreme cases may complete its life cycle in only a matter of days or a few short weeks. Thus, in a newly derived annual, certain genes may be free to evolve to other functions, become silenced, or lost. The shift to the annual habit would also require a substantial adjustment in terms of a major reallocation of resources toward a fail-safe, and much more efficient reproductive effort. Given these circumstances, a genome that was originally superbly adapted for a perennial life style would likely require a substantial reorganization of genetic factors, regulatory elements, and attendant position effects in order to yield a genome optimally suited to an annual life style. During this period of reorganization and "experimentation" it is likely that a great number and variety of chromosome alterations would arise spontaneously and be "tried out" for a time. Ultimately, from this extensive chromosome repatterning the most advantageous gene sequences and concomitant position effects would be attained. At this point, chromosome structural stability might be expected to prevail once again. Such a sequence of events may account for the genomic stability observed within the annual *Calycadenia multiglandulosa* - *C. hispida* complex, which so sharply contrasts with the extensive chromosome repatterning present in the extremely similar and very closely related *C. ciliosa* - *C. pauciflora* complex discussed earlier in this paper.

In conclusion, we have attempted to address two fundamental questions concerning the evolution of plant chromosomes. How is it that chromosomes do not evolve in certain situations where stochastic theory and models indicate they should readily and rapidly do so? Secondly, how is it possible that chromosomes evolve rapidly and extensively in certain other situations, where stochastic principles indicate chromosome evolution should be greatly impeded? In both of these contrasting, paradoxical situations, the action of natural selection, not chance factors, offers the more plausible explanation. We believe modern molecular genetics provides a credible explanation in the form of molecular level position effects that pervade the nuclear genome and upon which natural selection can effectively act. Also, genetic linkage effects are likely to play an important, but independent role in chromosome evolution, either reinforcing or opposing genetic position effects in various circumstances. In this paper we have cited a number of examples of plant groups with unexpected genomic stability and conversely, others with equally unexpected and extensive genomic variability; all of these cases sharply conflict with

stochastic concepts of chromosome evolution. Indeed, we believe there are too many of these contradictory examples to simply dismiss them as anomalies; they must be seriously addressed.

Ideally, experiments should be conducted to test current theories and models of chromosome evolution, as Bryant et al. (1986) did for bottlenecking and founder effect theory. Unfortunately, owing to the vast time spans needed to conduct experiments in chromosome evolution, it is impractical to attempt to experimentally test theories and models of chromosome evolution. Hence, the next best alternative is to critically analyze "nature's experiments", many of which have been in progress for millions of years. In this paper we have attempted to evaluate the unorthodox concepts we have proposed by providing a synthesis of molecular genetic, cytogenetic, and biosystematic research.

We believe that the evidence presented herein is totally inconsistent with predictions based on models of chromosome evolution that emphasize stochastic factors and underestimate the selective component of different chromosome arrangements. These inconsistencies disappear and apparent paradoxes of chromosome stability and lability in plants become explicable in light of the effects of gene repositioning associated with chromosome rearrangements. The molecular/physiological effects of gene position, together with gene linkage factors, appear to provide a coherent and logical explanation for otherwise irreconcilable patterns of chromosome evolution in higher plants.

It also appears likely that the new insights from molecular research will elicit an awareness of daunting and highly unpredictable complexities that may generally be as intractable to mathematical approaches as the genetically-based nonadditive variance revealed in the experiments on bottlenecking by Bryant et al. (1986). For instance, how could anyone foresee that a lethal molecular level position effect in the form of a lymphoma would result simply by translocating the c-myc gene to the vicinity of immunoglobulin genes and their enhancers?

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