Increased Chromosomal Mutation Rate After Hybridization Between Two Subspecies of Grasshoppers

Abstract. *Hybridization between two chromosomally distinct subspecies of the grasshopper* Caledia captiva *results in a high incidence of novel chromosomal rearrangements among the backcross progeny. Rearrangements are restricted to those chromosomes derived from the* F_1 *hybrid parent. Chromosomal involvement is nonrandom with the same rearrangement occurring repeatedly in different backcrosses. A single individual can also generate an array of different rearrangements among its offspring. Several of the rearrangements have also been found in natural populations. The nonrandom and recurrent nature of these chromosomal mutations at high frequencies provides a plausible explanation for the establishment and fixation of chromosomal rearrangements in natural populations.*

There is an increasing interest in natural phenomena that can perturb the stability of the genome and lead to the appearance of novel genetic variants at relatively high frequencies (*1–3*). This is exemplified by the "breakage-fusion-bridge" cycle in maize (*4*) and "mutator activity by hybrid release" in *Drosophila* (*5*). In both of these cases, genomic reorganization is activated by stress factors under unusual but natural circumstances. In maize, the breakage-fusion-bridge cycle relocates the X component transforming it from a quiescent to an active phase. In *Drosophila* increased mutation rates can be induced by hybridization between P and M strains of *D. melanogaster* (*6, 7*). Both can lead to profound genomic changes including genic and chromosomal novelties at frequencies that could have significant evolutionary potential (*8*). There are two important components of these phenomena. The first concerns the creation of new genetic variation at orders of magnitude above the conventional "spontaneous" mutation rates (*9, 10*). The second relates to their relevance in the evolution of natural populations and more specifically to the establishment of chromosomal mutations as homozygotes, an event that has not been clearly defined (*11, 12*). If it can be shown empirically that perturbation of the genome is also an extant phenomenon in natural populations, then some basic assumptions made in population genetics theory may need revision.

We have identified a system in the grasshopper *Caledia captiva* in which experimentally induced hybridity between two subspecies is correlated with a high rate of chromosomal mutation and, moreover, the same kind of hybridization is occurring in natural populations (*13, 14*). *Caledia captiva* is a taxonomic species that has undergone extraordinarily high levels of genomic reorganization resulting in the presence of four distinct chromosomal taxa (*15*). Two of these taxa—the Torresian and Moreton subspecies—differ by eight pericentric rearrangements and the presence or absence of a complex series of interstitial and terminal blocks of heterochromatin. In addition, they are substitutionally different at five enzyme loci ($\bar{D} = 0.18 \pm 0.02$) (*16*). The two subspecies form a narrow hybrid zone in southeast Queensland within which more than 80 percent of the individuals are derived hybrids. The F_2 generation between the subspecies is totally inviable as a result of embryonic breakdown, but backcrossing to either parent results in approximately 50 percent viability (*17*). The persistence of the hybrid zone and the presence of such a high frequency of hybrid individuals, despite the severity of hybrid breakdown, creates a paradox that is not readily resolved (*18*). During an analysis of chromosomal viabilities among the *surviving* progeny of a series of backcrosses, we discovered a wide range of novel chromosomal rearrangements not found in the pure parental taxa. In addition, an analysis of chromosomal variation among individuals from the hybrid zone has revealed the presence of two chromosomal fusions that themselves may have arisen as a consequence of hybridization.

Of the 1062 embryos obtained from five backcrosses (*19*), 439 had attained the same stage of development as the controls (*13*). These viable embryos were C-banded for chromosomal analysis. The wide array of chromosomal markers that distinguish these subspecies allows unambiguous identification of the haploid genome of the homozygous parent and the recombinant genome derived from the F_1 parent.

Fifty three (12 percent) of these viable embryos contained novel chromosomal rearrangements that included Robertsonian fusions (Fig. 1, a to k), pericentric rearrangements (Fig. 1, o to q), dicentric chromosomes (Fig. 1, n and t), centric fragments (Fig. 1i), novel bands of heterochromatin (Fig. 1, r and s) and several cases of complex, multiple rearrangements within the same individual which could not be accurately resolved (Fig. 1v).

The distinctive genomes of the F_1 parents provide a means of identifying the individual chromosomes involved in rearrangement and the precise mode of rearrangement. In every case chromosomal repatterning is restricted to the genome derived from the hybrid parent suggesting that the rearrangements had occurred prior to fertilization or that only chromosomes derived from the F_1 hybrid parent were susceptible to mutation events. Grasshoppers lay their eggs in pods containing 10 to 25 eggs and in two cases, it was observed that eggs derived from the same female (that is, within an egg pod) contained different rearrangements. An example is shown in Fig. 1 where a three-banded fragment of the Moreton metacentric X chromosome is fused with a Torresian chromosome 6 (Fig. 1f), whereas in Fig. 1g the same X fragment is now fused with a Torresian chromosome 9. In contrast, the same rearrangement has been found in several eggs within the same pod, but involving different recombinant forms of the same chromosome. For instance, Fig. 1e shows a Torresian chromosome 5 fused to a nonrecombined long arm of the Moreton X chromosome, whereas in Fig. 1b the 5 is now fused to recombined long arm of the Moreton X in two eggs derived from the same female.

In *Caledia* one can determine whether the involvement of members of the hybrid genome in chromosomal rearrangement is random or restricted to specific sites within and between chromosomes. In maize, chromosome 9 shows a marked influence on the occurrence of fusions, inversions, and arm loss. In *Caledia*, we find an analogous situation involving chromosome 5 which has been identified in nine independent fusion events with other nonhomologous chromosomes. The X chromosome shows a similar but less marked effect and has been identified in four separate fusions. Chromosome 10, which has been shown by in situ hybridization to carry the 18*S* and 28*S* ribosomal genes, also shows a disproportionate number of novel chromosomal variants involving pericentric rearrangements, increased size of heterochromatin segments, novel heterochromatic bands, and a fusion between two homologs (Fig. 1u). More than 20 different forms of chromosome 10 have been identified among the backcross progeny, including a fusion with chromosome 5 (Fig. 1i).

The unambiguous identification of the chromosomes involved in rearrange-

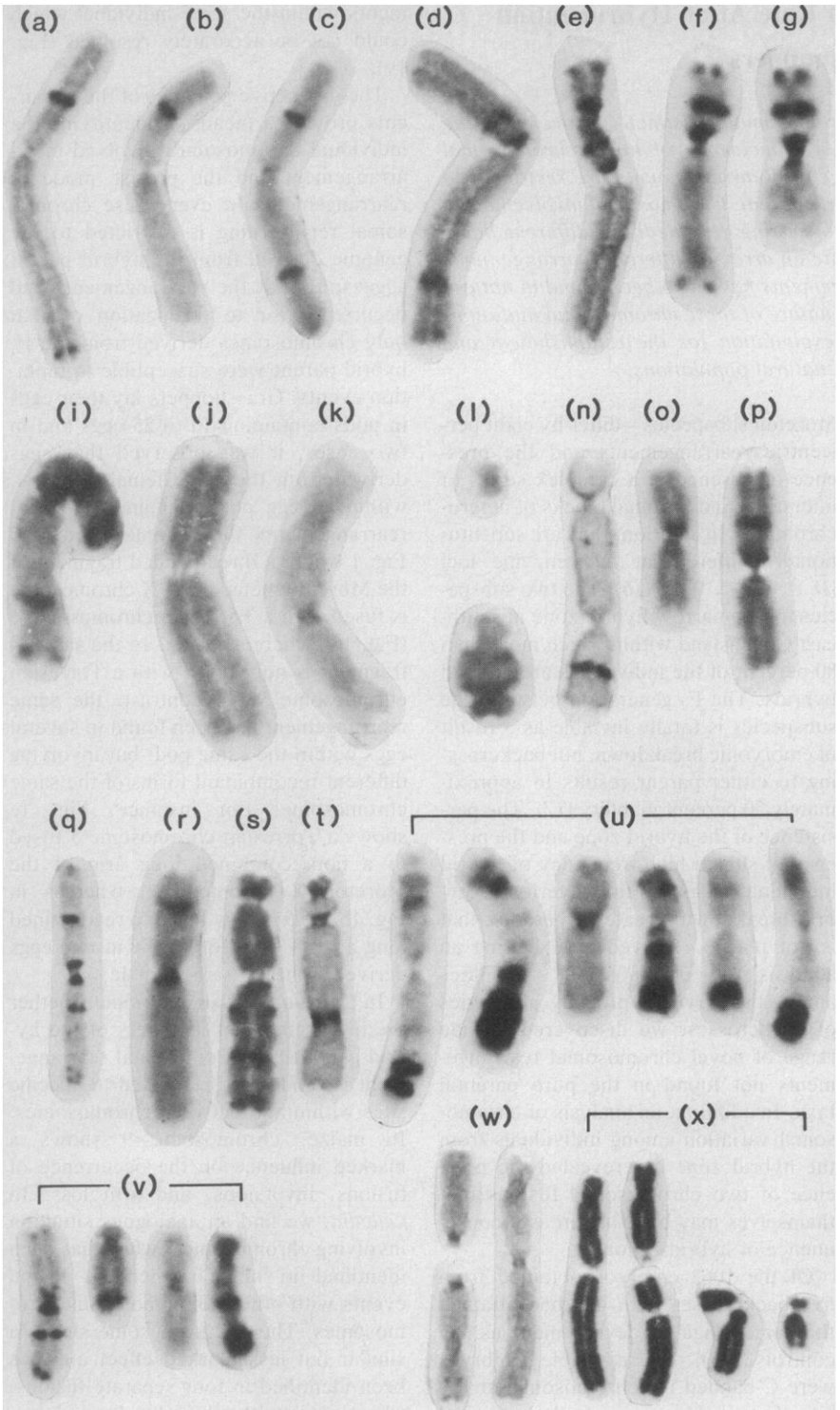

Fig. 1. Examples of the chromosomal rearrangements identified among viable backcross progeny of *C. captiva*. The interstitial and terminal bands of heterochromatin chracterize the Moreton chromosomes but can be recombined onto Torresian chromosomes in F_1 hybrids. Consequently the gametes of the F_1 parent contain both Moreton and Torresian nonrecombinant (MN and TN) and recombinant (MR and TR) chromosomes. (a) Fusion 4MN/4TR, (b) fusion 5MN/XTN, (c) fusion 2MN/1TR, (d) fusion 9MN/XTR, (e) fusion XMN/5TN, (f) fusion XMf/6TR, (g) fusion XMf/9TN, (i) fusion 10MN/5TR, (j) fusion 9T/5T, (k) fusion 4MR/5TN, (l) centric fragment, (m) dicentric chromosome, (n) complex rearrangement involving 5MN/5MR/9TN, (o) novel pericentric rearrangement on 6TN, (p) novel pericentric rearrangement on 9MN, (q) pericentric rearrangement on the XMN chromosome, (rs) new location of heterochromatic band on XMR, (s) dicentric XMN chromosome, (t) dicentric XTR chromosome, (u) examples of novel variants of the megameric chromosome 10 which carries the 18*S* and 28*S* ribosomal genes, (v) an example of multiple rearrangements within the same cell involving the XTR, 6TN, 8TN, and 10MN, (w) fusion heterozygote involving chromosomes 5T and 1T found within the hybrid zone, and (x) 5/1 fusion heterozygote found in a mixed population of two sibling species. This hybrid individual also contains two novel pericentric rearrangements and a novel small acrocentric chromosome.

ments is not possible in all cases. In some, complex multiple rearrangements have taken place. Fig. 1v is an example of such a genome in which chromosomes 6, 8, 10, and the X have been involved in rearrangement. It appears that the recombinant Torresian X chromosome has broken close to the C band which is proximal to the centromere. The acentric distal portion of the X is involved in a fusion with a centric fragment of chromosome 6, and chromosome 8 is now present as an unbanded metacentric chromosome. In addition, chromosome 10 carries an extra terminal block of heterochromatin which has also been observed in other backcross embryos (Fig. 1u).

The nonrandom nature of chromosome involvement is responsible for three important phenomena. First, the same fusion (that is, $5\widehat{X}$) occurred repeatedly among different backcrosses. Second, the same chromosomal fusion was present among several of the progeny of a single female. Third, different rearrangements were produced within the same germ line. Thus a single individual was capable of generating an array of the same or different rearrangements among its descendants. These three aspects of the mutation process have potentially important implications in the establishment and fixation of chromosomal rearrangements in natural populations.

Since these two subspecies form a narrow hybrid zone in nature it is a logical extension of the previous analysis to investigate the presence of novel chromosomal rearrangements in natural populations that contain high frequencies of hybrid individuals. Although a comprehensive survey of variation in the hybrid zone has not yet been completed, initial results have produced corroborative evidence which is directly relevant to the situation described above.

From a sample of 47 females collected from the center of the hybrid zone (maximal hybridity), two were identified as heterozygotes for different chromosomal fusions (Fig. 1w). These involved the long arms of Torresian chromosomes 1 and 4 each fused with a Torresian chromosome 5. The same fusions ($1\widehat{5}$, $4\widehat{5}$) were also found among the backcross progeny, which again indicates the nonrandom nature of chromosome involvement. Thus even with such a small sample size, some of the rearrangements are surviving in natural populations. In addition to these novelties within the hybrid zone, several of the novel backcross rearrangements have also been identified in chromosomally polymorphic popula-

tions of the Moreton subspecies. The polymorphic nature of these populations has been interpreted as evidence of past hybridization events during the westerly movement of the zone as predicted by the asymmetrical introgression of Torresian chromosomes (*15*). Again, it seems plausible that they may have arisen as a consequence of hybridization.

Another pertinent example has been found in a population sample from North Queensland where the Torresian and Daintree species are sympatric. One individual was heterozygous for a fusion involving chromosomes 5 and 1 (Fig. 1x), similar to the fusions seen in the backcrosses and in the hybrid zone. However, the most interesting feature of this individual was that in addition to the fusion, it was simultaneously heterozygous for two pericentric rearrangements involving chromosomes 1 and 6 and also contained a small acrocentric chromosome, the origin of which could not be ascertained. The karyotype of this individual suggests that it is a hybrid between the Torresian and Daintree species which has undergone a complex series of chromosomal rearrangements, similar to those observed in some of the backcross individuals.

From these data one can conclude that hybridity *per se* may indeed represent a dynamic evolutionary factor capable of generating extremely high levels of genetic variability. It may also provide a plausible explanation for the persistence of hybrid zones in nature. Thus it is conceivable that in these zones of hybridization a higher mutation rate may permit hybrid populations to attain new adaptive peaks. Studies on hybridization and hybrid zones provide substantial corroborative evidence to support this notion (*10, 20–26*). Quite clearly, the generality of the phenomenon of hybrid-induced chromosomal mutation would imply that previous hypotheses concerning the establishment of chromosomal rearrangements in the homozygous condition may no longer be appropriate (*12*). If chromosomal mutation is not a random event but one which occurs repeatedly at specific sites in the genome, as we have shown, then one can easily envisage a situation in which the establishment and fixation of a rearrangement could depend upon (i) the frequency of hybridization between two divergent taxa, (ii) the probability distribution of breakage-reunion events throughout the genome, and (iii) the relative fitness of the hybrid progeny. Thus, any reduction in population density due to hybrid inviability could produce a situation in which individuals carrying novel rearrangements may undergo positive assortative mating and hence facilitate the establishment of chromosomal homozygosity in small isolated communities. Furthermore, should the frequency of chromosome mutation events prove to be positively correlated with the intensity of hybrid breakdown then the relevance of the above scheme becomes even more plausible.

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19. The backcross technique is described in D. D. Shaw, P. Wilkinson, D. J. Coates, *Chromosoma (Berl.)* **86**, 533 (1982). Random samples of 5th instar nymphs were collected from a pure Torresian and pure Moreton population. Reciprocal F_1 generations were reared in the laboratory and backcrossed to wild-caught pure Torresian and Moreton individuals. For each backcross 20 males and 20 females were used making it improbable that those chromosomes involved in recurrent rearrangements were related by descent. No reciprocal differences in the frequencies of novel chromosome rearrangements were observed between the backcrosses (TT × MM) F_1♀ × TT♂ and TT♀X (TT × MM) F_1♂; the remaining three backcrosses [(MM × TT) F_1♀ × TT♂, (MM × TT) F_1♀ × MM♂, and (TT × MM) F_1♀ × MM♂] cannot be used to assess for reciprocal differences.
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Myosin Light Chain Phosphorylation Does Not Modulate Cross-Bridge Cycling Rate in Mouse Skeletal Muscle

Abstract. *An attempt was made to determine whether phosphorylation of the myosin light chain represents a thick filament–associated mechanism for modulating the rate of cross-bridge cycling in mouse skeletal muscle. When the degree of light chain phosphorylation was varied independently of tetanus duration, there was no correlation of phosphorylation with cross-bridge turnover rate, as measured by the shortening velocity of the muscle. It is concluded that in intact skeletal muscle phosphorylation of the myosin light chain does not in itself modulate cross-bridge cycling rate and that previously reported changes in cycling rate were due to other factors that may vary with tetanus duration.*

The regulatory light chain of skeletal muscle myosin in mammals can be phosphorylated by a calcium and calmodulin-dependent protein kinase (*1*), and cycles of phosphorylation and dephosphorylation have been observed in response to contraction and relaxation in whole muscle (*2*). Phosphorylation of the light chain is not essential for actin-activated adenosinetriphosphatase activity of myosin (*3*), but there are conflicting reports as to whether this phosphorylation may play a role in increasing or decreasing the rate of actomyosin adenosinetriphosphatase activity in isolated protein and skinned fiber systems (*4*).

Recently, Crow and Kushmerick (*5*) measured the maximum velocity of shortening and the degree of myosin light chain phosphorylation in intact mouse skeletal muscle after various periods of isometric tetanus. They found that, as the degree of phosphorylation increased with the duration of stimulation, the maximum velocity of shortening decreased. During prolonged stimulation, when light chain phosphorylation had increased to 50 percent there was a 50 percent decrease in the maximum velocity of shortening. Bárány (*6*) showed that the maximum velocity of shortening was proportional to the intrinsic rate of actin-activated adenosinetriphosphatase activity of myosin. Crow and Kushmerick (*5*) thus interpreted the relation between light chain phosphorylation and maxi-