A CYCLIC PATTERN OF R ALLELIC VARIATION

A
report of progress
on
a selected topic
for
Nov. 1, 1978 — Jan. 31, 1980

MASTER

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THE U.S. DEPARTMENT OF ENERGY
Contract DE-AS02-76EV01300

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RESUMÉ

Two R alleles vary in cyclic fashion. The original, intensely pigmenting forms change to weakly acting ones which revert in turn to the original. Neither direction of change is correlated with recombination of flanking markers. The reversion frequencies do not differ from the respective frequencies of change in the "forward" direction. The changes are restricted in the life cycle to about the time of meiosis. Modifying the incidence of crossing over in the R region altered the frequency of reversion proportionately. These features of instability could result from switching by intrachromosomal recombination between alternative arrangements of an R segment associated with an inverted duplication.

A fruitful approach to the analysis of gene complexes has been to identify and characterize the intragenic cross overs which fractionate the complex. A number of alleles in maize have been characterized in this way beginning with the pioneering studies of Laughnan (1949) on the A locus and of Stadler and Nuffer (1953) with R. Although rare, intralocus recombinants typically occur one to a few orders of magnitude more frequently than mutation. Thus our attention was drawn to a dichotomy among a collection of colored seed but green-plant (R-g) alleles tested for stability by Bray and Brink (1966). Two representatives yielded one variant for each 10^3 gametes tested, compared to one per 10^5 for the majority of R-g alleles studied.

One of the exceptional alleles derived from Peruvian strain 1304. A second, designated as "Harvard", has since been traced to Peruvian strain 1302. Both give uniformly intense pigmentation in R r r aleurone, thereby resembling other R-g alleles collected from the Andean highlands of South America. Because no essential difference between the Peru 1304 and 1302 alleles has been detected, they sometimes are treated collectively in the present discussion.

After incorporating the proximal flanking markers g (golden-1) and the distal flanking markers M-st (Modifier of R-stippled) and Lc (Leaf color), we tested for the possibility that the variants observed by Bray and Brink occurred in conjunction with recombination of flanking markers. This test, like theirs, was performed with the R-g alleles homozygous. The experiment was designed specifically to detect unequal crossing over, a characteristic feature of tandem duplication homozygotes. Variants, either colorless (r-g) or, more often, "nearly-colorless" (r-g:Nc) occurred in frequencies totaling 6.8 and 10.4×10^{-4} in the two stocks. The incidence of flanking marker recombination among the variants did not differ from a representative population of nonvariant bearing chromosomes 10.

However, reversion of r-g:Nc to a form resembling or equivalent to the parental R-g:Peru alleles was detected in the small population of heterozygous plants involved in testing the variants for heritability and linked marker composition. This observation was unexpected since reversion of r-g or r-g:Nc had not been established previously except for cases under influence of transposable controlling elements. Homozygous strains were prepared to measure reversion rate. The r-g:Nc $\rightarrow$ R-g reversion frequency of the three variants analyzed measured 5.7, 18.0 and 10.7×10^{-4} . Reversion frequency is comparable therefore to the incidence of the initial R-g $\rightarrow$ r-g:Nc change.

For R-g:Peru's pattern of variation to be considered cyclic the R-g revertants should have the same properties as the original R-g:Peru stock. The phenotypes are similar if not identical. Tests comparing the stability of three of the revertants with the ancestral tests are underway. Although quantitative results await the outcome of progeny tests, it is clear already that two of the three are unstable at a level approximating that of the ancestor.

Is the near-colorless phenotype exhibited by r-g:Nc simply an inhibited form of the strongly acting parental form? Alternatively, the weak phenotype could reflect action of a separate genic element whose expression normally is masked by the stronger one. The sorts of intralocus crossovers obtained when R-g:Peru 1302 and 1304 were paired with R-r:standard establish the second alternative.

Putative intragenic recombinants of R-g:Peru were selected as crossovers which had fractionated the compound allele R-r:standard in R-g:Peru/R-r:standard heterozygotes. The proximal member of the R-r:standard duplication specifies plant (P) pigmentation whereas the distal member specifies seed (S) pigment. Thirty-seven instances of (P) separation from

(S) where the product carried the distal flanking marker of R-g:Peru were established among 61,000 gametes tested. Of the 37 crossovers, 14 were colorless seed cases (r-r) whereas 23 were near-colorless (r-r:Nc), not unlike the seed color effects of r-g:Nc variants obtained from R-g homozygotes and 13 similar cases obtained from these R-r heterozygotes. Thus the R-g:Peru alleles evidently carry in a distal position a separable genic element which confers the near-colorless phenotype. Because this component is analogous to that isolated from R-stippled (Ashman, 1965) it can be designated (Nc). Similarly, the stronger acting proximal component can be designated (Sc), since its effects resemble that of the stable "self-colored" derivatives obtained from stippled (Kermicle, 1970). Thus a structure of (Sc)(Nc) is indicated for the R-g:Peru alleles even though the two do not behave as expected if associated with a tandem duplication.

If change of (Nc) to a strongly expressed state does not account for r-g:Nc $\rightarrow$ R-g reversion, then the (P)(Nc) intralocus variants should be stable. Indeed no revertant occurred among 63,000 gametes of three (P)(Nc) isolates tested as homozygotes. Also tested was the possibility that (Nc), although not itself unstable, incited instability in the proximal element. If such were the case in (P)(Nc) isolates, loss of (P) function would yield a product equivalent in phenotype to r-g:Nc. Seedling tests of 19,200 (P)(Nc) test-cross kernels gave no such case. Thus if (Nc) is involved in R-g:Peru instability, the interaction between (Sc) and (Nc) is specific to these genic elements.

We sought means of testing whether the R-g $\leftrightarrow$ r-g:Nc variation was due to crossing over but of such a nature as not to lead to recombination of flanking markers. One reason for re-examining the role of recombination was the restriction of switching between the two states to about the time of

meiosis - there being no evidence of instability in vegetative tissues.

Ideally, one would compare reversion frequency in the normal circumstance with a situation where crossing over was blocked. Although there is no means of blocking crossing over absolutely in maize, recombination rates often differ in the two sexes. Thus through reciprocal crosses we obtained an estimate of the frequency in the male which could be compared with 12.1×10^{-4} , the value cited above for tests through the female.

Our estimate of r-g:Nc to R-g reversion in the male comes to 36.4×10^{-4} (based on 123 cases), a three-fold increase relative to the female. A possible basis for the elevated frequency through the male would be the occurrence of premeiotic reversions, yielding clones of germinal tissue. Such events would yield clusters of variants. Yet, when the data are analyzed in terms of the number of variant kernels per ear compared to a random distribution described by Poisson theory, there is not a significant departure from randomness.

What evidence relates the increased reversion of r-g:Nc to R-g through the male to an elevated level of recombination? Our several previous analyses involving R intralocus recombination had all involved use of the female parent as the test heterozygote. We derived stocks appropriate for detecting recombination between (P) and (S) components of the R-r:standard allele in R-g:l heterozygotes when tested through the male. The test involved use of unlinked recessive mutants in combination with heterozygous flanking markers such that any false case resulting from foreign, contaminant pollen could be positively excluded. Tested through the female, the frequency of crossover r-r's was 6.34×10^{-4} ; through the male the frequency was 17.33×10^{-4} . The 2.71 fold excess through the male compares favorably with the 3.0 fold increase seen in r-g:Nc to R-g reversion.

Behavior of the two R-g:Peru stocks might be considered an isolated case were it not that an allele of contrasting phenotypic effects appears to be behaving similarly. R-r:cherry pigments not only the aleurone layer of the seed but a wide spectrum of plant parts, including the pericarp and nodes of the culm. It yields colorless-kernel, green-plant (r-g) variants relatively frequently (6.7×10^{-4} in our experiments), about half of which originate in association with recombination of flanking markers. In small scale tests, neither the crossover nor noncrossover r-g cases have reverted to R-r.

In addition to r-g variants, five pale-seeded, green-plants (R-g:pale) derivatives occurred among 75,600 gametes sampled (0.6×10^{-4}) from homozygous R-r:cherry plants. Only one was borne on a recombinant chromosome. Tests of R-g:pale stability, involving four separate isolates, yielded r-g variants in a frequency of 11.8×10^{-4} . Of more interest in the present connection was the establishment of seven colored-seed variants among a population totaling 99,900 kernels (0.7×10^{-4}). The seven proved to have the same spectrum of plant effects as parental R-r:cherry.

Do the R-r:cherry revertants possess the same stability and structural properties as the parental allele? Combination of R-r:cherry with R-g:l, an (S) derivative of R-r:standard, had earlier proved useful in understanding R-cherry structure. Two classes of r-g derivatives were frequent: one bearing the flanking markers contributed by R-r:cherry, another bearing the proximal marker of R-g but the distal marker of cherry. The two remaining marker classes together comprised only one-eighth of the r-g cases. Comparable tests on two revertants recently were completed. The frequencies of r-g variants were 7.1 and 6.4×10^{-4} for revertants compared with 6.1×10^{-4} for parental. More critically, the distribution of cases among the four marker classes did not differ from parental ($P = 0.3$). By the criteria so far

applied, therefore, the original structure of R-r:cherry has been restored.

Since R-r:cherry governs plant as well as seed pigmentation, its R-g:pale derivatives are more suitable than R-g:Peru for detecting reversion occurring at stages of the life cycle other than meiosis. Reversion during the differentiation of vegetative tissues would be expected to generate clones of tissue capable of expressing R-r:cherry as colored sectors. No instance of sectoring has been detected among the numerous R-g:pale carrying plants grown. Stability of R-g:pale in vegetative tissues is consistent with R-r:cherry and R-g:pale interconversion being based in an event uniquely associated with meiosis.

Although the frequencies of R-g:Peru and R-r:cherry changes to their respective reversible states differ by an order of magnitude, the underlying mechanisms could be similar. In an earlier report under this contract it was suggested that the initial change and its reversion might reflect positional alterations among components essential to R function. The possibility was considered that the cis-interacting region of an R allele might include a duplicated segment with the two members carried in reverse orientation. If so, one member would carry R information in the sense strand, one in the antisense strand. If the two members were identical, intrachromosomal crossing over between the members (visualized in Smithies, 1963) would be inconsequential. If one copy were mutant, however, only one arrangement would be functional. Switching between the two arrangements would result in alternating functional and nonfunctional states. A special recombination mechanism with site specific activity, such as demonstrated to account for phase variation in Salmonella (Zieg, et al., 1977) need not be postulated.

The evidence presented here is consistent with the inverted duplication model. Our further work is directed (1) at testing in various ways for recombinational dependence of switching, and (2) toward developing additional genetic criteria for detecting duplications of inverted orientation. Such structures have been shown by DNA renaturation studies (e.g., Wilson and Thomas, 1974) to be a common feature of eukaryotic genomes.

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