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RECOMBINANT-INBRED STRAINS

AN AID TO FINDING IDENTITY, LINKAGE, AND FUNCTION OF HISTOCOMPATIBILITY AND OTHER GENES[1]

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SUMMARY

The development of seven *recombinant-inbred* (RI) strains is described. The results of allele typing the seven strains at eight histocompatibility (*H*) loci are presented, and the use of such information in identifying *H* loci, searching for *H* gene function, and assisting linkage testing procedures is described.

Genetic segregation and recombination have provided the very foundation of classical genetics by revealing the particulate nature of the gene and the characteristics of linkage. On the other hand, inbreeding, which engenders the antithesis of segregation and recombination, has shown great utility as a means for producing replicable genotypes. The advantages of these processes can be exploited concomitantly by the use of RI strains.

RI strains are those which have been derived from the cross of two unrelated but highly inbred progenitor strains and which have been maintained independently under a regimen of strict inbreeding since the F_2 generation. This procedure genetically fixes the chance recombination of genes that occurs in the generations following F_1 in ever decreasing amounts as full homozygosity is approached. The resulting population of strains can be looked upon as a replicable recombinant population. The utility of a small group of such strains for analyzing histocompatibility (*H*) and other gene systems is described in this paper.

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[2] The principles of laboratory animal care, as promulgated by the National Society of Medical Research, are observed in this Laboratory.

STRAIN DEVELOPMENT

Twelve RI strains were derived from the cross of BALB/cBy (C) and C57BL/6By (B6) and were subsequently maintained as parallel strains by full sib mating. Of the 12 strains, 7 are extant after more than 30 generations of full sib mating. These have been named CXBD, CXBE, CXBG, CXBH, CXBI, CXBJ, and CXBK.

This group of RI strains was developed as an aid to identifying individual lines in a battery of newly developed B6 congenic *H* gene lines. The development and characteristics of the full battery of more than 18 lines will be given in greater detail in a separate paper. This paper must remain focused on the development and uses of the RI strains. The congenic lines must not be confused with the RI strains themselves. Briefly, however, each of the congenic lines was independently developed from an initial cross of B6 to C by a regimen of skin graft testing and backcrossing to B6 for at least 12 generations. This procedure resulted in a battery of congenic B6 lines, each differing from B6 itself by only an introduced C strain allele at a single distinctive *H* locus.

As each new congenic *H* gene line is backcrossed sufficiently to be deemed "congenic" for one *H* locus, it is tested against the RI strains to find which of the RI strains carry the C strain allele and which the B6 allele, at that particular *H* locus. This is done by grafting skin from donors of the congenic line onto the F_1 hosts which were produced by crossing each of the RI strains with the B6 strain. A surviving graft

TABLE 1. RI SDPs of BALB/c (C) and C57BL/6 (B6) alleles at 3 coat color loci and 8 histocompatibility loci

Locus[a]	SDPs in RI strain						
	CXBD	CXBE	CXBG	CXBH	CXBI	CXBJ	CXBK
a	C	C	C	B6	B6	B6	B6
b	B6	B6	C	C	C	C	B6
c	B6	B6	C	B6	C	B6	B6
H(w13)	C	B6	C	C	C	B6	B6
H(w17)	C	C	C	C	C	B6	C
H(w19) [*H-2*][b]	C	B6	B6	C	B6	B6	B6
H(w20)	B6	C	B6	C	B6	C	B6
H(w35)	B6	C	C	C	C	C	B6
H(w38)	B6	C	B6	C	C	C	B6
H(w80) [*H-1*][b]	B6	B6	C	B6	C	B6	B6
H(w96)	C	C	B6	B6	C	C	C

[a] Tentative designations of *H* loci are indicated by the letter *w* and a number enclosed in parentheses, to avoid confusion with names of established *H* loci.

[b] Recently identified by F_1 tests as the *H* locus indicated in brackets. The congenic lines distinguishing *H(w19)* and *H(w80)* will hereafter be designated as B6.C-*H-2*^{d} and B6.C-*H-1*^{b}, respectively.

indicates that the RI strain, parental to that F_1 host, carries the C strain allele; a rejected graft indicates the B6 strain allele. The resulting *strain distribution pattern* (SDP) is quite useful, as is explained. The SDPs for a sample of eight different *H* loci are presented in Table 1.

EXPERIMENTAL APPLICATION

Some potential uses of the RI strains are as follows.

1. To identify H loci. There is always a substantial possibility that new congenic *H* gene lines will duplicate one another or existing congenic *H* gene lines. Although this situation can be tested by directly exchanging skin grafts between congenic lines in all combinations, it is done more quickly by finding the SDP of the newly isolated gene in the RI strains. Identical patterns suggest that the locus is already represented. However, since the matching could have happened by chance, a followup graft exchange between the new line and its SDP-matching line is required for verification. On the other hand, distinctive SDPs can be taken as good evidence that one is dealing with different loci. Similar use of SDPs under genetically less controlled situations has been made for identifying blood group loci (*1*) and defining antigenic specificities at the *H-2* locus (*4*).

The 8 congenic *H* gene lines which represent the *H* loci listed in Table 1 have been shown to be distinctive by their SDPs. On the other hand, 4 other lines duplicated *H(w19)* and 1 duplicated *H(w38)*. Such duplicates were eliminated from the battery.

2. To search for H gene functions. The RI strains should also be useful for finding pleiotropic effects of the *H* genes. For example, the immune response genes have been associated with major *H* loci, and it has been hypothesized that *H* genes have an antigen recognition function (*3*). The utility of RI strains for testing this hypothesis, necessarily in regard to antigens to which B6 and C strains respond differently, is obvious. The location of a gene controlling the female immune response to the male antigen by this device will be presented in a separate paper (Bailey and Hoste, unpublished data).

3. To analyze traits that depend on replicative observations. Some traits, because of the nature of their expression, require measurements on several mice of the same genotype. Such traits are difficult, if not impossible, to evaluate in the genetically segregating populations usually required in genetic analyses. Resistance to tumor induction is an example in which the proportion of animals that bear no tumors is the measure of resistance. The differences and similarities in the RI and progenitor strain phenotypes, even if there should be no fit with any established SDP, would aid materially in evaluating the complexity (number of loci and interactions) of the controlling gene system and possibly would suggest the appropriate strain crosses one should make to isolate the effective genes.

4. To select marker genes most likely to succeed in locating a new gene by conventional linkage tests. When *H* genes have been located on the linkage map, they can then become markers. In that case, the RI strains will become useful in selecting the *H* gene markers most likely to be successful in locating a new gene. Highest priority would be given to those markers with SDPs having the fewest discrepancies with those of the new gene. The new genes with which this method can be used are limited to those by which the B6 and C strains differ, namely, the

extant polymorphic genes, such as those determining isozymes, immunoglobulins, and alloantigens. As an illustration (Table 1), where there is close linkage, as is known for *c* and *H-1*, the SDPs will be the same; when the linkage is not so close, as is known for *H*(*w13*) and *b*, the SDPs match in 5 out of 7 strains.

The latter suggested use points out the potential that a larger population of RI strains would have for *directly* determining linkages. Indeed, Haldane and Waddington (*2*) have considered the theoretical relationship between standard recombination values and those realized in a population of recombinant strains produced by full sib matings. Recombination that occurred since the F_1 generation of the RI strains, of course, is reflected by the proportion of strains in the SDP of one locus that fail to match those in the SDP of another locus. Once the RI strains are classified for a trait, the resulting SDP could be reemployed to compute the linkage relationships of every new trait examined.

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