Recessive Lethal Mutations and the Maintenance of Duplication-Bearing Strains of *Dictyostelium discoideum*

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ABSTRACT

Recessive lethal mutations have been isolated and used to maintain $n + 1$ aneuploid strains of *Dictyostelium discoideum* carrying a duplication of part or all of linkage group VII. The recessive lethal mutations, *relA351* and *relB352*, arose spontaneously in diploids; no mutagenic treatment was used in the isolation of these mutations. The probable gene order on linkage group VII is: centromere, *relB*, *couA*, *bsgB*, *cobA*, *relA*. Maintenance of aneuploids disomic for linkage group VII was made possible by complementation of a *rel* mutation on each linkage group VII homologue by the corresponding wild-type allele on the other linkage group VII homologue. The duplication-bearing disomic strains were slow-growing and produced faster-growing sectors on the colony edge. Haploid sectors probably arise by a combination of mitotic recombination and subsequent loss of one homologue, diploid sectors may be formed by chromosome doubling to $2n + 2$, followed by chromosome loss to return to $2n$, and aneuploid sectors may arise by deletion or new mutation.

DICTYOSTELIUM discoideum is a eukaryotic microorganism that exists as uninucleate amoebae which feed by phagocytosis of other microorganisms. On starvation the amoebae aggregate and differentiate to form multicellular fruiting bodies consisting of spore, stalk and basal disc cells (LOOMIS 1982). This species is normally haploid with a karyotype containing seven acrocentric chromosomes (ROBSON and WILLIAMS 1977), although diploids are easily maintained. While transient aneuploidy is involved in the segregation of haploid strains from diploids (BRODY and WILLIAMS 1974), stable aneuploid strains of *D. discoideum* are not normally encountered. The only previous report of aneuploidy is that of WILLIAMS, ROBSON and WELKER (1980) in which aneuploid strains containing a fragment chromosome corresponding to part of linkage group II were found. These arose from diploids constructed using pairs of haploid strains that were derived from different wild isolates and that are thought to have chromosome rearrangements affecting linkage group II. In *D. discoideum* the growth of strains bearing duplications is subject to adverse gene dosage effects (WELKER, METZ and WILLIAMS 1982); the inability to recover aneuploids is undoubtedly due to such effects.

Duplication-bearing strains are of great value in molecular genetic analysis of the *D. discoideum* genome since they allow linkage determinations based on comparisons of the copy number of sequences in the DNA from duplication-bearing and nonduplication-bearing strains (WELKER *et al.* 1986). If duplications covering the entire genome could be developed, then genetic analysis of cloned DNA probes could proceed without the need for time-consuming genetic crosses. In other species, duplication-bearing strains are maintained by complementation of recessive lethal mutations. Here, we present the first report of recessive lethal mutations in *D. discoideum*. These mutations allowed the isolation and characterization of aneuploid strains that carry a duplication of all or part of linkage group VII.

MATERIALS AND METHODS

Strains and culture conditions: All strains of *D. discoideum* described in this report were derived from the NC4 wild isolate; genotypes are summarized in Table 1. Stock cultures were passaged weekly in association with *Klebsiella aerogenes* at 21 ± 1° on SM agar (SUSSMAN 1966; WELKER and WILLIAMS 1982a). Aneuploid strains were maintained either by streak cloning or by dilution cloning (WILLIAMS, ROBSON and WELKER 1980; WELKER, METZ and WILLIAMS 1982). Suspension cultures used pregrown *Escherichia coli* B/r ($\sim 10^9$/ml) as a food source (WELKER and WILLIAMS 1982b).

Parasexual genetics: Standard diploid formation techniques involving complementation of recessive conditional-lethal growth mutations were employed (NEWELL 1982). Haploid segregants were usually obtained from diploids using the haploidizing agents benlate (35 μg/ml; WILLIAMS and BARRAND 1978) or thiabendazole (2 μg/ml; WELKER and WILLIAMS 1980a); segregants of DU1980 were selected on the basis of a recessive methanol resistance mutation (*acrA371*). Determination of genotypes followed established procedures (NEWELL 1982). Mitotic recombinants of heterozygous diploids were obtained by selection for homozygos-

TABLE 1

Genotypes of strains

Strain	Linkage group I	II	III	IV	VI	VII	Parent	Reference
HU526	*cycA1*	+	*bsgA5*	*bwnA1*	*manA2*	+	DU740	1
HU886	*cycA1*	*acrA371*	*radB13*	*bwnA1*	+	*couA351* *frtB353*	DU1244	2
HU1133	*cycA1*	*axeA1?* *axeC1?* *oaaA1?*	*whiB500* *axeB1?* *acrC388* *radC44*	+	+	*bsgB500* *cobA353*	DU1443	2
HU1456	*cycA1*	*acrA371*	*axeB1?* *acrC388* *radC44*	+	+	*couA351/couA*$^+$ *frtB353/frtB*$^+$ *bsgB*$^+$*/bsgB500* *cobA*$^+$*/cobA353* *relA*$^+$*/relA351* *relB352/relB*$^+$	DU1980	3

Haploid and aneuploid strains are designated HU, and diploids are designated DU. Phenotypes of mutations at these loci are as follows: *acrA*, resistance to acriflavin (100 μg/ml), methanol (2%) and benzimidazole carbamates; *acrC*, resistance to acriflavin (100 μg/ml) and benzimidazole carbamates; *axe*, ability to grow in axenic media; *bsg*, inability to grow using *Bacillus subtilis* as a food source; *bwnA*, production of brown pigment during development; *cobA*, resistance to cobaltous chloride (300 μg/ml); *couA*, sensitivity to 1.3 mM coumarin with pleiotropic temperature sensitivity (formerly designated *tsgK21*, see Figure 2); *cycA*, resistance to cycloheximide (500 μg/ml); *frtB*, distribution of fruiting bodies in concentric rings; *manA*, α-mannosidase-1 deficient; *oaaA*, absence of development in the presence of ω-aminocarboxylic acids; *rad*, sensitivity to UV or gamma rays; *rel*, recessive lethal mutation; *whiB*, white spores, absence of the normal yellow spore pigment. Wild-type alleles are denoted by +. The *frtB353* mutation was not scored in this work. For simplicity, the cobalt-resistance mutation derived from HU1133 has been designated *cobA353*, although it is possible that cobalt resistance in strain HU1133 may be due to the *cobA358* rather than the *cobA353* allele. In earlier work (WILLIAMS 1978), *cobA353* was associated with a semidominant cobalt resistance; this characteristic varies with genetic background and in the present work led to a low level of cobalt resistance in heterozygous diploids and aneuploids. Strain HU886 also contains a mutation *supE368*, which leads to the suppression of cobalt resistance (WELKER and WILLIAMS 1982a); this mutation appears to map to linkage group III or VI of HU886 and not to be present in HU1456. Diploid DU1443 was previously reported as heterozygous at the *acrC* locus (WELKER and WILLIAMS 1982a); it is now known to be homozygous for the *acrC388* mutation. References for the origin of the strains are (1) WELKER and WILLIAMS 1982b, (2) WELKER and WILLIAMS 1982a, and (3) this work.

ity for mutations leading to drug resistance (MOSSES, WILLIAMS and NEWELL 1975), in particular resistance to 300 μg/ml cobaltous chloride (WELKER and WILLIAMS 1980b; WALLACE and NEWELL 1982).

Nomenclature: Following the conventions utilized for *D. discoideum* genetics, the recessive lethal mutations have been given the three-letter code *rel*, followed by a capital letter locus and a numberical allele designation (DEMEREC *et al.* 1966).

Cytological examination: Amoebae taken from stock plates were arrested in metaphase using the benzimidazole carbamate derivative thiabendazole (10 μg/ml) as described previously (WELKER and WILLIAMS 1980a; WELKER and WILLIAMS 1981).

RESULTS AND DISCUSSION

Origin of the recessive lethal mutations: Both of the recessive lethal mutations that were isolated in this work, *relA351* and *relB352*, arose spontaneously in diploid *D. discoideum* strains. No specific selective techniques or mutagenic treatments were utilized in the recovery of the recessive lethal mutations. The diploids in which *relA351* and *relB352* arose (DU1769 and DU1980, respectively) were closely related to strains that exhibited a high frequency of spontaneous unselected mitotic recombination events. The original strain bearing this phenotype, DU1443, was shown to have unselected recombination events affecting linkage groups III and VII (WELKER and WILLIAMS 1982a). One expectation of strains with higher than normal recombination frequencies is that they may produce derivatives with chromosome rearrangements such as translocations, insertions or deletions. Recessive lethal mutations will arise when chromosome rearrangements interrupt or delete genes required for vegetative growth.

Isolation of strains carrying the *relA351* mutation: The recessive lethal mutation *relA351* arose in diploid DU1769 (HU1133 × HU886) and was identified on the basis of the skewed distribution of the genotypes of haploid segregants derived from DU1769 (Table 2). While segregation of linkage groups II, III and IV appeared normal, all but one segregant out of a total of 69 independent benlate-induced segregants bore the linkage group VII markers *couA351*, *bsgB*$^+$ and *cobA*$^+$ derived from the parental haploid HU886. The almost complete absence in the segregants of the linkage group VII markers *couA*$^+$, *bsgB500* and *cobA353* from the parental haploid HU1133 indicated the presence of the *relA351* mutation on the chromosome corresponding to the linkage group VII derived from HU1133. The single segregant that carried the *couA*$^+$, *bsgB500* and *cobA353* markers probably arose by mitotic recombination; as described above, the genetic background of

TABLE 2

Segregation analysis of diploid DU1769 which is heterozygous for the *relA351* recessive lethal mutation on linkage group VII

	Linkage group					
	II		III		IV	
VII	+	*acrA371*	*whiB500* *radC44*	*radB13*	+	*bwnA1*
couA$^+$ *bsgB500* *cobA353*	0	1	1	0	1	0
couA351 *bsgB*$^+$ *cobA*$^+$	15	53	37	31	48	20

Diploid DU1769 (HU1133 × HU886) carries a recessive lethal mutation, *relA351*, on the linkage group VII homologue bearing the *bsgB500* and *cobA353* mutations. The single *couA*$^+$, *bsgB500*, *cobA353* segregant is most likely the product of a mitotic recombination event that created a *couA*$^+$, *bsgB500*, *cobA353* but *relA*$^+$ homologue of linkage group VII. All segregants were obtained by benlate-induced haploidization. Only partial genotypes are presented; complete genotypes can be determined from Table 1. Markers from HU1133 are presented in the left column of each pair for linkage groups II, III, and IV and for linkage group VII on the top row.

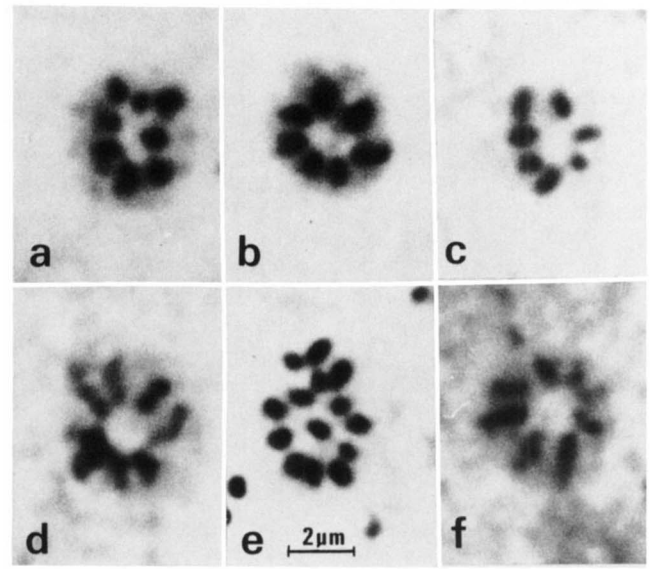

FIGURE 1.—Mitotic figures of HU1456 (a) and of representative sectors of each identified class [class 1, haploid, HU1466 (b); class 2, haploid, HU1463 (c); class 3, aneuploid, HU1449 (d); class 4, diploid, DU2147 (e); and class 5, aneuploid, HU1495 (f)]. The haploids (b, c) have seven chromosomes, the aneuploids (a, d, f) eight chromosomes and the diploid (e) 14 chromosomes.

strains used in this work apparently has a higher than normal mitotic recombination frequency.

Isolation of the *relB352* mutation: The *relB352* mutation was identified on the basis of the segregation pattern of DU1980. This diploid was selected from DU1769 as a mitotic recombinant homozygous for the linkage group III markers *acrC388* and *radC44*, but this recombination event is apparently unrelated to the origin of *relB352*. Diploid DU1980, which was known to contain the *relA351* mutation, was expected to have a segregation pattern for linkage group VII markers similar to that of DU1769. Its haploid segregants were anticipated to display the coumarin-sensitivity and temperature-sensitivity phenotypes associated with the *couA351* mutation and to have normal growth rates. However, the majority of the segregants obtained from DU1980 exhibited a slow growth rate and were coumarin-resistant and temperature-resistant.

Cytological examination of one segregant from DU1980, HU1456, established that it was an $n + 1$ aneuploid with a karyotype containing eight chromosomes (Fig. 1a). In two experiments, more than 50% of metaphases had eight chromosomes as expected for an $n + 1$ aneuploid. On the basis of the cytological and phenotypic data, HU1456 bears two chromosomes corresponding to linkage group VII. Like other duplication-bearing strains, the vegetative growth of HU1456 was severely restricted. After 7 days its colonies on SM agar had a diameter of only 1–2 mm, whereas those of normal laboratory strains (*e.g.*, HU526) were 1–2 cm in diameter. The growth of HU1456 was also slow in suspension culture; its doubling time was approximately 8 hr under conditions in which normal strains (*e.g.*, HU526) doubled every 4 hr. HU1456 produced fruiting bodies with spores, stalks and basal discs, albeit smaller than normal, presumably due to a smaller than normal aggregation territory size. The spore size of HU1456 was within the range for normal haploid strains.

Since most of the other segregants of DU1980 were similar to HU1456 in their growth rates and expression of group VII markers, they are probably aneuploids which, like HU1456, carry two homologous chromosomes corresponding to linkage group VII. The few faster-growing segregants of DU1980 that were obtained are likely to be sectors from aneuploids (see below). A segregation pattern of this type has not been reported previously for *D. discoideum*.

Characterization of sectors derived from HU1456: The aneuploid HU1456 spontaneously produced faster-growing derivatives which could be isolated as sectors on the edge of the slow-growing HU1456 colonies, and indeed, it is difficult to maintain the original strain. Genetic analysis of these sectors established that at least the portion of linkage group VII between the *couA* and *cobA* loci was present in two copies; the duplication must also cover the *relA* and *relB* loci. Sectors of HU1456 fell into at least five major classes: (1) coumarin-resistant (*couA*$^+$), cobalt-resistant (*cobA353*) haploids unable to use *B. subtilis* as a food source (*bsgB500*), *e.g.*, strain HU1466; (2) coumarin-sensitive (*couA351*), cobalt-sensitive (*cobA*$^+$) haploids that grew on *B. subtilis* (*bsgB*$^+$), *e.g.*, strain HU1463; (3) coumarin-sensitive (*couA351/couA351*?), cobalt-sensitive (*cobA*$^+$/cobA353?) aneuploids that

TABLE 3

Sectors of aneuploid strain HU1456

Class	Sector	Apparent genotype on linkage group VII			Ploidy	No.	Frequency
1[a]	HU1466	*couA*$^+$	*bsgB500*	*cobA353*	Haploid	32	0.10
2[b]	HU1463	*couA351*	*bsgB*$^+$	*cobA*$^+$	Haploid	(251)	(0.78)
3[b]	HU1449	*couA351*	*bsgB*$^+$	*cobA*$^+$	Aneuploid		
		couA351?	*bsgB500?*	*cobA353?*			
4[c]	DU2147	*couA*$^+$	*bsgB500?*	*cobA353?*	Diploid	34	0.10
		couA351?	*bsgB*$^+$	*cobA*$^+$			
5[c]	HU1495	*couA*$^+$	*bsgB500?*	*cobA353?*	Aneuploid	5	0.02
		couA351?	*bsgB*$^+$	*cobA*$^+$			

Genetic tests to confirm the presence of the markers indicated by the question marks were not performed. In some sectors these markers may be absent due to deletions, since their presence, in particular of a second copy of the *couA351* mutation in class 3 sectors, would require additional recombination events. Sectors of classes 3, 4 and 5 had a low level of cobalt resistance associated with the semidominance of *cobA353*.

In addition to the sectors shown above, a single diploid sector (on the basis of spore size) that was apparently homozygous for the *couA351*, *bsgB*$^+$ and *cobA*$^+$ alleles was obtained.

[a] Since few sectors of class 1 were examined cytologically, this class might also include aneuploids.

[b] Because sectors of classes 2 and 3 were not distinguishable on the basis of marker phenotypes, the actual number of sectors in each class was not determined; however, on the basis of slower growth rates, less than 10% of sectors were of class 3.

[c] Sectors of classes 4 and 5 were distinguished on the basis of spore size; aneuploidy was, in some cases, confirmed by cytological examination of the karyotype.

grew on *B. subtilis* (*bsgB*$^+$/*bsgB500?*), *e.g.*, strain HU1449; (4) coumarin-resistant (*couA*$^+$/*couA351?*), cobalt-sensitive (*cobA*$^+$/*cobA353?*) diploids that grew on *B. subtilis* (*bsgB*$^+$/*bsgB500?*), *e.g.*, strain DU2147; (5) faster-growing coumarin-resistant (*couA*$^+$/*couA351?*), cobalt-sensitive (*cobA*$^+$/*cobA353?*) aneuploids that grew on *B. subtilis* (*bsgB*$^+$/*bsgB500?*), *e.g.*, strain HU1495. The relative frequencies at which these sector classes were obtained are given in Table 3. Mitotic figures of representative strains of each sector class are presented in Figures 1b–f. Although strains HU1449 (Figure 1d) and HU1495 (Figure 1f) were designated as $n + 1$ aneuploids, in both cases only 10–20% of metaphases were aneuploid, the remainder having 7 chromosomes. The incidences of eight chromosome metaphases is rare in normal haploid strains, so this frequency of aneuploids is highly significant. Spore sizes of the haploid and aneuploid sectors were similar to that of normal haploids, whereas the spore of the diploid sectors was about twice this size, similar to normal diploids. This distinction enabled easy identification of the diploid sectors.

The simplest explanation for the production of the haploid sectors (classes 1 and 2) is that they arise by single mitotic recombination events between the *relA351* and *relB352* mutations, followed by loss via nondisjunction of the chromosome now carrying both recessive lethal mutations. The diploid sectors (class 4) probably arose by chromosome doubling and subsequent loss of one pair of linkage group VII homologues to give a $2n = 14$ karyotype. The aneuploid sectors, in particular those of class 3, probably arose by deletion of part of one homologue; class 5 aneuploids might have arisen by deletion or by mutation elsewhere in the genome.

Localization of the recessive lethal mutations within linkage group VII: Since the recessive lethal mutations arose on a pair of well-marked chromosomes, it was possible to obtain mitotic recombination data for mapping the mutations within linkage group VII. Diploid mitotic recombinants were obtained from DU1769, which was heterozygous for *relA*, by selection for cobalt resistance (*cobA353*) followed by screening for the inability to grow using *B. subtilis* as a food source (*bsgB500*). Selection for the expression of both the *bsgB500* and *cobA353* phenotypes ensured that these diploids arose by recombination and not by new mutation at *cobA*, whereas the presence of the *relA351* mutation on the original *bsgB500*, *cobA353* linkage group VII homologue of DU1769 ensured that these diploids did not arise by nondisjunction. In this manner, 90 independently derived diploid recombinants were obtained at a frequency of about one per 10^7 cells. Taking into account the gene order (centromere, *bsgB*, *cobA*) and the relative map positions proposed for these loci (Figure 2a), this recombination frequency is consistent with an origin for the recombinants involving double crossing-over, with the proximal crossover point between the centromere and the *bsgB* locus and the other crossover point distal to the *cobA* locus but proximal to the *relA* locus. Alternatively, the *relA* locus could be located proximal to the *bsgB* locus. In this case *relA* must be close to *bsgB*, since cobalt-resistant mitotic recombinants due to single crossovers between the centromere and the *bsgB* locus arise in diploids lacking recessive lethal mutations at frequencies of 10^{-3} to 10^{-5} (WALLACE and NEWELL 1982).

In DU1769 the *couA351* mutation was present on the linkage group VII homologue that did not carry

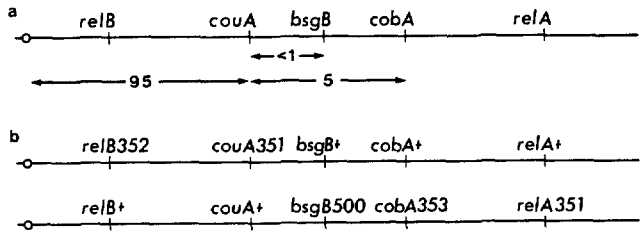

FIGURE 2.—A map of linkage group VII, adapted from WALLACE and NEWELL (1982) and this work (a), and the linkage group VII pair in HU1456 (b). The position of the *couA* locus given here corresponds to that of the *tsgK* locus in the map proposed by WALLACE and NEWELL. Since coumarin sensitivity (*couA351*) and temperature sensitivity (*tsgK21*) corevert (WELKER and WILLIAMS 1982b), and since a strain considered to be recombinant in the *couA* to *tsgK* interval by WALLACE and NEWELL (1982) was shown to carry a new *cou* mutation (WELKER and WILLIAMS 1982), we conclude that these phenotypes represent pleiotropic effects of a single mutation, which we designate *couA351*. Moreover, in the work reported here, no separation of the coumarin-sensitivity or temperature-sensitivity phenotypes was observed. The positions of *relA* and *relB* inferred from the data are unaffected by whether *couA* and *tsgK* are the same locus or different loci. The figure is not drawn to scale; the positions of *relA* and *relB* are indicated relative to the other markers, but no estimate of map distance can be made. The relative map positions indicated for the other markers are based on mitotic recombinants selected on the basis of homozygosity at *cobA* (WALLACE and NEWELL 1982).

the *relA351*, *bsgB500* and *cobA353* mutations; hence, the positions of the proximal crossover points relative to *couA* could be determined by screening segregants from each of the 90 recombinants for expression of the *couA351* coumarin-sensitivity and temperature-sensitivity phenotypes. All haploid segregants obtained from the 90 recombinants were $couA^+$, since they were coumarin-resistant and temperature-resistant. These segregants must, in each case, carry the linkage group VII produced by the recombination event, since the parental linkage group carrying the $couA^+$, *bsgB500* and *cobA353* alleles also carries the recessive lethal mutation *relA351*. Thus, the proximal crossover point in all the recombinants is between the centromere and the *couA* locus. Hence, if *relA* is proximal to *bsgB* it must also be proximal to *couA*. The complete absence of recombination events between *couA* and *bsgB* among the 90 recombinants provides supporting evidence for the location of *relA351* distal to the *cobA* locus. If *relA351* were close but proximal to *couA*, then, from the map distance between the centromere and *couA* and the map distance between *couA* and *bsgB*, a large proportion of the crossover points should be in the *couA* to *bsgB* interval.

The types of haploid sectors produced from HU1456 provide further information on the location of the recessive lethal mutations relative to the other markers. Haploid sectors almost certainly arise by single recombination events followed by nondisjunction, and most potential gene orders can be excluded since with these gene orders certain classes of haploid sectors that were observed could not be produced by single crossovers. If the *relA* locus is distal to the *cobA* locus, then the most likely location for *relB* is between the centromere and the *couA* locus. Assuming acrocentric chromosomes (ROBSON and WILLIAMS 1977), the linkage group VII pair in HU1456 is as shown in Figure 2b.

This gene order is consistent with all the available data, but predicts that, in addition to class 1 and 2 haploids (Table 3), one may also obtain coumarin-resistant ($couA^+$), cobalt-sensitive ($cobA^+$) haploid sectors unable to grow on *B. subtilis* (*bsgB500*) and coumarin-resistant ($couA^+$), cobalt-sensitive ($cobA^+$) haploid sectors able to grow on *B. subtilis* ($bsgB^+$). These should arise by recombination in the *bsgB* to *cobA* and the *couA* to *bsgB* intervals, respectively. The frequencies with which sectors of each class are recovered depend on the map positions of the *relA* and *relB* loci relative to the other loci and on the differences in growth of the sectors relative to HU1456. The *couA* to *cobA* interval has been shown to be short compared to the centromere to *couA* interval (WALLACE and NEWELL 1982), and the *relB* locus may map anywhere in the centromere to *couA* interval. A large *relB* to *couA* interval will favor the recovery of sectors of class 2 relative to those involving recombination between *couA* and *cobA*, whereas a large *cobA* to *relA* interval will favor recovery of sectors of class 1. Haploids of the predicted but unrecovered classes may be less able to take over the HU1456 colony edge than those sector haploids that have been recovered. Hence, the absence of the two classes of sectors that would arise by recombination in the *couA* to *bsgB* and *bsgB* to *cobA* intervals does not exclude the proposed gene order.

This work indicates that aneuploid strains carrying two copies of most or all of one *D. discoideum* chromosome can be isolated, provided that suitable selective pressure can be applied. Previously reported duplication-bearing strains carry only a portion of one chromosome in duplicate. These involve a tandem duplication of a sequence from linkage group III (WELKER, METZ and WILLIAMS 1982) and a chromosome fragment corresponding to the centromere proximal part of linkage group II (WILLIAMS, ROBSON and WELKER 1980). Duplication-bearing strains that are maintained by complementation of recessive lethal mutations are expected to prove useful in molecular genetic analysis based on comparison of gene copy numbers in haploid vs. duplication-bearing genomes. Such comparisons have already been utilized in *D. discoideum* for analysis of the genome organization of the actin gene family (WELKER *et al.* 1986).

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