With the current interest in genetic exchange whether there are any plasmids of a specific in the environment there is a need for efficient populations from diverse microcosms such as epilithon, rhizosphere, phylloplane or lake sediment. One approach is to determine are capable of lateral spread of genes between broad host range plasmids (6) and since they diverse bacterial species, it is important to evolution of this plasmid family, about which conserved genetic regions which could serve as IncPcr plasmids and R751 from the IncPp [reviewed in subgroups of IncP plasmids, RK2 from the].

Our study of plasmids from the two major subgroups of IncP plasmids, RK2 from the IncPa plasmids and R751 from the IncPj plasmids, has allowed us to define highly conserved genetic regions which could serve as the basis for PCR primers that have the best chance of working on new IncP plasmids. The genes chosen are \textsc{ntf-a}, which is essential for vegetative replication and therefore must be functional in any IncP replicon (3) and \textsc{katA}, the key regulator which stops lethal over-expression of a variety of IncP genes (1). The primers have been designed to give products in the 250 to 300 bp size range and are complementary to segments which show least divergence between RK2 and R751. For \textsc{ntf-a} we recently reported that the C-terminus is very highly conserved (5) and this fits with the observation that deletion of even two or three amino acids from the C-terminus results in loss of DNA replication initiation capacity (2). The 3' end of each primer is chosen to correspond to nucleotides one or two of conserved codons for amino acids with a minimum of degeneracy in the genetic code. For example, primer 1 for \textsc{ntf-a} ends in a codon for tyrosine (\textsc{Uac}) which has a minimum of degeneracy. Similarly, the codons concerned for \textsc{katA} are GAA (glutamate) and GAC (aspartate). A change at position one or two cannot occur without loss of acidic character of the side-chain. Additional sequence information is available but as yet unpublished. Anyone wishing to help in designing further primers, particularly with increased degeneracy, is welcome to contact us.

**Guidelines**

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

Approval for publication rests with the Editor-in-Chief, who reserves the right to edit letters and/or to make a brief reply. Other interested persons may also be invited to reply. The Editors of *Microbiology* do not necessarily agree with the views expressed in *Microbiology* Comment.

Contributions should be addressed to the Editor-in-Chief via the Editorial Office.
recombination intervals (the origin and terminus of replication were preferentially involved) and rec functions (red-independent terminus of replication were preferentially recombination) (3, 11, 15, 16).

The so-called CD (complementing diploids) were selected as prototrophs due to the complementation of several allelic mutations carried by the parental strains; they were by far the most unstable, and stabilized rapidly as complementation of several allelic mutations prototrophic or auxotrophic recombinants of cells can be considered as true diploids, secondary, in contrast with the directly selected, only a few of them are still of the CD type unstable and rapidly lead to recombinants (10).

(ii) Abundant experimental data support the diploidy of all the fusion types. However, only CD cells can be considered as true diploids, according to criteria based on physiological (complementation), genetical and biochemical approaches (identical recovery of both alleles by transformation); they are, however, highly unstable and rapidly lead to recombinants (10). The rest of the progeny recombinants, and NCD, are diploids of a novel type in prokaryotic genetics, with several inactive alleles or an entire inactive chromosome. This DNA is poorly accessible to routine genetical and biochemical analyses such as transformation (1, 4), hybridization or other techniques applied to detect it (12). In this situation the number and proportion of haploid cells contained in one of any of the fusion products cannot be established; this conclusion has been pointed out in the majority of the previous papers. Every previous worker in this field has searched for the usual signs of diploidy without success: the size of colonies or of cells, the culture generation time, the presence of increased amounts of DNA or of distinctive peaks of nucleoids, did not reveal any unique identifying properties.

Our response. Hauser & Karamata (6) did not take into account the above considerations. In addition, we feel that they made the following points which were invalid.

(i) They consider the inactive chromosome to be as efficiently accessible as active DNA using the same biochemical techniques whose inactivity had been shown in the previous papers. They searched for the presence of inactive alleles by quantitative estimations of hybridization. They did not analyse the variations of any colonial phenotype. No novel molecular approach or technique, e.g. PCR, was applied to obtain a novel result.

(ii) While Hauser and Karamata did not determine the number of haploid cells in a clone, they derive conclusions from quantitative determinations of the amount of DNA per cell from only 50 individual cells, obtained from one NCD clone, and the same number of cells from three prototrophic clones, considered by them to be of CD type only by checking for prototrophy and not from their unstable phenotype. From our experience (10) these CD clones were certainly recombinants.

With respect to the criticisms raised by Hauser and Karamata, let us analyse a previous paper of ours (15) concerning the diploidy of the 'recombinant progeny' or the so-called 'phenotypically recombinant progeny' (15).

Their suggestion that Sanchez-Rivas and co-workers were dealing with syntrophic mixtures cannot be substantiated, since the primary and secondary recombinants described in that paper were all analysed after several purification protocols (re-isolation on minimal medium and sporulation) that are considered acceptable by Hauser and Karamata.

The phenotype of these recombinants cannot be explained by a simple transformation occurring in the plate. In fact such phenomena could not be understood unless both entire parental chromosomes were in the same cell, because of the intervals involved and the frequencies of recombination. Among the recombinants resulting from the same number of cross-overs, those involving the origin and terminus of replication were 100 times more frequent. When these recombinants were analysed by transformation, diploidy was demonstrated for eight markers distributed around the chromosome. In addition, although the recombinants were phenotypically Trp+, the inactive wild-type trp allele was also shown to be present.

Of the recombinants obtained from fusions in which only one parent was lysogenic for the F105 prophage, all (40/40) were lysogenic. The simple explanation of their occurrence by prophage induction is ruled out by the fact that induction of the prophage was not observed during the fusion procedure (14, 16). Regarding the criticism of the poor burst-size reported with NCD clones containing the thermoinducible repressor mutation t223 of prophage F105 (5), data from this (13) and other laboratories (J. Errington (2), during his construction of F105 cloning vectors) indicate that this mutation is poorly inducible.

On changing the above comments we feel justified in concluding that the experimental support of Hauser & Karamata (6) is insufficient to dismiss the polyploidy of the fusion progeny.

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II. No experimental support for diploidy and chromosomal silencing

In their letter (above), C. Sanchez-Rivas and C. Levi-Meyrueis take as granted that the published evidence in favour of the diploidy of Bacillus subtilis exquisits is compelling and propose to rediscuss some specific experiments already extensively dealt with by us (4). While being prepared to enter into discussion, we feel that a detailed refutation of the con-