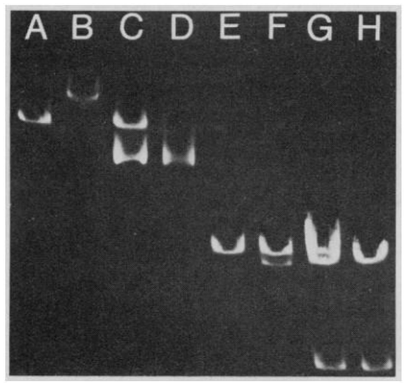

Fig. 2. Agarose slab gel electrophoresis of supercoiled and Eco RI-digested DNA. Plasmid DNA was prepared by the procedure described in Fig. 1. Portions of DNA were diluted into TEN buffer containing 0.01*M* $MgCl_2$. Eco RI restriction endonuclease was added to the samples to be restricted, and digestion was carried out for 15 minutes at 37°C. The samples were heated to 65°C for 5 minutes to dissociate the cohesive termini of the fragments, then placed on ice. The DNA was subjected to electrophoresis through a 0.7 percent agarose gel in tris-borate buffer (*4*) for 15 minutes at 40 volts, then for 3 hours at 150 volts. After the gel was stained with ethidium bromide (0.1 μg/ml) in tris-borate buffer, the bands were illuminated with ultraviolet light and photographed through a contrast filter (Ultraviolet Products J-344) and a gelatin filter (Kodak Wratten No. 16). (A) Unrestricted supercoiled RK2; (B) unrestricted supercoiled RK2trp2(pRM2); (C) unrestricted supercoiled DNA from W3110 Δ*trpE5*(RK2, pVH103); (D) unrestricted supercoiled pVH103; (E) restricted RK2; (F) restricted pRM2; (G) restricted plasmid DNA from W3110 Δ*trpE5*(RK2, pVH103); and (H) restricted pVH103.

Electrophoretic patterns of Eco RI–digested DNA are shown in Fig. 2, E to H. The restriction of pVH103 yields two fragments (*9*), the fast-migrating band corresponding to the ColE1 portion of the molecule and the other, slower band corresponding to the λtrp$^+$ fragment. Restriction of supercoiled DNA from W3110 Δ*trpE5*(RK2, pVH103) shows these two bands and, in addition, the single linear species of restricted RK2. We observed, in contrast, only two DNA fragments from restricted pRM2 DNA, one comigrating with linear RK2 and the other with the λtrp fragment of restricted pVH103. The relative amount of the λtrp fragment recovered from W3110 Δ*trpE5*-(pRM2) is less than from W3110 Δ*trpE5*-(RK2, pVH103); this would be expected if the fragment were no longer replicated as part of the relaxed ColE1 replicon (*12*). These results suggest that pRM2 was formed by the insertion of the λtrp restriction fragment at the RK2 restriction site during the restriction and ligation procedure. This conclusion is supported by examination of the sedimentation values in sucrose of the various supercoiled species. pVH103 gave the expected value of 47*S*, corresponding to a molecular weight of 22×10^6 (*9*). Since the molecular weight of the ColE1 fragment is 4.2×10^6 (*13*) the λtrp segment must be approximately 18 million. Accordingly, pRM2 should exhibit a molecular weight of 58×10^6 which is in good agreement with the molecular weight calculated from the value of 73*S* obtained for this plasmid.

We tested for the inactivation of other, nonessential genes in pRM2. The W3110 Δ*trpE5*(pRM2) strain remained fully resistant to ampicillin, kanamycin, and tetracycline. Plasmids of the P group confer sensitivity to the phages PRR1 (*14*) and PRD1 (*15*). A portion of a phage suspension was spotted onto fresh lawns of W3110 Δ*trpE5* strains carrying the plasmids pRM2, RK2, or RK2 and pVH103. All three strains remained sensitive to both phage.

W3110 Δ*trpE5*(pRM2) was fully effective as a donor in conjugation and could transfer drug resistance at a level comparable to W3110 Δ*trpE5*(pRM2) and W3110 Δ*trpE5*(RK2, pVH103) (Table 1). As expected, in the case of pRM2 all transconjugants initially selected for kanamycin resistance were also Trp$^+$. When the primary selection was for Trp-independence, then all the transconjugants tested were also resistant to kanamycin. Tryptophan independence and kanamycin resistance (*kan^r*) were not linked when W3110 Δ*trpE5*(RK2, pVH103) was the donor.

The linkage of the *trp* and *kan^r* genes in pRM2 is an expected result of their being located on the same piece of DNA, as suggested by the gel and sucrose gradient analysis. Alternatively, cotransfer of the genes could result from some intimate but noncovalent association during conjugation. To check this point, we transformed W3110 Δ*trpE5* with supercoiled DNA from both W3110 Δ*trpE5*(pRM2) and W3110 Δ*trpE5*(RK2, pVH103). Again, linkage of the *kan^r* and *trp* genes was observed for pRM2, while no linkage was observed in the transformation with RK2 and pVH103 DNA.

In summary, we have demonstrated that RK2 has a single Eco RI site, and that a stable, hybrid plasmid may be constructed in vitro by the insertion of a DNA fragment into this site. This hybrid retains self-transmissibility and all other tested characteristics of the parental plasmid. Because of the extraordinarily wide host range of RK2, hybrids constructed in this way may be used to create new genotypes in bacteria previously intractable to genetic manipulation. However, full exploitation of this system will have to await the elimination of the antibiotic resistance genes in RK2, due to the potential hazards associated with the transfer of drug resistance into novel backgrounds.

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Social Class and Frequency of XYY and XXY

Abstract. *The karyotype and paternal social class were determined for 10,348 consecutively born males. No significant difference in paternal social class was associated with the occurrence of the XYY or the XXY karyotype. This argues against the suggestion that socioeconomic factors significantly affect the frequency of the nondisjunctional events leading to these chromosome abnormalities.*

In 1965 it was reported that individuals with the XYY karyotype appeared with unexpectedly high frequency in an institution for the confinement of persons with combined mental defect and antisocial behavior (*1*). This raised the possibility that the XYY chromosome complement might be associated with deviant behavior. The interest aroused by this study resulted in a large number of investigations. The avail-

Table 1. Occurrence of XXY, XYY, and trisomy 21 among 13,751 males from a survey of consecutively born infants.

Race	Karyotype			Total tested
	XXY	XYY	Trisomy 21	
Caucasian	9*	13†	16	10,817
Non-Caucasian	2	0	4	2,756
Unclassified	0	0	0	178

*Includes one infant with a 47,XY,Xp karyotype and one 46,XY/47,XYY mosaic. †Includes one 46,-XY/47,XYY mosaic and one infant born of a non-Caucasian mother and a Caucasian father.

Table 2. Comparison of paternal social class, maternal age, and maternal racial classification for families with newborn males having various karyotypes.

Social class	Infants with indicated karyotype							
	Caucasian				Non-Caucasian			
	XY*	XXY†	XYY‡	+21	XY*	XXY	XYY	+21
	Maternal age ≤ 30 years							
I	1746	2	3	1	99	1	0	0
II	1228	0	2	2	74	0	0	0
III	1306	1	0	1	136	0	0	0
IV	1456	2	2	1	359	0	0	0
V	535	0	2	0	276	1	0	2
	Maternal age > 30 years							
I	1140	3	2	5	76	0	0	0
II	603	0	0	3	27	0	0	0
III	489	0	0	1	38	0	0	0
IV	428	1	1	1	108	0	0	0
V	145	0	0	1	79	0	0	0

*Includes 23 infants with autosomal rearrangements, 5 with a "supernumerary," small, metacentric chromosome and 6 with 46,X,inv(Yp+q-). †Includes one infant with a 47,XY,*Xp*- karyotype and one with 46,XY/47,XXY mosaicism. ‡Includes one infant with 46,XY/47,XYY mosaicism.

able data were judged consistent with the original suggestion that individuals with the XYY karyotype are found in certain behavioral settings with a greater frequency than would be predicted from the incidence of this karyotype among newborn males (*2*). It should be emphasized that only a small fraction of the total number of XYY individuals ever appear in a mental-penal institutional setting (*3*).

There is as yet insufficient published information to determine whether the XYY karyotype is associated with a behavioral pattern that increases the chance for some affected individuals to run afoul of society's rules. In the absence of such information, other explanations for the observed concentration in mental-penal settings are at least equally valid. It has been suggested, for example, that the XYY karyotype might occur more commonly among the progeny of lower socioeconomic groups and that the concomitant social and economic factors might account for their incarceration (*4*). However, in chromatin and chromosome surveys of newborns conducted in Edinburgh, no difference in social class distribution was found between the families of infants with sex chromosome abnormalities (XYY, XXY and XXX) and the families of all live births in Scotland during "the middle year" of each survey (*5*).

In the study described here, social class information was obtained (where it was available) for the fathers of all infants tested (both chromosomally normal and chromosomally abnormal infants). A more critical comparison is thus possible than could be accomplished in the Edinburgh survey. A total of 14,206 male newborns surviving beyond 24 hours was recorded by the hospital participating in the survey during the period covered by this report. A karyotype was obtained on 13,751 (96.8 percent) of these infants (Table 1) with procedures described previously (*6*). In the remaining 3.2 percent, a karyotype was not obtained either because of technical difficulties or because the parents declined to participate in the study.

Infants were assigned to social classes on the basis of the father's educational and occupational status as recorded in the mother's hospital chart. The classes were determined according to published procedures (*7*). Fathers in class V are considered least advantaged in terms of income and education; those in class I are most advantaged. Because social class is correlated with parental age and ethnic group, comparisons were made only among infants for whom all three of these characteristics were known (75.6 percent of the karyotyped population). In most instances where the infants were excluded, social class could not be calculated, either because the mother was single (so that the father's occupational and educational status was unavailable) or because the father was a full-time college student (so that no occupational status could be assigned). One XYY infant was born to a single mother and therefore was not assigned to a social class.

The number of chromosomally abnormal infants in the non-Caucasian group was very small (Table 2) and no attempt was made to analyze further the data for this group. Among Caucasians, trisomy 21 infants occurred with significantly greater frequency among the older mothers ($P < .01$), as would be expected from prior experience (*8*). The frequency of XYY and XXY karyotypes in infants born to women over 30 years of age was not significantly different from that for women 30 years of age and under. The average maternal age at delivery for the XYY group was 27.1 years; for the XXY group, 27.5 years; and for the total karyotyped population, 26.9 years. Comparisons of relative frequency within social class groups was therefore made for XYY and XXY without regard to maternal age.

The practice of Ratcliff *et al.* (*5*) was followed and classes I, II, and III were pooled, as were classes IV and V. The frequencies of the XYY karyotype in these two social class pools are not significantly different ($.5 > P < .3$). The XXY frequencies in the two social class pools likewise are not significantly different. The results of this survey are thus in agreement with the Edinburgh survey (*5*) in showing no significant effect of social class upon the frequency of XYY, and of XXY, among newborn males.

We are aware of only one study which suggested that socioeconomic factors might affect the frequency of nondisjunctional events (*9*). However, after further data were collected, the same investigators judged their findings "not conclusive" (*10*). Although social and economic factors may play a role in the confinement of those few XYY individuals who appear in mental-penal settings, there is no evidence that these factors exert their influence primarily by affecting the frequency of nondisjunction.

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