and females (two samples) was digested with Pronase for 6 to 7 hours at 37°C. The resulting solution elicited the appropriate responses.

4) Polysaccharides in four male and two female water samples were hydrolyzed in 1*M* HCl at 100°C for 5 hours in sealed vessels. The hydrolyzate was neutralized with KOH to a *p*H 7 to 7.2. The products of the hydrolysis when tested on six crayfish gave negative results—there was neither aggressive behavior elicited by the hydrolyzate from male water, nor was there courtship (or any overt change in behavior) elicited by the hydrolyzate from female water. The experimental animals responded to untreated male and female water appropriately.

From the above tests, we can infer that the chemical factor is a carbohydrate, although we have not yet identified it.

The response of male antennules to male and female water were studied electrophysiologically. Antennulogram recordings (*5*) were made from ablated antennular flagella (Fig. 1). The eight male subjects responded similarly. The characteristic shape of the wave (Fig. 1c) resulting from stimulation with both male and female water was also seen as a response to male or female water treated with Pronase (Fig. 1d). The responses to the acid hydrolyzates from male and female water (Fig. 1e) and to distilled water treated with acid (Fig. 1f) are probably responses to the chemical treatment of the water. The outer flagella of the antennules did not respond to test water, although the basis for the differential responses of the inner and outer flagella is not yet known.

Among the ways the sexes recognize and respond to one another are through differences in morphology (as in sexually dimorphic species), behavior (posture, sound production), or chemicals (pheromones). A combination of these may be found in the same species, as in the genus *Uca*, in which morphology and behavior (sound production and posture) are used (*6*).

In crayfish it has been assumed that the male recognizes the female only through the submissive behavior of the latter (*7*). In species without clear sexual dimorphism, it is extremely difficult to investigate the features peculiar to the male that cause the female to adopt a submissive posture during an encounter. There must be other cues involved, especially since these animals are nocturnal. The presence of a sex pheromone is an obvious sexual identification mechanism.

The possible presence of a sex pheromone has been reported for several crustaceans (*3, 8, 9*), although experimental verification has been found for only a few (*10*). Prior to copulation, the male of a number

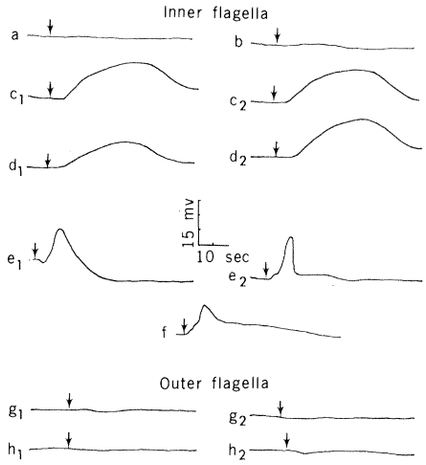

Fig. 1. Representative electroantennulogram records from the inner and outer antennular flagella. Arrows indicate the introduction of stimulus water. The solutions used were: a, distilled water; b, aged tap water; c_1, normal water from females; c_2, normal water from males; d_1, Pronase-treated female water; d_2, Pronase-treated male water; e_1, acid-hydrolyzed female water; e_2, acid-hydrolyzed male water; f, acid-treated distilled water; g_1, distilled water; g_2, aged tap water; h_1, normal female water; h_2, normal male water.

of species can detect the female before she molts or just afterward at a distance (*3, 9*). In crayfish, the female does not molt just before copulation.

While a chemoreceptive function of the antennules of crayfish during feeding has been demonstrated in some species (*11*), *P. clarkii* does not rely on antennules for distance chemoreception during feeding (*12*). Presumably the structures are used in contexts other than feeding, and one such context is intraspecific communication.

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Molecular Vehicle Properties of the Broad Host Range Plasmid RK2

Abstract. *The plasmid RK2 is stably maintained in a broad range of gram-negative bacteria. The RK2 DNA has a single Eco RI restriction site. The insertion of a DNA fragment into this site does not interfere with either plasmid maintenance or self-transmissibility. Because RK2 has a broad host range, it should be useful for the construction in vitro of hybrid plasmid molecules capable of being established by conjugal transfer or transformation into many genera of gram-negative organisms.*

Bacterial plasmids of compatibility class P may conjugally transfer into many different host genera of gram-negative bacteria in which they are stably maintained (*1, 2*). These plasmids are therefore useful for introducing new genes into these organisms. For this reason, we examined the molecular vehicle properties of the P plasmid RK2, in the hope that it may be used to construct in vitro new plasmids that would retain the broad host range of the parental molecule.

The plasmid RK2 specifies resistance to the antibiotics ampicillin, kanamycin, and tetracycline (*2*). Supercoiled, tritium-labeled RK2 DNA was purified by preparative scale ethidium bromide–cesium chloride equilibrium centrifugation of "cleared" (*3*) lysates of JC411(RK2), a strain of *Escherichia coli* K-12 carrying the plasmid. The DNA was exhaustively digested by the restriction endonuclease Eco RI (*4*) and analyzed both by sedimentation through a neutral sucrose gradient and by agarose gel electrophoresis. Undigested RK2 DNA is homogeneous in size (Figs. 1A and 2A), with $s_{20,w}$ values of 60 and 43, corresponding to the covalently closed and nicked circular forms of the plasmid, and a calculated molecular weight of 40×10^6 (*5, 6*). Restricted RK2 DNA migrated at a single position on the

gel (Fig. 2E), and has a sedimentation value in a sucrose gradient (Fig. 1B) expected of a linear molecule with a molecular weight of 40 million (*7*). Therefore, RK2 is cleaved only once by the Eco RI restriction enzyme, a result we obtained for this plasmid isolated from several strains of *E. coli* K12. While this work was in progress another P plasmid, RP4, also has been reported to possess a single site sensitive to Eco RI (*8*).

To determine whether the Eco RI cleavage site is in a region essential for the maintenance of RK2, we constructed a hybrid plasmid in vitro. The plasmid pVH103 is a ColE1-trp hybrid that was constructed by the insertion of an Eco RI restriction fragment of λ*trpED10T* DNA containing the promoter, operator, and genes *E* and *D* of the tryptophan operon of *E. coli*, into the Eco RI site of the ColE1 molecule (*9*). Purified RK2 and pVH103 DNA were mixed and digested with Eco RI for 15 minutes as previously described (*4*), and the product of the digestion was further incubated with bacteriophage T4 ligase (Miles). Incubation with the ligase was carried out in 66 m*M* tris (*p*H 7.5), 33 m*M* NaCl, 15 m*M* $MgCl_2$, 10 m*M* dithiothreitol, 6 m*M* adenosine triphosphate (ATP), and 3.3 m*M* EDTA at 15°C for 2 hours. The resulting mixture was used directly to transform the *E. coli* strain W3110 Δ*trpE5*, according to the procedure of Cohen, Chang, and Hsu (*10*) modified as described (*11*). Cells were plated onto medium lacking tryptophan (trp) to select for Trp^+ transformants, and emerging colonies were screened for drug resistance and sensitivity to colicin E1 in order to detect hybrid molecules which do not have the ColE1 portion of the pVH103 molecule. A single drug-resistant Trp^+ colony that was sensitive to colicin E1 was designated W3110 Δ*trpE5*(RK2trp2) and saved for further study. For a control, we also analyzed W3110 Δ*trpE5*(RK2, pVH103), prepared by transforming W3110 Δ*trpE5*(RK2) with unrestricted pVH103 DNA.

The gel electrophoretic pattern of undigested, supercoiled DNA is shown in Fig. 2, A to D. As expected, we observed two migrating species for the supercoiled DNA of W3110 Δ*trpE5*(RK2, pVH103), one comigrating with RK2 DNA, and the other with pVH103. However, supercoiled DNA from W3110 Δ*trpE5*(RK2trp2) migrated as a single band. We confirmed by neutral sucrose gradient analysis that only one species of supercoiled DNA was obtained from W3110 Δ*trpE5*(RK2trp2) and that this sedimented more rapidly than the supercoiled DNA from W3110 Δ*trpE5*(RK2, pVH103) (Fig. 1, C and D). The RK2trp2 plasmid was designated pRM2.

Table 1. Conjugal transfer of plasmids. Plasmids were transferred from the various donor strains to the recipient W3110 Δ*trpE5 nal*^{r} by conjugal mating between log-phase cultures of cells in L broth at 37°C for 60 minutes. The donor to recipient cell ratio was 1 : 10. The mating mixtures were plated on L broth containing kanamycin (25 μg/ml) plus nalidixic acid (25 μg/ml) and on a minimal salts medium (*16*) containing glucose (0.4 percent), thiamine (10 μg/ml), casamino acids (0.5 percent), and nalidixic acid (25 μg/ml). The numbers in parentheses indicate the number of clones examined for the Trp^+ and Kan^r characteristics.

Donor strain	Frequency of transfer per donor cell		Percent Kan^r clones that are Trp^+	Percent Trp^+ clones that are Kan^r
	kan^r	*trp*$^+$		
W3110 Δ*trpE5*(RK2)	2.6×10^{-5}	$<10^{-7}$	0(30)	
W3110 Δ*trpE5*(pRM2)	8.0×10^{-6}	3.0×10^{-4}	100(30)	100(45)
W3110 Δ*trpE5*(RK2, pVH103)	1.8×10^{-5}	1.1×10^{-4}	72(97)	49(93)

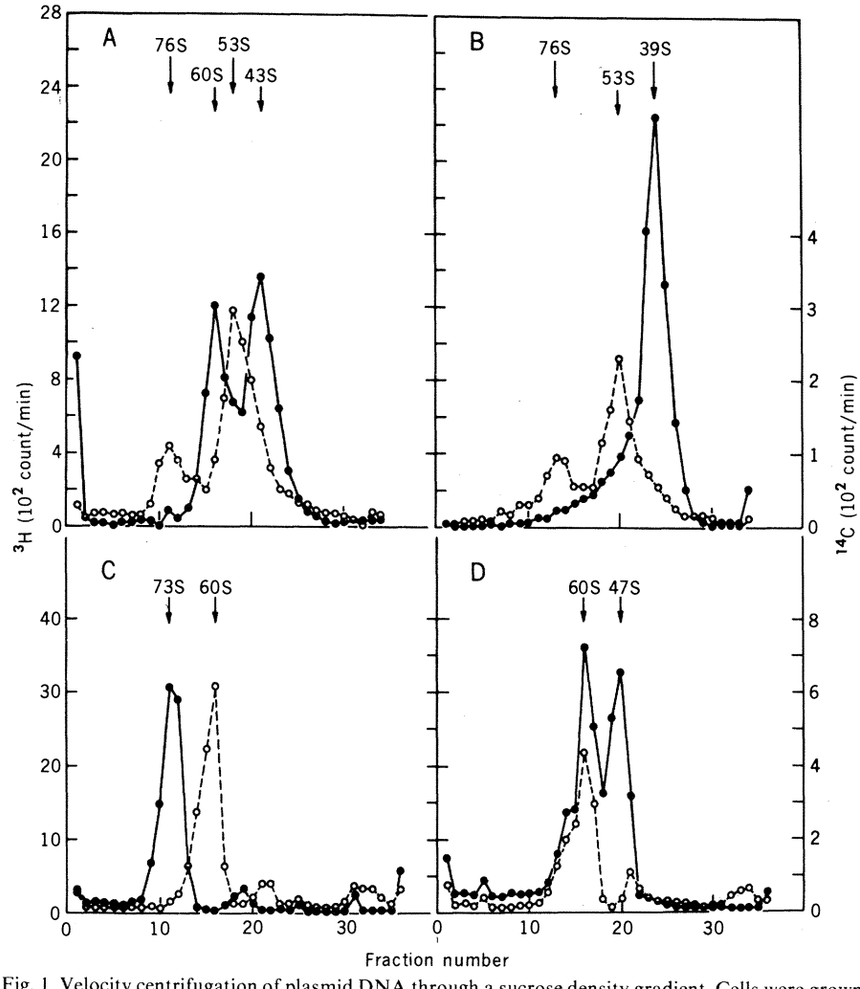

Fig. 1. Velocity centrifugation of plasmid DNA through a sucrose density gradient. Cells were grown in phosphate-buffered minimal medium (*16*) containing [methyl-^{3}H]thymidine (2 μc/ml) or [^{14}C]thymine (0.1 μc/ml); the cultures were lysed in mid-log phase of growth (approximately 5×10^8 cell/ml). Plasmid DNA was prepared by equilibrium centrifugation of cell lysates in an ethidium bromide–cesium chloride gradient. The supercoiled DNA, banding at a denser position in the gradient, was drawn off with a needle, the dye was removed with isopropanol saturated with CsCl, and the preparations were dialyzed against TEN buffer (0.10*M* tris, 0.005*M* EDTA, 0.05*M* NaCl, *p*H 7.5) to remove the CsCl. The DNA preparations were precipitated by ethanol after the addition of yeast transfer RNA as carrier to 50 μg/ml, and resuspended in 50 to 100 μl of TEN prior to use. Restriction by Eco RI and purification of the enzyme was according to the procedure of Green *et al.* (*4*). Centrifugation was at 189,000*g* through a sucrose density gradient (5 to 20 percent), containing 0.05*M* tris, 0.005*M* EDTA, and 0.5*M* NaCl (*p*H 7) for 60 minutes at 15°C. Fractions were collected directly onto filter paper and precipitated with 5 percent trichloroacetic acid, and the radioactivity was counted. The sedimentation values of supercoiled and open-circular R100 were calculated (*5*) from a molecular weight of 67 million (*17*). (A) Unrestricted supercoiled and open-circular RK2 ^{3}H-labeled DNA (●——●); supercoiled and open-circular R100 ^{14}C-labeled DNA marker (○---○). (B) Eco RI restricted RK2 ^{3}H-labeled DNA (●——●); R100 ^{14}C-labeled DNA (○----○). (C) Supercoiled ^{3}H-labeled DNA from W3110 Δ*trpE5*(pRM2) (●——●); RK2 ^{14}C-labeled DNA (○----○). (D) Supercoiled ^{3}H-labeled DNA from W3110 Δ*trpE5*(RK2, pVH103) (●——●); RK2 ^{14}C-labeled DNA (○----○).

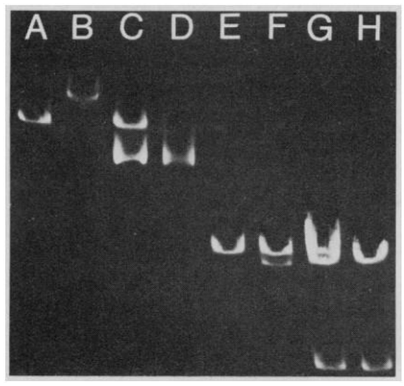

Fig. 2. Agarose slab gel electrophoresis of supercoiled and Eco RI-digested DNA. Plasmid DNA was prepared by the procedure described in Fig. 1. Portions of DNA were diluted into TEN buffer containing 0.01*M* $MgCl_2$. Eco RI restriction endonuclease was added to the samples to be restricted, and digestion 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-