The vole *A. scherman exitus*, $2n = 36$ is characterized by a most unusual sex chromosome situation as was determined by autoradiographic studies.

The X comprises 6.80% of $(nA + X)$; it is of a complex composite type. The functional part is divided into 2 regionally divided sections, the short arm and the distal third of the long arm. The proximal third of the long arm is apparently structurally heterochromatic, the intermediate third is not late replicating, it may be a euchromatic segment, remaining so in the allocyclic X as well.

The Y-chromosome is unusually long ($^2/_3$ of the X), and completely structurally heterochromatic. Thus, the male possesses more structural heterochromatin than the female sex[12].

Zusammenfassung. Autoradiographische Untersuchungen mit H^3-Thymidin deckten bei der Wühlmaus *A. scherman exitus* ($2n = 36$) eine ungewöhnliche Geschlechtschromosomensituation auf. Das X-Chromosom misst 6,8% von $(nA + X)$. Sein funktioneller Anteil ist zweigeteilt, daneben verfügt es über einen strukturell heterochromatischen und einen stets euchromatisch bleibenden Anteil. Das Y ist für ein Säugetier aussergewöhnlich gross ($^2/_3$ des X).

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Studies on r_{II} Region of T_2-Phage

Considerable efforts have been made over the last decade and a half to analyse more incisively the structure and function of the gene. Many reports[1-3] have appeared about the divisibility of the gene and its subunit structure, thus demolishing the concept of the gene being the ultimate unit of heredity.

By selecting a suitably marked microorganism as an experimental tool in the study of recombination, one can subdivide the gene almost down to a single nucleotide pair[3-4]. While studying the internal structure of the gene, BENZER was able to elucidate the unit of function of the r_{II} region of the T_4 phage and has shown that r_{II} mutants of this phage fall into 2 groups r_{II} A and r_{II} B[3-6].

We report here a similar genetic analysis using mutants falling in the r_{II} region of T_2 L (LURIA, 1945) phage in order to determine to what extent the findings concerning functional unit within the r_{II} region of T_4 phage could be generalized to the r_{II} region of T_2 phage.

The strains used were *E. coli* B, *E. coli* $K_{12}S$ and *E. coli* K_{12} (λ) and bacteriophage T_2 L (wild type) which were obtained from the Microbial Genetics Research Unit, Hammersmith Hospital, London W.12.

The different cultures of *E. coli* were maintained on nutrient agar slants; phage stocks were maintained in M_9 medium[7] and stored at 4 °C. Chloroform was added to act as a preservative.

T_2 L phages were used for the isolation of '*r*' mutants and *E. coli* B as a host bacterium. 5-Bromouracil and acridine orange were used as mutagens.

Mutants in the '*r*' region were isolated by the method of LITMAN and PARDEE[7]. 5-Bromouracil (50 μg/ml) and acridine orange (4 μg/ml) were used for the isolation of mutants[8]. The percentage of mutagenesis was calculated under standard conditions for both the cases. Each mutant was isolated from a separate plaque and freed from contaminating wild type particles by replating. The isolated '*r*' mutants were further differentiated according to BENZER's scheme[9]. Some of the r_{II} mutants had a tendency to revert spontaneously to wild type, so only stable h^+r mutants[10] were selected for further study.

All T_2 r_{II} mutants were tested in possible pairs for the cistrons test as described in the case of T_4 phage[9]. The recombination test was carried out by the method of HERSHEY and ROTMAN[11] by infecting a culture of *E. coli* B with equal multiplicities of each type and incubating at 37 °C for 90 min. Recombinants were detected on *E. coli* K_{12} (λ).

The results in Table I show that acridine orange (AO) acts as a better mutagenic agent than 5-bromouracil (5-BU). The mutagenic activity of AO being $1^1/_2$ times that of 5-BU.

Table I. Mutagenic effect of 5-bromouracil and acridine orange

Mutagens	Plaque counts obtained		% Mutagenesis
	wild	mutants	
5-Bromouracil	148	190	56
	109	106	50
Acridine orange	51	149	74
	49	200	79

E. coli B grown in supplemented M_9 medium to 10^8 cells/ml. 10^8 particles of T_2 L phage were added to it and aerated for 120 min. Mutants were isolated on B + B/2L mixed indicator bacteria (2:1).

[1] G. PONTECORVO, Adv. Enzymol. *13*, 121 (1952).
[2] G. PONTECORVO, *Trends in Genetic Analysis* (Columbia University Press, New York 1958).
[3] S. BENZER, Proc. natn. Acad. Sci., U.S.A. *45*, 1607 (1959).
[4] S. BENZER, Proc. natn. Acad. Sci., U.S.A. *45*, 403 (1961).
[5] S. BENZER, in *The Chemical Basis of Heredity* (Ed. W. D. MCELORY and E. GLASS; The Johns Hopkins Press, Baltimore 1957).
[6] S. BENZER, Proc. natn. Acad. Sci., U.S.A. *41*, 344 (1955).
[7] R. M. LITMAN and A. B. PARDEE, Nature *178*, 529 (1966).
[8] R. DEMARS, Nature *172*, 964 (1963).
[9] W. HAYES, *The Genetics of Bacteria and Their Viruses* (Blackwell Scientific Publications, Oxford 1964).
[10] G. STENT, *Molecular Biology of Bacterial Viruses* (W. H. Freeman and Company, San Francisco and London 1963).
[11] A. D. HERSHEY and R. ROTMAN, Genetics *34*, 44 (1949).

In addition mutants isolated with AO showed less reversion than with 5-BU (Table II). This suggests that in BU mutants only a single base pair is altered which has the possibility of easy reversal, whereas in AO mutants the mutation is effected by insertion or deletion where the reversion would be less.

Table II. Reversion rate of mutants used in the crosses

Mutant No.	Reversion frequency with 5-BU	Mutant No.	Reversion frequency with AO
51	0.07	109	–
52	0.02	110	–
53	0.06	111	0.001
54	0.01	112	–
55	0.02	113	–
56	0.01	114	–
		115	–
		210	–
		211	0.001
		212	0.001
		213	–
		214	0.002
		215	–

Reversion rate was determined by plating 10^5 particles on *E. coli* K-12 (λ).

Table III. Recombination frequencies in acridine orange mutants

Cross between mutant No.	% Recombination frequency
210 × 113	0.105
113 × 109	0.075
113 × 215	0.130

Crosses between 2 mutants were made by infecting a culture of *E. coli* B with equal multiplicities of each type. Incubation was at 37 °C for 90 min and recombinants were detected on *E. coli* K_{12} (λ).

None of the mutant pairs used showed lysis when complementation tests were done, indicating the presence of only one functional unit in the case of the r_{II} region of the T_2 L phage. This correlates with the work of STREISINGER and FRANKLIN[12], who found only one cistron in the case of the '*h*' region of the T_2 phage.

Tests carried out for the detection of 3 cistrons yielded negative results. This suggests the possibility of one cistron, and this was substantiated by the results obtained by recombination between different pairs of AO mutants (Table III).

These results indicate that the r_{II} region in the T_2 L phage seems to act as a single unit.

Zusammenfassung. Untersuchungen der r_{II}-Region des T_2-Bakteriophagen. Es wurde eine genetische Analyse der r_{II}-Region des T_2-Bakteriophagen durchgeführt zur Feststellung, ob die Befunde, die sich auf die Funktionsteile innerhalb der r_{II}-Region des T_4-Phagen beziehen, in bezug auf die r_{II}-Region des T_2-Phagen verallgemeinert werden können. Die Mutanten wurden mit Hilfe von 5-Bromouracil und Akridineorange isoliert und die h^+r-Mutanten nach demselben Schema wie für T_4-Phagen weiter differenziert. Sechzig ausgewählte Mutanten wurden nach funktionaler Identität geprüft, was zur Feststellung führte, dass die gesamte Region als eine Einheit funktioniert.

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Department of Microbiology, M.S. University of Baroda, Baroda-2 (India), 9 October 1967.

[12] G. STREISINGER and N. C. FRANKLIN, Cold Spring Harb. Symp. quant. Biol. *21*, 103 (1956).

Initial Cytotaxonomic Data on Certain Families of Amphibious *Anura* (Diplasiocoela, after NOBLE)

The phyletic relationships between the families of *Anura* that NOBLE[1] includes in the sub-order of Diplasiocoela, or Ranidae, Hyperolidae (= Polypedatidae = Rhacophoridae) and Microhylidae (= Brevicipitidae), are still a matter of discussion. To these 3 families, certain authors add that of the Phrynomeridae, created by PARKER[2,3] for the single genus *Phrynomerus*, with about 5 species, which differs from the typical Microhylidae in the presence of intercalary phalanges, which are absent in the latter. Many systematicians[4], however, maintain that the differences existing between the Microhylidae and *Phrynomerus* are of no very great taxonomic value and assign this genus to a sub-family of the Microhylidae (Phrynomerinae).

The most important theories on the phyletic relationships between the above-mentioned families are essentially 3 in number. According to the first of these theories, largely attributable to NOBLE and taken up by various authors, the Ranidae constitute the ancestral stock from which were differentiated first the Microhylidae (including *Phrynomerus*) and later the Hyperolidae. According to PARKER's theory, accepted by various other authors[5], the Microhylidae constitute an initial differentiation from a ranoid stock, from which there later derived the Ranidae in the Holarctic realm and the Hyperolidae in

[1] G. K. NOBLE, *Biology of the Amphibia* (Dover Inc., New York 1931).
[2] H. W. PARKER, Archo zool. ital. *16*, 1239 (1932).
[3] H. W. PARKER, *Frogs of the Family Microhylidae* (British Museum, London 1934).
[4] M. K. HECHT, Syst. Zool. *12*, 20 (1963).
[5] I. GRIFFITHS, Biol. Rev. *38*, 241 (1963).