

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 mu-

tants the mutation is effected by insertion or deletion where the reversion would be less.

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 FRANKLIN¹², 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 Funktions-teile 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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Baroda-2 (India), 9 October 1967.

¹² G. STREISINGER and N. C. FRANKLIN, Cold Spring Harb. Symp. quant. Biol. 27, 103 (1956).

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 10^6 particles on *E. coli* K-12 (λ).

Table III. Recombination frequencies in acridine orange mutants

Cross between mutant No.	% Recombination frequency
210 \times 113	0.105
113 \times 109	0.075
113 \times 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* K₁₂ (λ).

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

The phyletic relationships between the families of *Anura* that NOBLE¹ 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 systematists⁴, 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 authors⁵, 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).

the Ethiopian region; the Phrynomeridae would constitute a collateral branch derived from the Microhylidae. According to a third theory, prevalent among those authors^{4,6,7} that attribute great value to the systematization of the larval forms of the *Anura* as established by ORTON⁸, the Microhylidae, including *Phrynomerus*, constitute one of the most primitive groups of *Anura*, which evolved separately from the Ranidae.

To make a contribution to the classification of the *Anura* of these families, I have studied the chromosome set of certain species of the various families; these are: *Breviceps gibbosus* (Africa): $2n = 24$ and *Kaloula pulchra* (Asia): $2n = 28$ (Microhylidae); *Phrynomerus bifasciatus* (Africa): $2n = 26$ (Phrynomeridae or Microhylidae); various European species of the genus *Rana* (all with $2n = 26$, except *R. arvalis*, with $2n = 24$) and *Mantella aurantiaca* (Madagascar): $2n = 26$ (Ranidae)⁹; *Hyperolius argentivittis* (Africa): $2n = 26$ and *Kassina senegalensis* (Africa): $2n = 24$ (Hyperolidae).

Figures 1-5 show mitotic metaphases of some of the species studied, prepared by means of the technique of squashing.

In none of the species studied are morphologically recognizable sex chromosomes present. The meiotic bivalents of the male line normally show 2 terminal

chiasmata each (Figures 6-8), save for a few exceptions of which I shall speak in other works.

The morphological relationships between the sets of the species studied are summarized in Figure 9, which shows a comparison between the haploid karyotypes of species of the various families, constructed by using a single homologue of each pair of a mitotic figure of each species.

From an examination of this Figure, certain observations may be made.

The 2 Microhylidae, belonging to 2 different subfamilies, differ widely in their karyotypes, which do not reveal any characteristic of karyological primitiveness, such as the presence of acrocentric chromosomes or microchromosomes, which is, on the other hand, proper to the set of species belonging to the older families of *Anura* (Ascaphidae, Discoglossidae, Pipidae¹⁰⁻¹²).

⁸ R. F. INGER, *Evolution* 21, 369 (1967).

⁷ R. LAURENT, personal communication.

⁶ G. L. ORTON, *Syst. Zool.* 6, 79 (1957).

⁹ A. MORESCALCHI, *Caryologia* 20, 65 (1967).

¹⁰ A. MORESCALCHI, *Att. Soc. pelorit. Sci. fis. mat. nat.* 13, 23 (1967).

¹¹ A. MORESCALCHI, *Riv. Biol.* 59, 3 (1966).

¹² A. MORESCALCHI, *Experientia* 23, 1071 (1967).

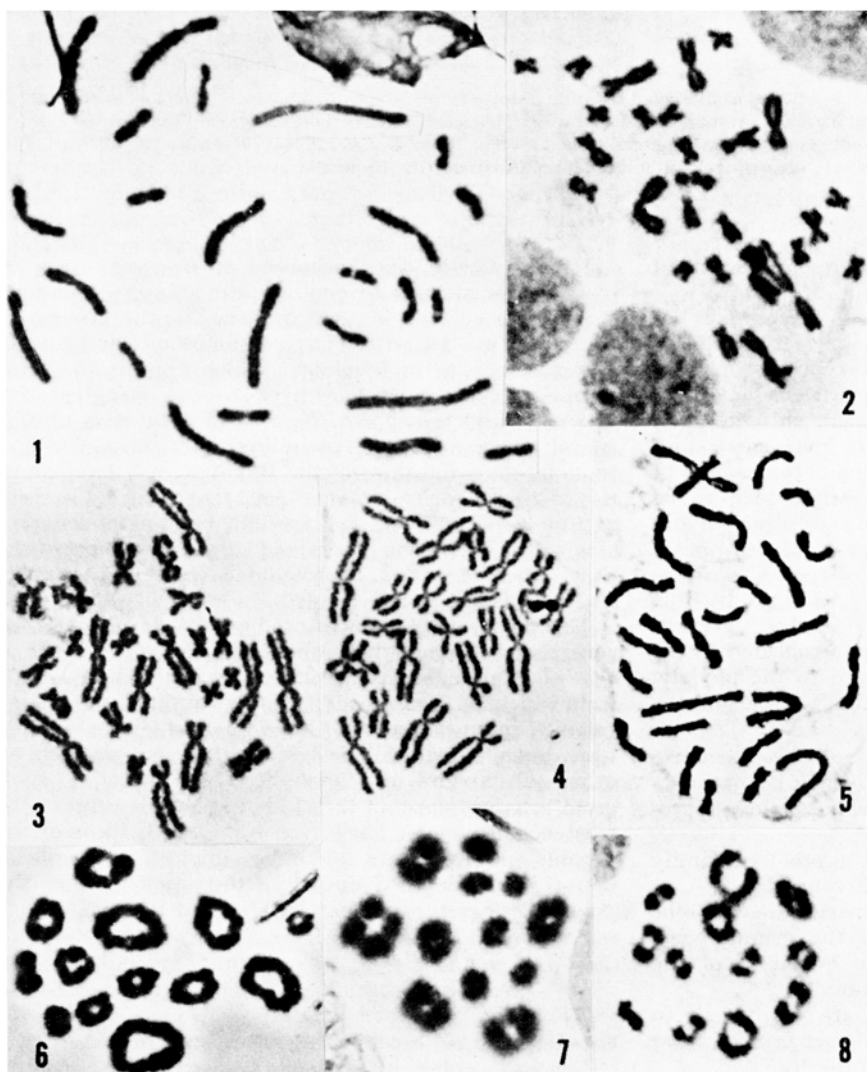


Fig. 1. Spermatogonial metaphase of *Breviceps*. $\times 2000$.

Figs. 2-5. Intestinal metaphases of *Kaloula* ♀ (Fig. 2), *Phrynomerus* ♂ (Fig. 3), *Kassina* ♂ (Fig. 4), *Hyperolius* ♂ (Fig. 5) $\times 2000$.

Figs. 6-8. Spermatocyte metaphases I of: *Phrynomerus* (Fig. 6), *Breviceps* (Fig. 7), *Kassina* (Fig. 8) $\times 2000$.

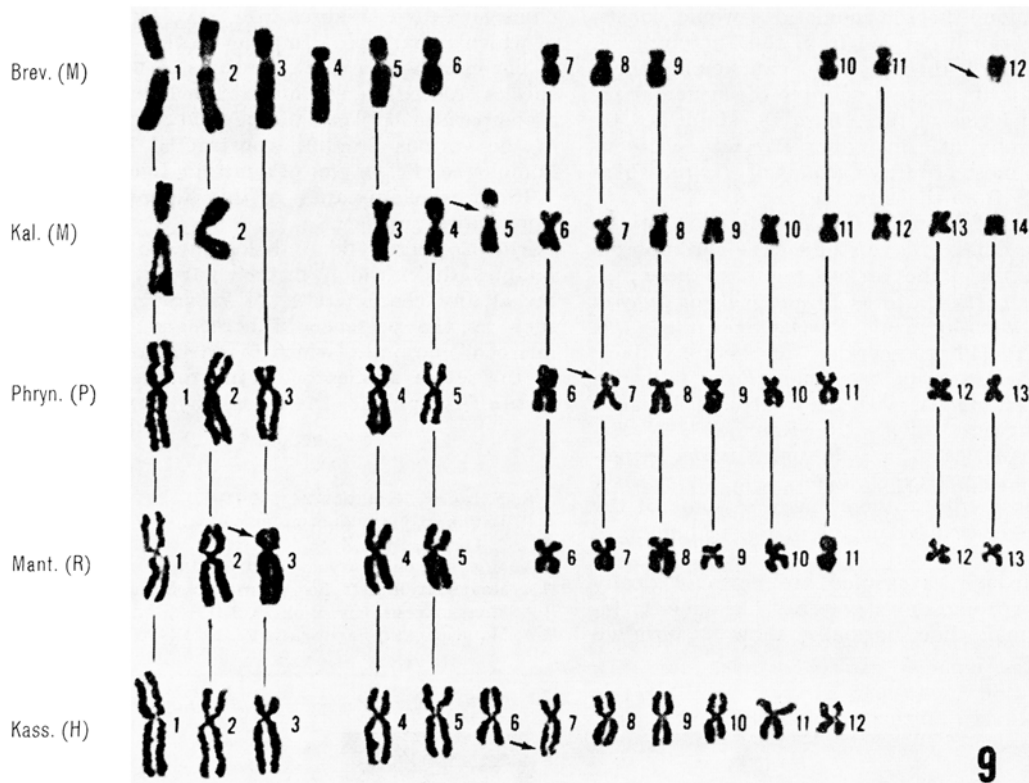


Fig. 9. Haploid karyotypes of some of the species studied, constructed by using a single chromosome of each pair of homologues of a mitotic metaphase. The lines joining chromosomes of various karyotypes indicate a morphological affinity between them; the arrows indicate the chromosomes most frequently provided with heterochromatic areas in each species. Brev., *Breviceps*; Kal., *Kaloula*; (M), Microhylidae; Phryn., *Phrynomerus*; (P), Phrynomeridae; Mant., *Mantella*; (R), Ranidae; Kass., *Kassina*; (H), Hyperolidae.

The karyotype of *Phrynomerus* close resembles that of certain Ranidae of the genera *Rana* and *Mantella*⁹.

The karyotype of *Hyperolius* ($n = 13$) possesses an additional small metacentric chromosome as compared with that of *Kassina* ($n = 12$); the karyotypes of the 2 Hyperolidae differ from the general karyotype of the Ranidae in the morphology of the small chromosomes, which, in the former, are nearly all metacentric and relatively larger than the corresponding chromosomes of the Ranidae. The 2 families are therefore seen to be karyologically well differentiated from each other; in any case, for further cytotaxonomic observations, it appears necessary to study a greater number of species, particularly of other geographical areas.

The karyological reports so far described, even if scanty, may provide certain data for reconsidering the various theories formulated with regard to the phyletic relationships between the families of the Ranidae and the Microhylidae.

In this respect, the data collected on *Phrynomerus* are of the greatest interest: this Anuran, which is anatomically a specialized type of Microhylidae, has a close karyological affinity with the Ranidae.

This fact may perhaps constitute a proof of family relationships between Ranidae and Microhylidae.

In this case, the karyological data would appear to be in disagreement with the theory of the primitiveness (previously mentioned) and separate evolution of the Microhylidae as compared with the Ranidae^{4,6,8}.

However, the data just described are insufficient to clarify the question of the greater or lesser primitiveness of the Microhylidae as compared with the Ranidae.

Hypothetically, the karyotype of *Breviceps* ($n = 12$) may be traced back to one of a ranoid type, assuming that it has evolved in the same way as the genome of *R. arvalis* ($n = 12$, with 6 large chromosomes and 6 small chromosomes, as in *Breviceps*), which appears to derive from the generalized karyotype of the Ranidae with $n = 13$ ⁹; the karyotype of *Kaloula* ($n = 14$) may also be traced to a ranoid type, assuming that 1 large chromosome (such as the third of the Ranidae) may have given rise to 2 smaller chromosomes (such as the fifth and sixth of *Kaloula*). However, the possibility of the inverse process, or the derivation of a ranoid karyotype from 1 of the karyotypes of the 2 Microhylidae (especially that of *Kaloula*), is not to be excluded.

The theory may be advanced that a karyotype of a generalized form, perhaps similar to that of *Kaloula* or that of *Rana*, may have been present in the ranoid stock from which, as is assumed by various authors, the 2 large families in question may have evolved. This working hypothesis, which will be tested with new research on other sub-families of Microhylidae and Ranidae, is of great interest, since, in the case of a confirmation of the existence of a single karyotype from which those of the various species of the 2 families might have evolved, doubt would be shed upon the theories regarding the polyphyletic nature of Microhylidae and Ranidae^{6,13}.

¹³ Research carried out through a contribution from the C.N.R. (Genetics Enterprise).

Riassunto. Lo studio carilogico di alcune specie delle famiglie dei Microhylidae, Phrynomeridae, Ranidae e Hyperolidae, le cui relazioni filetiche sono tutt'ora assai discusse, fornisce dati a favore dell'ipotesi di rapporti di parentela tra i Microhylidae (i quali non appaiono carilogicamente primitivi) e i Ranidae, in quanto *Phrynomerus*, che fenotipicamente è assai vicino ai Microhylidae e da vari autori è considerato un membro di questa

famiglia, ha un cariotipo assai somigliante a quello dei Ranidae; gli Hyperolidae studiati sono carilogicamente abbastanza differenziati da quest'ultima famiglia.

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Phototactic Choice Between Two White Light Sources of Various Intensity in Blowfly, *Calliphora erythrocephala* Meig.

A group response of freely moving invertebrates to 2 white light sources of varying relative intensities seems to be presently a neglected problem in the study of animal behaviour. We can hardly say whether it has ever been thoroughly studied at all. A single paper on honeybees¹ may be considered the bulk of the literature on the problem. A formula put forward in it:

$$\log R = m \log E + b \quad (1)$$

(where E is the ratio of the intensity of the variable white light to that of the constant, standard white light, R the ratio of the number of bees attracted by the constant light, m the tangent of the angle of inclination, and b a constant) claimed for about 35 years to describe the fate of insect population in the Y maze under the influence of 2 lights of various intensity. An attempt to verify this hypothesis on house-flies, made recently by the senior author², failed to confirm it unequivocally. Those results, however, provided no explanation for another mathematical description of the behaviour of flies.

In the light of this, it seems to be justified to present the results obtained by one of us, recently complemented by the senior author, which concern a similar problem in blowflies.

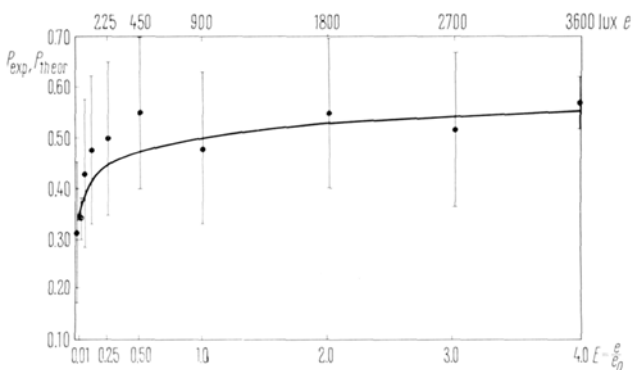
Material and methods. The applied method was the same as in the paper of CHMURZYŃSKI². A mixed population of both sexes of dark-adapted blowflies of the 'wild strain' were allowed to penetrate a wooden Y maze illuminated from the ends of the 2 arms by approximately parallel white light beams coming through water heat-filters from 50 W 12 V C. Zeiss (Jena) bulbs. Intensity of a standard light at the distant end of one arm amounted to 900 lux (e_0), a relative intensity of the other light assumed values, E , within the limits from 1/100 to 4, as presented in the Table; (e are corresponding absolute values of illumination of variable light). After each 10 min run, the lights were switched off, and the flies collected from the ends of the arms, and counted. Then the experiment was resumed. The procedure was repeated until all the flies made choice between the 2 arms of the maze. Each pair of lights was tested at least twice, each time with another group of flies, the mutual position of lights being changed. All the experiments were performed in similar conditions as to the age of flies, the time of day, temperature, humidity, and so on.

Results. The numbers of flies attracted to the experimental lights, n , in all experiments are presented in the Table; N are the numbers of flies used for each test. Respective proportions, P_{exp} , and the ratios, R_{exp} , of the flies approaching the variable light are also given. These data indicate that the greater the difference between intensities of the pair of experimental lights, the less flies

enter the darker arm compared with those attracted to the other one.

Discussion. This general tendency is similar to that observed by BERTHOLF¹ in honeybees and by the author² in house-flies. However, when compared in details, this correlation (Figure) does not coincide in all these cases. The important feature of our recent results is that at higher intensities of variable light its attractiveness rises only very slowly, unlike this in lower intensities. Thus one can expect that the proportion of flies attracted by the brighter light tends towards a maximal value of about 0.60. Further experiments are needed for a decisive solving of this view. And here is a possible explanation of the mechanism of photic reactions of insect population such as are observed under the condition of choice of 2 white lights of various relative intensities in a Y maze. The assumed mechanism consists of 2 processes independent of each other.

(a) A certain proportion of insect population, P_i , makes choice by chance with the probability of $1/2$, independently of relative intensities of lights. This group of 'indifferent'³ insects, n_i , seems to be directly proportionally correlated with the logarithm of the sum of absolute values of both



Correlation of the proportion ($\pm 1.96 \sigma$) of flies approaching variable white light with the relative intensity of it. Further explanations in text.

¹ L. M. BERTHOLF, J. agric. Res. 42, 379 (1931).

² J. A. CHMURZYŃSKI, Bull. Acad. pol. Sci. Cl. II Sér. Sci. biol. 15, 415 (1967).

³ Some of them, however, can be sensitive but they might have entered a 'wrong' arm due to geometrical characteristic of the maze. It will be, of course, a statistically constant proportion (for a given type of maze) which contributes to the parameter a in the formula (2).