measurement errors, a distinction was made according to the criteria suggested by Levan at al.[12], also between metacentrics and submetacentrics. It was thus possible to divide the chromosomes of the bikirs investigated into 4 groups: A) large metacentric chromosomes; B) medium size metacentric chromosomes; C) medium size submetacentrics; D) small chromosomes. From this it can be seen that in all 3 species analyzed in this report, as well as in *Calamoichthys calabaricus*[2], the karyotype is basically the same and comprises the same number of chromosome pairs in each group, i.e. 5 in the group A, 6 in B, 2 in C and 5 in D (figure 2). Also comparison with the figures of the karyogram of the bikirs given by Denton and Howell[1] conforms to the basic similarity existing between all the polypterines whose karyotype is now known. In fact, the differences pointed out[1] mainly concern the position of the centromere in small chromosomes, in which centromeric calculation is found to be debatable, together with slight differences in arm ratio in the medium size chromosomes, as revealed by a karyometric method. Considering the high degree of variability in the material presented, as the authors themselves point out in their table II, these differences are not particularly significant.

In the light of these considerations, the cytological evidence for a separation between primitive Actinopterygia and Polypteriformes (Brachipterygia) can be said no longer to depend on an exceptional find in 2 species, but it is supported by evidence of an uniform and presumably stable and ancient karyotype found in 4 species belonging to 2 living genera, that is in all the living genera and one third of the living species comprising this group.

We do not claim that these observations will put an end to the longstanding dispute over Polypterine relationships, but we can be said to have added to the 'confusing mass of embryological, morphological physiological and chemical data'[10] cytotaxonomic evidence whose significance in any phyletic reconstruction, although open to criticism[13], may be considerable.

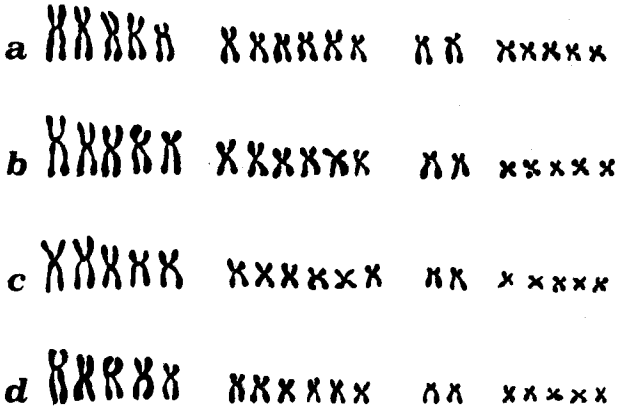

Fig. 2. Comparison among the karyotypes of 4 Polypterine fishes: *a Calamoichthys calabaricus, b Polypterus palmas, c Polypterus endlicheri congicus, d Polypterus delhezi.*

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Progressive resistance against the male recombination factor 31.1 MRF acquired by *Drosophila melanogaster*

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Summary. A genetically abnormal but structurally normal second chromosome (31.1 MRF) which induces recombination in the male sex, was introduced by outcrossing into the cytoplasm of a normal strain. In this new combination, male recombination frequency induced by females dropped from 3.81% to 0.17% whithin 10 generations, indicating a gradual acquisition of resistance against the activities of the 31.1 MRF.

In Dipterans, meiotic recombination is normally absent in the male sex. Various mutant types have been found especially in *Drosophila*, where this restriction is removed to some extent. Nearly all male recombination systems examined in *Drosophila melanogaster* since 1971[2] have shown a reciprocal-cross effect with respect to male recombination frequencies, as well as to sterility, lethal and visible mutations, segregation distortion and chromosomal abnormalities at male meiosis[3-11]. However, neither chromosomal abnormalities[10,11] nor sterility (unpublished data) have been observed in our inbred stock showing male recombination.

A 2nd chromosome (symbol 31.1 MRF) was isolated, by means of the Cy L^4/Pm method, from a large natural population from Southern Greece (Peloponnesus) during autumn 1971. The chromosome was found to be inversion free and kept balanced with an In(2L)Cy + In(2R)Cy, Cy L^4 sp^2 chromosome designated below as Cy L^4, for it is lethal in homozygotes. This 31.1 MRF chromosome was found to induce male recombination, both in the II- and III-chromosomes. Moreover, we have observed that male recombination was always associated with chromosomal abnormalities at male meiosis. It is also worthwhile mentioning that the phenomenon was temperature-sensitive in larval stages and that the 31.1 MRF expresses its effect independently of sex. Our previous data favour the assumption that the reciprocal-cross effect is due to a cytoplasmic factor[9,11].

The aim of the present investigation was to study whether the ability of suppressing male recombination and chromosomal abnormalities at male meiosis could be acquired by

Second chromosome male recombination in 31.1 MRF/dp b cn bw; ve/+ males from 31.1 MRF/SM5 mothers A and fathers B*

Generation G	A			B		
	No. of males tested	No. of progeny	Male recombination frequency (%)	No. of males tested	No. of progeny	Male recombination frequency (%)
G Null	19	2284	3.81	20	3553	2.93
G2	20	4762	1.93	20	3852	2.78
G4	20	5171	0.91	18	4636	2.65
G5	30	8235	0.64	18	3635	3.77
G7	22	7329	0.27	20	2736	2.23
G9	23	8236	0.18	21	4200	2.42
G10	20	7850	0.17	20	4082	2.53
a**	20	2600	2.27			
b	20	2531	2.49			

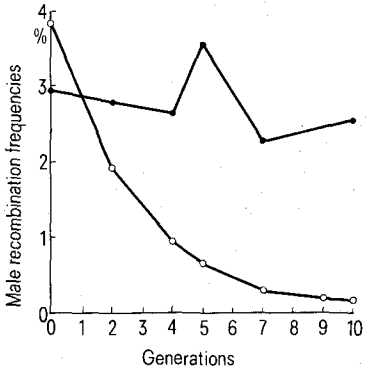
* All cultures were maintained in vials kept at 25±0.5 °C and an 'Instant' *Drosophila* medium (Philip Harris Biological Ltd) was used.
** See text.

means of a new combination bearing the 31.1 MRF and a cytoplasm which has derived from a different stock than the 31.1 MRF/Cy L^4. In order to test this hypothesis, males 31.1 MRF/Cy L^4 were mated to Sp/SM5 (= In(2LR)SM5, al^2 Cy lt^v cn^2 sp^2) virgin females (G Null). From this cross 31.1 MRF/SM5 virgin females and males were separately crossed to dp b cn bw; ve males or virgin females respectively (F_1). Heterozygous 31.1 MRF/dp b cn bw; ve/+ males were then individually crossed to dp b cn bw; ve virgin females (F_2) and their progeny were scored for recombinants up to the 17th day after setting up the crosses. The results presented in the table (G Null) clearly show that the 31.1 MRF acts independently of sex and that the cytoplasm of the Sp/SM5 stock does not suppress the male recombination induced by the 31.1 MRF. Furthermore, from the cross G Null (Sp/SM5 φ^v × 31.1 MRF/Cy L^4 ♂) 31.1 MRF/SM5 males and virgin females were mated in a brother-sister mating (G1) and thus a new balanced stock was established. The latter stock derives its cytoplasm from the Sp/SM5 strain. After the establishment of this stock, designated 31.1/Cy, male recombination frequencies were estimated (in generations G) both from females and males crossed with either dp b cn bw; ve males or virgin females (see table). The estimation of male recombination was carried out with the same procedure as used for G Null.

Our findings presented in the table clearly show a progressive decline of male recombination frequencies in the heterozygous 31.1 MRF/dp b cn bw; ve/+ sons which have inherited the 31.1 MRF from their mothers. On the contrary, we have not been able to observe the same phenomenon in the 31.1 MRF/db b cn bw; ve/+ sons which have inherited the factor from their fathers. The figure presents diagrammatically our results.

In order to assess whether or not the 31.1 MRF/dp b cn bw; ve/+ F_1 sons from the cross G10A induce male recombination to their progeny, 31.1 MRF/dp b cn bw; ve/+ and 31.1 MRF/dp b cn bw; ve/ve males were separately collected at random from the F_2-generation and were individually mated to dp b cn bw; ve virgin females (F_3); the progeny of the F_3 was scored for recombinants. The results of these crosses, which are shown in the table (lines a and b, respectively), indicate that, while the 31.1 MRF still exists in the 31.1/Cy females of the G10, it seems that it has been inactivated by a suppressor, which, very probably, the 31.1/Cy stock has progressively acquired. Moreover, these results show that the 3rd chromosome of the 31.1/Cy stock does not affect the male recombination frequency of the 2nd chromosome.

The progressive decline of male recombination frequency induced from 31.1/Cy females has as a result an increase of female fertility. Thus, in the G1 the reproductivity of



Observed differences in male recombination frequencies, induced by 31.1 MRF/SM5 females (○) and males (●), crossed with dp b cn bw; ve males or virgin females.

females was low and the sterility was high (55%), a gradual increase of the reproductivity was observed while a decrease in the female sterility was found; the decrease was restricted to only 5% in the G10 (in both G1 and G10 60 females were examined for sterility by means of crosses with 5 dp b cn bw; ve males).

In addition, while in the G1 male pupae have shown bridges and/or fragments at anaphase I and II with respective frequencies 36.56% and 31.68%[11], in the G10 only 2% of the examined anaphases I and 2% of the anaphases II were found to be abnormal (the estimation in the G10 was made on the basis of 97 and 149 anaphases I and II examined from 32 male pupae).

The results presented here clearly suggest that the new 31.1/Cy strain has acquired a progressive resistance against the activities of the 31.1 MRF. This resistance seems to be cytoplasmic, as its effect is transmitted through the females; however, its nature is up to now unknown.

It has been found[9] that the cytoplasm of a Cy L^4/Pm stock suppresses males recombination induced by the 31.1 MRF. However, this stock induces neither male recombination in the 3rd chromosome nor female sterility (unpublished data). The reason why this stock possesses the ability to suppress male recombination remains still rather obscure. On the other hand, it is impossible to test whether or not this strain bore the factor in the past, while now it has become free of it.

We think that our findings may offer an explanation to the fact that the MRF systems generally show the reciprocal-cross effect in outcrosses, as well as to why they do not affect their progeny in inbred crosses. Furthermore, if the

same phenomenon (acquisition of progressive resistance to MRF) occurs in natural populations, then the high frequencies of MRF factors observed in several populations[2,8,12-16] may be at least partially attributed to acquired resistance. It has been suggested[14-18] that male recombination factors may be viruses or episomes. If this hypothesis is true, one cannot help thinking that our results provide evidence that *Drosophila melanogaster* has the potentiality to acquire some kind of resistance against such factors.

1 Part of this work was carried out in the Dept of Genetics, University of Cambridge, England, and was supported by a grant from the Greek State Scholarship Foundation. I am greatly indebted to Drs M. Ashdurner and G. Richards (Dept of Genetics, University of Cambridge, England) for helpful advice, as well as to Prof. M. Pelecanos (Dept of Genetics, University of Patras, Greece) for constructive criticism and corrections of the manuscript.

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The karyotypes of the Corsican and Sardinian mountain salamanders, *Euproctus montanus* and *E. platycephalus* (Urodela: Salamandridae)[1]

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Summary. The chromosomes of *Euproctus montanus* and *E. platycephalus* were studied by means of the C-banding method and the AS-SAT technique which are useful for identifying the single pairs of the complement and for recognizing nucleolar organizer regions. According to the morpho-structural characteristics shown by the specific karyotypes, it has been possible to draw some cytotaxonomic deductions concerning the karyological evolution within the insular group.

Euproctus Gené, 1838, is a salamandrid genus confined to Europe and consists of 3 species highly adapted to cold running waters and geographically isolated: *E. asper* (Dugès, 1852) has a range entirely restricted to the Pyrenees at high altitude; *E. montanus* (Savi, 1838) lives in the mountain streams of Corsica and *E. platycephalus* (Gravenhorst, 1829) in those of Sardinia. Their morpho-anatomical and functional characteristics are well known[2-6]; instead the karyological picture is still rather limited[7-9], there being known only the karyotype of *E. asper*[9]. Therefore, we have started a more extensive work on the fine configuration of chromosomes of the insular *montanus-platycephalus* group to find possible karyotypic differentiations related to speciation process. The present report deals with the morpho-structural characterization of the specific karyotypes carried out by means of the C-banding method and the AS-SAT technique.

Materials and methods. 20 alive specimens of both sexes of *E. montanus*, collected near Zonza, and of *E. platycephalus*, collected on the M. Limbara, near Berchidda, were injected with 0.15 ml of Colcemid (Ciba; 1 mg/ml), followed by 2 or 3 additional doses at intervals of 48 h when somatic tissues, such as gut and spleen, were to be excised for cytological preparations. Fragments of tissues and organs, treated with a hypotonic solution for 10 min, were fixed in 1:3 glacial acetic acid-absolute ethanol for 20–30 min, then dissociated and squashed in acetic acid 45%, following the usual dry-ice method. Then a few preparations were simply stained with Giemsa diluted in phosphate buffer pH = 7; several other preparations were treated according to a) the C-banding method by Gall and Pardue[10] with the omission of radioactive RNA; or b) the ammoniacal silver staining technique (AS-SAT) by Howell et al.[11] with a denaturation time of only 2 min. The nomenclature here followed to classify the single chromosomes is that proposed by Levan et al.[12].

Results and discussion. The chromosome number is confirmed to be $2n = 24$, $n = 12$[7,8] in both species. The lengths of the single pairs are gradually decreasing and their distinction into 3 groups follows the same conventional scheme as already made for the karyotypes of the European Salamandrids studied so far[13].

E. montanus. Group A consists of the 4 largest chromosome pairs which are all metacentric. Centromere indices of pairs I, II and III are 0.461; 0.476 and 0.446 respectively. Pair IV is the most heterobrachial (i.c. = 0.398) (figure 1). Group B consists of the 4 medium-sized chromosome pairs, of which 3 elements (V, VI and VIII) are metacentric, although pair VI is at the limit between metacentric and submetacentric (i.c. = 0.452; 0.380; 0.418 respectively); pair VII, the most heterobrachial in the group, is submetacentric (i.c. = 0.293) (figure 1). Group C consists of the 4 shortest pairs in the complement: pair IX, the most heterobrachial, is submetacentric (i.c. = 0.294) and shows a secondary constriction subterminally on the long arm; pairs X, XI and XII are metacentric, but pair XI is at the limit between metacentric and submetacentric (i.c. = 0.426; 0.376 and 0.493 respectively) (figure 1).

E. platycephalus. Group A consists of the 4 largest chromosome pairs, which are all metacentric. Pair II is the most