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.

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2 Y. Hiraizumi, Proc. nat. Acad. Sci. USA *68*, 268 (1971).
3 M.G. Kidwell and J.F. Kidwell, Nature *253*, 755 (1975).
4 M.G. Kidwell, J.F. Kidwell and P.T. Ives, Mut. Res. *42*, 89 (1977).
5 M.G. Kidwell, J.F. Kidwell and J.A. Sved, Genetics *86*, 813 (1977).
6 M.G. Kidwell, Genet. Res., Cambridge *30*, 77 (1977).
7 J.A. Sved, Aust. J. biol. Sci. *29*, 375 (1976).
8 R.C. Woodruff and J.N. Thompson, Jr, Heredity *38*, 291 (1977).
9 G. Yannopoulos and M. Pelecanos, Genet. Res., Cambridge *29*, 231 (1977).
10 A.S. Henderson, R.C. Woodruff and J.N. Thompson, Jr, Genetics *87*, in press (1977).
11 G. Yannopoulos, Genet. Res., Cambridge, in press (1978).
12 G. Broadwater, L.V. Owens, E. Winfrey and F.R. Waddle, D.I.S. *50*, 99 (1973).
13 J.A. Matthews and Y. Hiraizumi, Genetics *83*, 48 (1976).
14 O. Yamaguchi, Mut. Res. *34*, 389 (1976).
15 F.R. Waddle and I.I. Oster, J. Genet. *61*, 177 (1974).
16 M.M. Green, Proc. nat. Acad. Sci. USA *74*, 3490 (1977).
17 R.A. Voelker, Mut. Res. *22*, 265 (1974).
18 P.A. Roberts, in: The Genetics and Biology of *Drosophila*, vol. 1a, p. 67. Ed. M. Ashburner and E. Novitski, 1976.

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

metacentric (i.c. = 0.486), while pair IV is the most heterobrachial (i.c. = 0.408). Pairs I and III have i.c. = 0.462 and 0.439 respectively (figure 2). Group B consists of the 4 pairs of elements of medium length, among which it is possible to distinguish 2 pairs (V and VII) made up of metacentric chromosomes (i.c. = 0.437 and 0.401 respectively) and 2 pairs (VI and VIII) made up of submetacentric chromosomes, of which pair VI is at the limit between metacentric and submetacentric and pair VIII is the most heterobrachial (i.c. = 0.374 and 0.299 respectively) (figure 2). Group C consists of the 4 shortest pairs in the complement. Pair XII is easily identifiable, because it is the smallest and the most metacentric (i.c. = 0.488). Pairs X and XI are submetacentric (i.c. = 0.353 and 0.305 respectively), while pair IX is metacentric (i.c. = 0.451) (figure 2).

The C-banding method is particularly effective in inducing

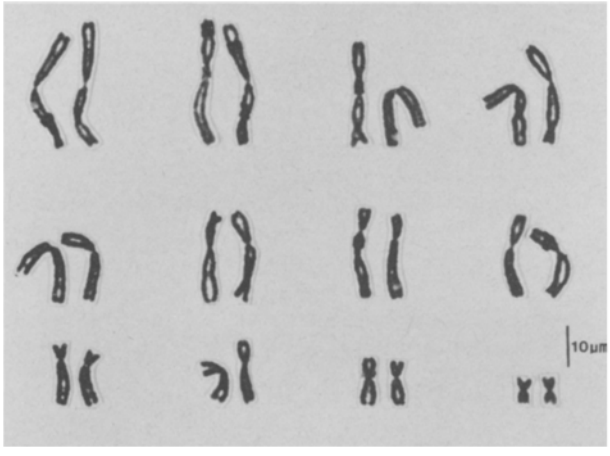

Fig. 1. The karyotype of *E. montanus* (gut, Giemsa in phosphate buffer pH = 7).

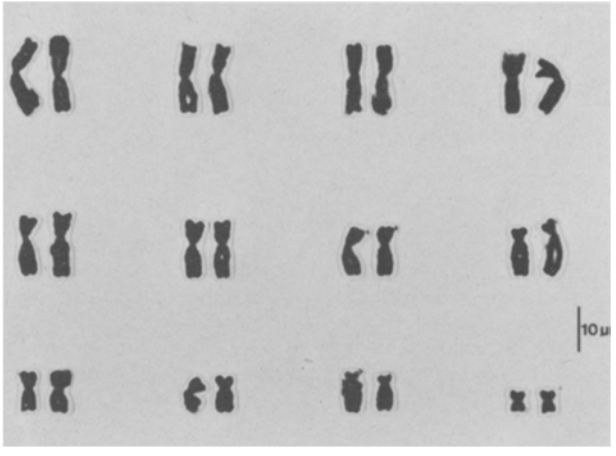

Fig. 2. The karyotype of *E. platycephalus* (spermatogonium, Giemsa in phosphate buffer pH = 7).

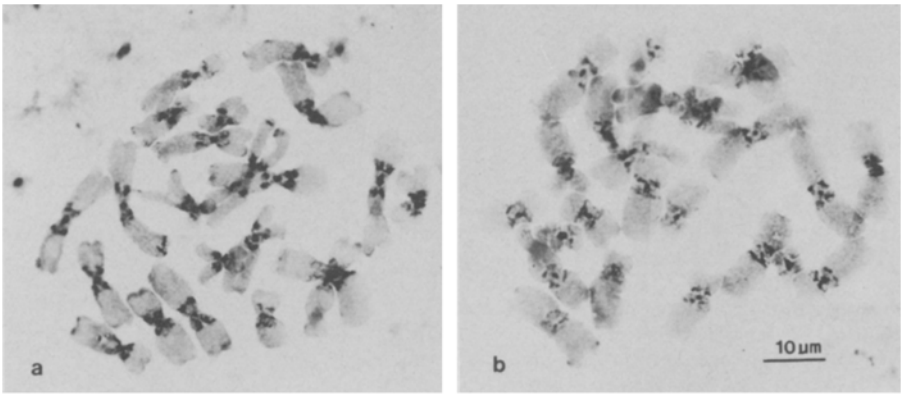

Fig. 3. Spermatogonial metaphases of *a E. montanus* and *b E. platycephalus* (C-banding method).

Fig. 4. Mitotic metaphase of ♂ *E.m.*5. ➡ point out the homozygous NORs on chromosome pair IX; → show the heterozygous additional sites of coloration on chromosomes II and IX (gut, AS-SAT technique).

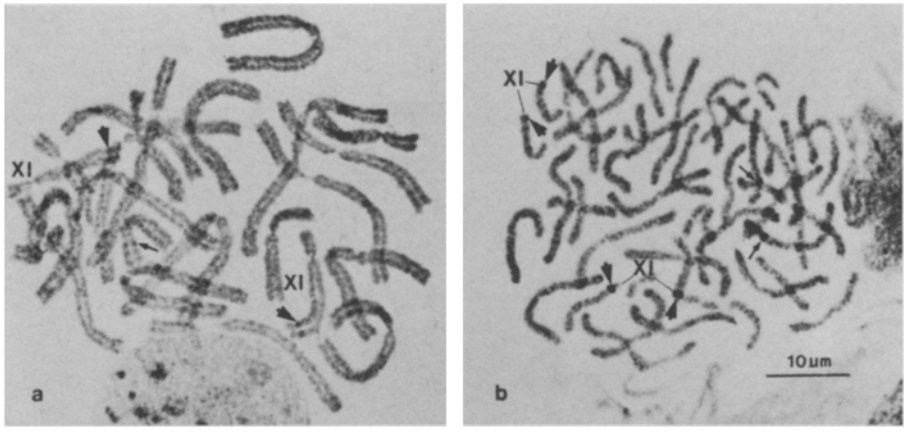

Fig. 5. A normal mitotic metaphase (a) and a C-mitosis (b) showing homozygous black spots on chromosome pair XI (➡). → point out heterozygous additional sites of coloration on chromosomes IX (♂*E.p.*1; spleen: AS-SAT technique).

a dark stain of the centromere region on chromosomes of both species (figure 3): this makes it possible to confirm the localizations of centromeres. In addition, this technique produces a heavy coloration of both pericentric regions of all the chromosomes, except pair XII of only *E. montanus* (figure 3). However, the thickness and the intensity of staining on the pericentric regions seem to be higher in *E. montanus*. A subterminal dark band or spot characterizes pair II in both species. In the most favorable preparations, the long arm of pair IX of *E. montanus* (in a region corresponding to the above-mentioned secondary constriction) and the long arm of pair XI of *E. platycephalus* show a darkly stained C-band.

Applying the technique by Howell et al.[11] to chromosomes of *E. montanus*, the 2 chromosomes of pair IX constantly show a black spot subterminally on the long arm (figure 4). Additional sites of coloration may be evident on various elements of the complement, but they are always heterozygous. Such sites are constant for any one specimen, but vary between different individuals. For instance, spermatogonial and somatic metaphases of ♂*E.m.* 5 show homozygous granules on pair IX and additional heterozygous sites subterminally on the long arm of one homologue of pair II, and procentrically on the long arm of one homologue of pair IX (figure 4). ♂*E.m.* 2, besides the homozygous black spots on pair IX, shows an additional site of coloration on the long arm of both homologues of pair VII, but one is intercalary, the other one is procentric. In *E. platycephalus*, the AS-SAT technique induces a black subterminal spot on the long arm of both homologues of pair XI (figure 5). Additional sites of coloration are also evident, distributed on various elements of the complement, but they are always heterozygous. For instance, spermatogonial metaphases of ♂*E.p.* 1 show homozygous granules on pair XI and an additional heterozygous site of coloration, procentrically on the short arm of pair IX (figure 5). Keeping in mind the results of previous authors[11,14,15], the homozygous site on pair IX of *E. montanus* and on pair XI of *E. platycephalus* can be assumed as to be the nucleolus organizer region (NOR).

In conclusion, the karyotypes of the Sardinian and Corsican mountain salamanders seem to be largely overlapping as to usual morphometric factors. Some discrepancies seem to concern the arrangement of the single chromosome pairs in groups B and C; however, these groups contain the same number of metacentrics and submetacentrics in both species, and, in group C, the NORs are present on the most heterobrachial pair in subterminal position. The karyotypes of both insular species, mainly that of *E. montanus*, appear to be similar also to the karyotype of *E. asper* given by Jaylet[9]. Again, some discrepancies have been noted concerning the form of chromosomes belonging to group C: e.g. pair XII is less metacentric in the Pyrenean mountain newt. However, the karyotypes of the 3 *Euproctus* species cannot be considered so highly differentiated as one could expect according to the specific anatomical, physiological and ethological characteristics. Then, as previously supposed for most European salamandrids[13], we can admit that a series of karyotypic events, mainly minute rearrangements, had occurred during the karyological evolution of the genus, which led to the present configuration and structure of the 3 karyotypes. Minute rearrangements are believed to cause speciation in a large number of animal groups[16] and to support the recent hypothesis of the so-called 'unity of genotype'[17]. Thus *Euproctus* can represent a particularly suitable biological system where detailed studies on chromosome evolution can be accomplished also by means of interspecific hybridization[18].

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2 R. Despax, Thesis Sc. nat. Toulouse 1923.
3 L. Goux, Vie Milieu, suppl. *8*, 223 (1959).
4 L. Goux, Vie Milieu, suppl. *8*, 259 (1959).
5 R. Thorn, Les Salamandres. Ed. P. Lechevalier, Paris 1968.
6 J.W. Steward, The tailed Amphibians of Europe. Eds. David and Charles, Newton Abbot 1969.
7 G. Mancino, Rc. Acad. naz. Lincei *39*, 540 (1965).
8 G. Mancino and G. Barsacchi, Rc. Accad. naz. Lincei *41*, 396 (1966).
9 A. Jaylet, Chromosoma *18*, 79 (1966).
10 J.G. Gall and M.L. Pardue, in: Methods in enzymology, vol. 31, p. 470. Ed. L. Grossman and K. Moldave. Academic Press, New York 1971.
11 W.M. Howell, T.E. Denton and J.R. Diamond, Experientia *31*, 260 (1975).
12 A. Levan, K. Fredga and A.A. Sandberg, Hereditas *52*, 201 (1964).
13 G. Mancino, M. Ragghianti and S. Bucci-Innocenti, in: The Reproductive Biology of Amphibians, p. 411. Ed. D.H. Taylor and S.I. Guttman. Plenum Press, 1977.
14 C. Goodpasture and S.E. Bloom, Chromosoma *53*, 37 (1975).
15 M. Ragghianti, S. Bucci-Innocenti and G. Mancino, Experientia *33*, 1319 (1977).
16 M.J.D. White, The Chromosomes. 6th ed. Chapman and Hall, 1973.
17 E. Mayr, Biol. Zbl. *94*, 377 (1975).
18 G. Mancino, M. Ragghianti and S. Bucci-Innocenti, Rc. Accad. naz. Lincei *61*, 661 (1976).