indistinguishable from the satellited acrocentric pair described in the African ground squirrel *Xerus rutilus*[8] (Figure 2). A single band located in the proximal half of the chromosome characterized the satellited pair from both *Callosciurus* and *Xerus*.

Discussion. Among the very few species of *Callosciurus* from scattered localities examined so far, no interspecific or geographic variation in karyotypes has been observed. Moreover, all possess a distinctive satellited pair of acrocentric chromosomes, similar to those found in the ground-dwelling callosciurine, *Dremomys rufigenis* ($2n$ = 38). In addition, this satellited acrocentric pair appears to be morphologically identical, on the basis of Giemsa-band comparisons, with that of the satellited acrocentric chromosomal pair found in the African xerine ground squirrel, *Xerus rutilus* ($2n$ = 38). This supports the suggestion[8] that xerines and callosciurines may be related. At the same time, the Asian palm squirrels, *Funambulus*, may also be related to callosciurines, according to immunological evidence[9]. However, neither *F. palmarum* ($2n$ = 46)[10] nor *F. pennanti* ($2n$ = 54)[11] appear to have satellited acrocentrics, and their karyotypes are quite different from those described for xerine and certain callosciurine squirrels.

Although the evolutionary history of *Calloscuirus* is poorly understood, fossil xerine ground squirrels are known from the late Oligocene of Europe (*Heteroxerus*), and the Miocene of Morocco and Spain (*Getuloxerus*)[12]. The satellited acrocentric autosomes of extant *X. rutilus* may have been retained within the xerine lineage since that time, a view that is supported by the appearance of seemingly identical chromosomes in *Callosciurus* and in the more recently evolved xerine genus *Spermophilopsis*[8].

Zusammenfassung. Die Karyotypen von *Callosciurus notatus, C. finlaysoni* und *C. flavimanus* besitzen alle $2n$ =40 Chromosomen: 6 metazentrisch, 10 submetazentrisch und 3 akrozentrisch. Eines der akrozentrischen Paare trägt einen auffälligen Satelliten; akrozentrische Satellitenchromosomen von *Callosciurus* und *Xerus rutilus* besitzen identische G-Bandmuster. Akrozentrische Chromosomen mit Satelliten wurden auch von den Callosciurinen *Dremomys rufigenis* und der Xerine *Spermophilopsis leptodactylus* beschrieben.

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7 October 1974.

[8] C. F. Nadler and R. S. Hoffmann, Experientia, *30*, 889 (1974).
[9] M. E. Hight, M. Goodman and W. Prychodko, Syst. Zool. *23*, 12 (1974).
[10] K. L. Satya Prakash and N. V. Aswathanarayana, Mammal. Chromos. Newslett. *12*, 86 (1971).
[11] M. D. L. Srivastava and V. S. Bhatnagar, Mammal. Chromosomes Newslett. *12*, 51 (1971).
[12] C. C. Black, Evolut. Biol. *6*, 305 (1972).
[13] Supported by National Science Foundation Grants No. GB 32114X and 29131X. We thank Dr. T. C. Hsu, M. D. Anderson Hospital, Houston, Texas, for Giemsa-band chromosome preparations from skin biopsies. Dr. R. W. Turner and Dr. Illar Muul provided us with live specimens.

Cytotaxonomical Consideration of the Genus *Blennius* (Pisces-Perciformes)

Within the complicated suborder of the Blennioidea, in which various benthonic fish families are gathered[1,2], the Blenniidae comprise a large family whose ecological valence has allowed it to colonize the coastlines of all seas and to penetrate into brackish and even fresh waters. One of the problems of Blenniidae classification is the division into genera and subgenera[3,4].

The purpose of the present paper is to make some contribution to solving the above problem by means of the karyological data concerning several Mediterranean species of blenny of the genus *Blennius* L., 1758. As already demonstrated in one of our previous works[5], karyotype analysis can be useful if carried out on species belonging to taxonomically homogeneous groups whose classification is, however, complicated because of their great size and wealth of specialized forms.

One example of this is the genus *Blennius* L., a complicated and subdivided taxon including numerous species

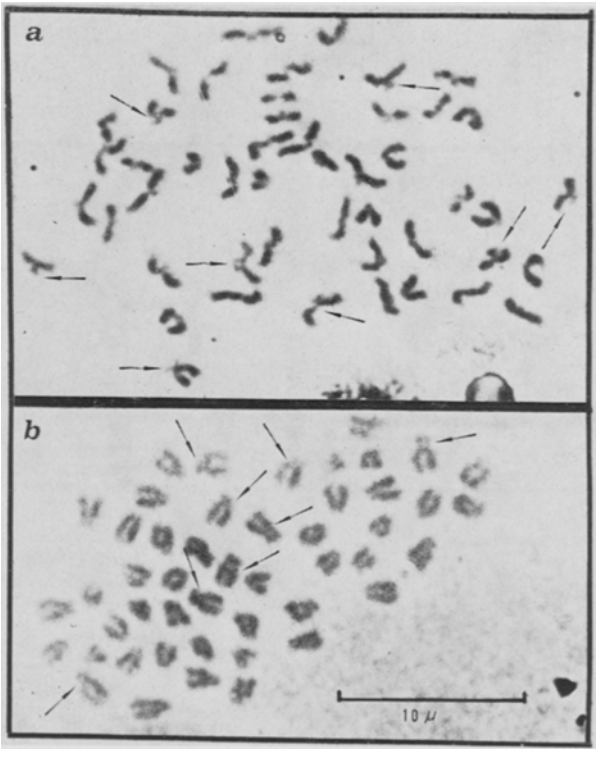

Fig. 1. Metaphase plate of *Blennius sanguinolentus* (a) and of *Blennius pavo* (b). Arrows indicate the subtelocentric chromosomes.

[1] C. T. Regan, Ann. Mag. nat. Hist. Ser. 8, *10*, 256 (1912).
[2] L. S. Berg, Trudy Inst. Zool. Kyyiv *2*, 87 (1940).
[3] J. R. Norman, Ann. Mag. nat. Hist., Ser. 11, *10* (1943).
[4] V. G. Springer, Bull. Am. Mus. nat. Hist. *284*, 1 (1968).
[5] S. Cataudella, M. V. Civitelli and E. Capanna, Caryologia *27*, 93 (1974).

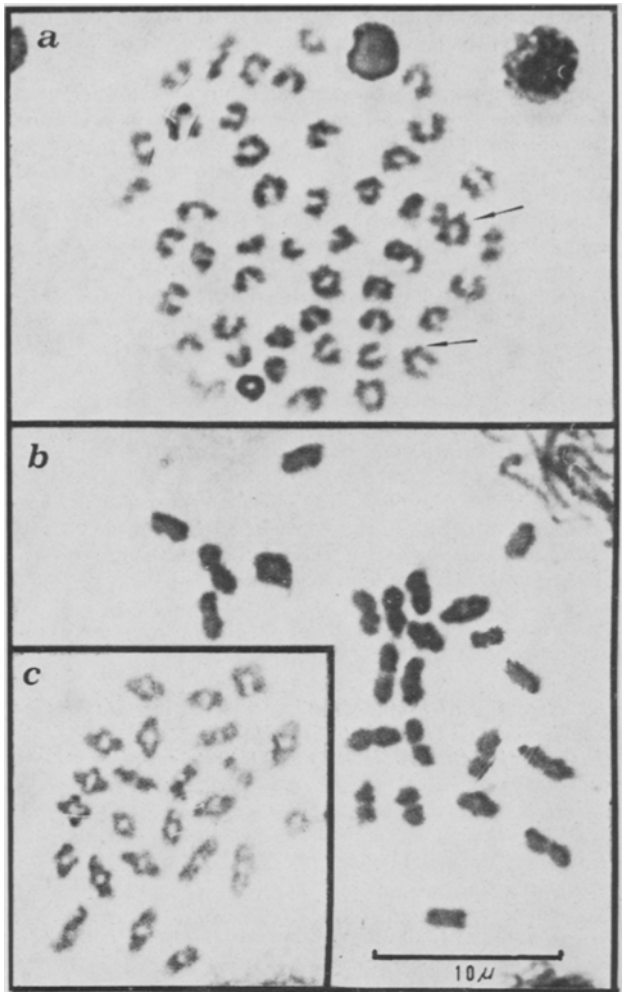

Fig. 2. Metaphase plates and meiotic diakinesis of *Blennius incognitus* and *Blennius sphinx*. a) Metaphase of *B. incognitus*, b) metaphase of *B. sphinx*, c) diakinesis of *B. incognitus*, d) diakinesis of *B. sphinx*. Arrows indicate the subtelocentric chromosomes.

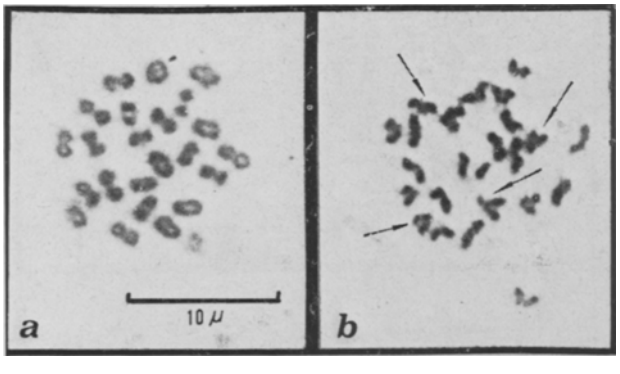

Fig. 3. Meiotic pattern of *Blennius fluviatilis* and *Blennius canevae*. a) Diakinesis of *B. fluviatilis*, b) diakinesis of *B. canevae* and c) 2nd metaphase of *B. canevae* in which 4 subtelocentrics are shown (arrows).

among which there are 20 described for the Mediterranean fauna. The genus is usually divided into several subgenera, i. e. *Blennius* L., *Salaria* Forskal and *Lipophrys* Gill. The *Salaria* subgenus, the richest in species, is in turn subdivided into species groups: *gattorugine, cristatus* and *fluviatilis*.

We have now studied 7 species of Mediterranean blenny, namely *Blennius sanguinolentus* Pallas, *Blennius pavo* Risso, *Blennius fluviatilis* Asso, *Blennius sphinx* Valenciennes, *Blennius incognitus* Bath, *Blennius trigloides* Valenciennes and *Blennius canevae* Vinciguerra; the first 5 species are ascribed to the *Salaria* subgenus while *B. trigloides* and *B. canevae* are referred to the *Lipophrys* subgenus.

The technique used is the one proposed by HITOTSUMACHI[6] which we have suitably modified. Somatic metaphase slides were prepared from cephalic kidney and gill tissue. Meiotic diakinesis observations were carried out on testicular tissue.

In all the species studied, the diploid number was found to be $2n = 48$. However, in spite of the technical difficulties involved it was found possible to demonstrate the morphological peculiarities of the karyotype and thus characterize the various cytotaxonomic groups.

Blennius pavo and *Blennius sanguinolentus* display an identical karyotype consisting of 40 acrocentric chromosomes, plus 4 pairs of subtelocentric chromosomes with very small short arms (Figure 1). *Blennius sphinx* and *Blennius incognitus* display 48 chromosomes, all of which are acrocentric except for a pair of large acrocentrics with a very short arm. All that we have obtained for *Blennius fluviatilis* and *Blennius canevae* are the meiotic images, whose 24 bivalents clearly display a diploid number of $2n = 48$. For *Blennius canevae*, however, it was possible to find some second meiotic metaphases (Figure 3) in which 4 subtelocentric chromosomes could be discerned among the 24 of the haploid system. It was thus not difficult to place also the karyotype of *B. canevae* back in the cytotaxonomic group of *B. sanguinolentus*.

Blennius trigloides, on the other hand, displays a quite different karyotype to that of the other blennies studied, although the diploid number is still $2n = 48$. The karyotype (Figure 4) displays 1 pair of large metacentric chromosomes, 3 pairs of submetacentrics, 9 pairs of subtelocentrics and 11 pairs of acrocentrics. The diploid number as derived from the meiotic bivalents was checked in all the species examined in the somatic metaphases.

The results obtained once again confirm the mode of the diploid number of $2n = 48$ of the chromosome complements of the perciform Teleostei. They also show up the necessity of carrying out a more accurate cytotaxonomic analysis, taking account of all the morphological features of the karyotype and not limited to the diploid number. The blennies studied fall into 3 cytotaxonomic categories; the first of these comprises *B. sanguinolentus*, *B. pavo* and *B. canevae*, the second, still somewhat similar to the first, comprizing *B. sphinx* and *B. incognitus*, and a third comprizing *B. trigloides* alone.

It thus transpires that the cytotaxonomic data observed by us do not agree entirely with the division at the level of subgenera[3] according to which *B. canevae* and *B. trigloides* are assigned to the same subgenus *Lipophrys*. According to our data, on the contrary, it would appear that *B. canevae* belongs to a cytotaxonomic group containing *B. sanguinolentus* and *B. pavo* that differs only slightly from that comprizing *B. sphinx* and *B. incognitus*.

In the light of the above considerations, the absence or presence of supraorbital tentacles, on which the division

[6] S. HITOTSUMACHI, M. SASAKI and Y. OJIMA, Jap. J. Genet. *44*, 157 (1969).

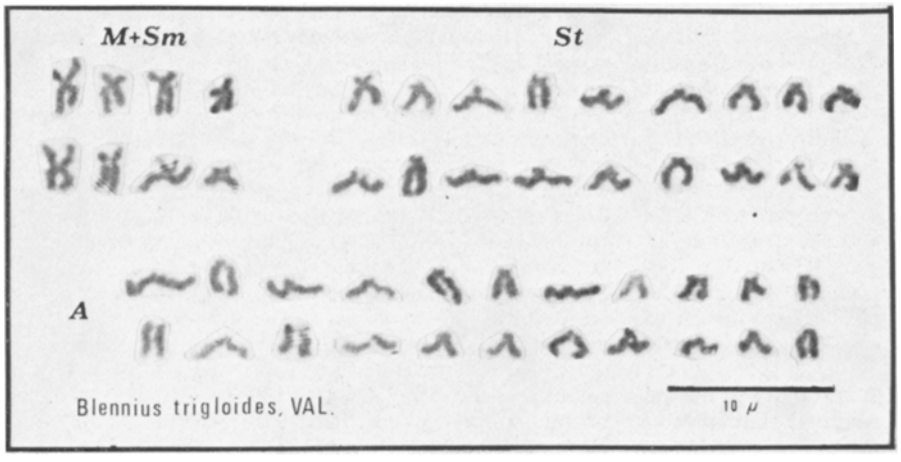

Fig. 4. Metaphase plate and karyotype of *Blennius trigloides*. M + SM, metacentric and submetacentric chromosomes; St, subtelocentric chromosomes and A, acrocentric chromosomes.

into the subgenera *Salaria* and *Lipophrys* is based, does not seem to be sufficient ground for such a subdivision. On the other hand, this cytotaxonomic feature shows up the peculiarity of the *B. trigloides* karyotype, thus calling for an accurate taxonomic review of this species and a more extensive analysis of all the biological characters in order to ascertain its natural taxonomic position in the *Blennius* L. genus[7].

Riassunto. È stato descritto il cariotipo di 7 specie di Blennidi Mediterranei appartenenti al genere *Blennius* L.: *B. sanguinolentus*, *B. pavo*, *B. sphinx*, *B. trigloides*, *B. canevae*, *B. fluviatilis* e *B. incognitus*. Il numero diploide di tutte le specie è $2n = 48$ tuttavia la morfologia del cariotipo presenta alcune differenze tra le specie; *Blennius trigloides* si distingue infatti nettamente da tutti gli altri *Blennius* poichè il suo cariotipo mostra un alto numero di metacentrici e sub-metacentrici mentre il cariotipo delle altre specie studiate è formato fondamentalmente da acrocentrici tra i quali fanno spicco quattro coppie (*sanguinolentus*, *pavo* e *canevae*) o una coppia (*incognitus* e *sphinx*) di subtelocentrici.

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The Generation of the Superoxide Radical by 2-Amino-4-Hydroxy-6,7-Dimethyl-5,6,7,8-Tetrahydropteridine ($DMPH_4$)

Superoxide dismutase (SOD) is a copper-zinc containing protein of molecular weight 33,000 whose enzymatic activity was first described by McCord and Fridovich[1]. SOD catalyzes the dismutation of the superoxide anion radical (O_2^-) to form hydrogen peroxide and oxygen:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Recently, Petrack and Chertock[2] reported that SOD partially protected the labile activated state of tyrosine hydroxylase. This effect of SOD suggested that O_2^- was inactivating tyrosine hydroxylase. Petrack and Chertock[2] did not identify a source of the O_2^- in their experimental system. A likely origin of O_2^- was the reduced pteridine cofactor $DMPH_4$, a compound known to generate H_2O_2[3]. Many compounds which autoxidize to form H_2O_2 (the two-electron reduction product of oxygen) also generate O_2^- (the one-electron reduction product of oxygen) as an intermediate[4].

Some autoxidations are catalyzed by O_2^- which is generated during the reaction. SOD can diminish the overall rate of such autoxidation reactions. For example, it has been shown that SOD inhibits both the base catalyzed and the metal ion catalyzed autoxidation of epinephrine[5], and the spontaneous autoxidation of 6-hydroxydopamine at pH 7.4[6]. We now present evidence that $DMPH_4$ generates O_2^- which catalyzes the autoxidation of $DMPH_4$.

Materials and methods. Experiments were run on a Biological Oxygen Monitor (Clark Oxygen Electrode, Yellow Springs Instruments, Yellow Springs, Ohio) connected to a Honeywell Electronik 19 Recorder at 37 °C in 1 ml of a modified Krebs-Ringer phosphate buffer at pH 7.4[6] or a 0.05 *M* sodium acetate buffer at pH 6.5. The $DMPH_4$ was dissolved in O_2 free water and was added to the oxygen electrode by means of an Oxford automatic pipette. In some experiments 100 μg SOD (frozen liquid, 3,000 units per mg, Truett Laboratories, Dallas, Texas) was added prior to the addition of $DMPH_4$.

[1] J. M. McCord and I. Fridovich J. biol. Chem. *244*, 6049 (1969).
[2] B. Petrack and H. Chertock, Fedn. Proc. *33*, 535 (1974).
[3] R. Shiman, M. Akino and S. Kaufman, J. biol. Chem. *246*, 1330 (1971).
[4] H. Taube, *Oxygen*, (Little Brown and Co., Boston 1965), p. 29.
[5] H. P. Misra and I. Fridovich, J. biol. Chem. *247*, 3170 (1972).
[6] R. E. Heikkila and G. Cohen, Science *181*, 456 (1973).