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A(

QU'EST-CE QUE LA SPÉCIATION?

EUGEN V. NICULESCU

La spéciation est un terme entré dans le vocabulaire de tous les zoologistes et qui signifie l'apparition d'une espèce nouvelle.

Le mécanisme de la spéciation relève selon les zoologistes de conceptions bien différentes.

Dans ce travail, nous présenterons les opinions de deux zoologistes distingués — Ernst Mayr et Georges Bernardi — et ensuite notre propre conception qui diffère de celles de ces deux chercheurs.

E. Mayr (3), critiquant le critère morphologique de l'espèce, dit : « Le degré de différenciation morphologique manifesté par une population naturelle n'est qu'un produit accessoire de la divergence génétique résultant de l'isolement reproductif ». Il en découle que, selon Mayr, une espèce nouvelle apparaît lorsque s'est développé un seul mécanisme d'isolement : le facteur reproductif. De cet isolement reproductif naîtra *ultérieurement* la divergence génétique. Enfin, la différenciation morphologique ne constitue qu'un *produit accessoire* de cette dernière divergence.

La définition unilatérale proposée par Mayr se résume donc comme suit : « Les espèces sont des groupes de populations naturelles capables d'intercroisement et reproductivement isolés d'autres groupes semblables ». Comme on le voit, l'espèce n'a qu'un seul attribut : l'isolement reproductif !

Nous ne sommes pas d'accord avec cette définition unilatérale (5) puisque la spéciation est un phénomène plus complexe et que l'espèce possède plusieurs attributs (6—9).

Georges Bernardi (1) est, quant à lui, beaucoup plus proche de notre conception qu'il avait critiquée par le passé : « Tous les zoologistes s'accordent à reconnaître comme bonnes espèces les taxons qui sont à la fois génétiquement isolés, morphologiquement différenciés et sympatriques ». Si l'introduction du critère morphologique parmi les attributs de l'espèce est pertinente, et à ce chapitre nous sommes entièrement d'acord avec l'auteur français, qui s'est enfin décidé à nous suivre, il reste encore un point de divergence. Selon Bernardi « La spéciation proprement dite est accompagnée ou suivie de deux autres phénomènes : l'apparition de différences morphologiques et acquisition de différences écologiques suffisantes pour que la sympatrie soit possible ». Si nous interprétions bien ces lignes, l'espèce nouvelle apparue ne diffère pas, selon Bernardi, morphologiquement, des autres espèces, la distinction morphologique se manifestant a posteriori, donc la nouvelle espèce se caractérise uniquement par l'isolement génétique.

A l'encontre de Mayr et Bernardi nous admettons que la spéciation est achevée lorsque se sont installés tous les mécanismes d'isolement : morphologique, génétique, écologique et chimique, phénomènes in globo qui ont pour résultante l'isolement reproductif (8). Les individus d'une espèce ne se croisent plus avec les individus d'une autre espèce (isolés reproductivement) parce qu'ils sont isolés sur les plans morphologique

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génétique, écologique et chimique (les stimuli chimiques) *en même temps;* alors seulement la spéciation est achevée et nous pouvons parler, à bon droit, d'une espèce nouvelle qui ne s'intercroise plus avec aucune des espèces anciennes.

Donc, pour répondre à la question de notre intitulé nous poserons que la spéciation est un phénomène complexe qui consiste en un ensemble de phénomènes: morphologique, génétique, écologique, éthologique et chimique dont la conséquence est l'isolement reproductif qui maintient la pureté de l'espèce, ses individus étant interstériles par rapport aux individus des autres espèces.

Si tel est le cas, alors les espèces jumelles ne constituent pas des [6] espèces achevées » puisque, chez ces «espèces» la distinction morphologique manque. Dans ce problème aussi notre conception s'écarte de celle de Bernardi qui est un adepte de la notion d'espèces jumelles dépourvue de sens à notre avis. Mais Bernardi ne demeure pas conséquent avec ses propres idées lorsqu'il admet, d'une part, la « distinction morphologique » de l'espèce et d'autre part que « des formes morphologiquement semblables puissent être rangées dans des espèces distinctes »! Comme exemple d'espèces jumelles il cite *Amphipyra pyramidea* et *A.berbera*, mais cet exemple est mal choisi vu que les deux espèces diffèrent morphologiquement tant par le graphisme que par les genitalia mâles et femelles. Pourquoi ces deux espèces seraient-elles « jumelles »? Si elles se distinguent si bien par le graphisme et le coloris ainsi que par les genitalia. elles constituent deux espèces « normales » dotées des deux attributs habituels des espèces; leur classement dans la catégorie des espèces jumelles est, partant, arbitraire, tout a fait gratuit et dénué de sens (4).

Mais Bernardi, conscient de la faiblesse et du manque de poids de la notion d'espèces jumelles, émet, pour la sauvegarder, l'idée de l'existence chez ces espèces, de certains caractères « plus ténus » que ceux qui séparent habituellement les espèces du même groupe. Mais ces « caractères » n'ont maintenu en rien le « prestige » des espèces jumelles. Quels sont ces caractères « ténus »? En quoi les caractéres « ténus » différent-ils des caractères normaux? Les espèces ont-elles vraiment deux sortes de caractères : ténus et normaux? Où placer la limite entre ces deux types de caractères? L'arbitraire s'y manifeste au gré de chacun : rien de précis, rien de scientifique!

A la question de Bernardi « pourquoi certaines espèces ne présentent-elles que peu ou pas de différences morpologiques », nous répondons : pour le simple motif qu'elles ne sont pas en fait des espèces, elles ne sont pas arrivées au stade final de la spéciation. Toutes les espèces achevées différent morphologiquement l'une de la autre. Il n'y a pas (chez les Lépidoptères) d'espèces qui se différencient peu (par des caractères «ténus » vis-a-vis des autres espèces.

Mayr lui-même admet six catégories d'espèces inachevées et parmi celles-ci se trouvent aussi les espèces jumelles. Si ces « espèces » ne sont pas parvenues au stade final de la spéciation, est-il juste et logique de les considérer comme des espèces? Pour celles-ci il y a un grand nombre de catégories : semispecies, vice species, quasi species, etc., mais non pas species car les bonnes espèces « sont celles qui sont non seulement bien

isolées par une incompatibilité génétique, mais encore bien différenciées à la fois sur la plan morphologique et sur le plan écologique » (Jean Génermont et Maxime Lamotte).

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MESAPAMEA SECALELLA REMM DANS LA R. S. DE ROUMANIE ET QUELQUES RÉFLEXIONS SUR LA VALIDITÉ DE CE TAXON

LÁSZLÓ RÁKOSY

The author points out the existence in Romania of the taxa *Mesapamea secalella* Remm by investigating the genitalia of 89 individuals of *Mesapamea secalis* L. coming from different zones of the country.
On the basis of a critical interpretation of the works referring to *M. secalella*, it is considered rather premature to accept a specific taxonomic status as the differences pointed out in genitalia are not sufficient for the reproductive isolation.
The author moots, but at the same time, asks questions, the answers of which might clarify the taxonomic statute of *M. secalella*.

La description, en 1983, d'une nouvelle espèce du genre *Mesapamea*, un genre très bien étudié jusqu'au présent par de nombreux lépidopthérologues, parmi lesquels Heinicke [3], [4] dans ses travaux de révision, basés aussi sur les genitalia, a attiré l'attention des spécialistes consacrés dans l'étude des Noctuidae. Peu de temps après, la nouvelle espèce *Mesapamea secalella* Remm a été signalée dans nombreux pays européeens [1, 2, 5, 8].

Cette espèce a déclenché l'intérêt de nombreux spécialistes, mais personne n'a trouvé de caractères constants dans l'habitus, sur lesquels *M. secalella* puisse être séparée de *M. secalis* L., en faisant la détermination seulement sur les petites différences dans la structure des genitalia ♂ et ♀ (fig. 1, 2, 3, 4).

En vérifiant le matériel de notre collection, celui du Musée d'Histoire Naturelle de Sibiu, et de quelques collections privées (K. Bere, R. Nemes, E. Schneider), nous avons constaté aussi la présence du nouvel taxon en Roumanie. Nous avons examiné le graphisme et les genitalia chez 89 spécimens appartenant au genre *Mesapamea* et avons constaté que seulement huit appartiennent (d'après la genitalia) à l'espèce *M. secalella*.

Le matériel pour *M. secalis* L. provient de vingt habitats (19 de Roumanie et un de Deining — R.F.A.)

M. secalella Remm a été collecté à Hagieni (8.IX.82; 8.IX.84), sur le Mont Domogled (Banat) (16.VII. 1982), à Someşul Rece (14.VII. 1978), et Deining (R.F.A.) (13.VII. 1981).

On a pu collecter peu d'exemplaires et nous ne pouvons pas préciser si l'espèce est plus fréquente dans le Sud ou dans le centre du pays. Bien sûr que beaucoup d'habitats contiennent les deux taxons conspécifiques.

Bien que les différences trouvées dans l'armature génitale de H. Remm [7], bien complétées par L. Rezbanyai-Reser[8], permettent en général, la détermination exacte, dans le matériel étudié, nous avons trouvé aussi des formes intermédiaires, spécialement des femelles.

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La signalisation de *M. secalella* Remm au Nord [1], [5], au centre, [2], et au Sud de l'Europe (Mt. Baldo — Italie) (M. Gerstberger in. litt) consolide la position du nouvel taxon, mais nous considérons encore prématuré l'accordage du statut taxonomique spécifique.

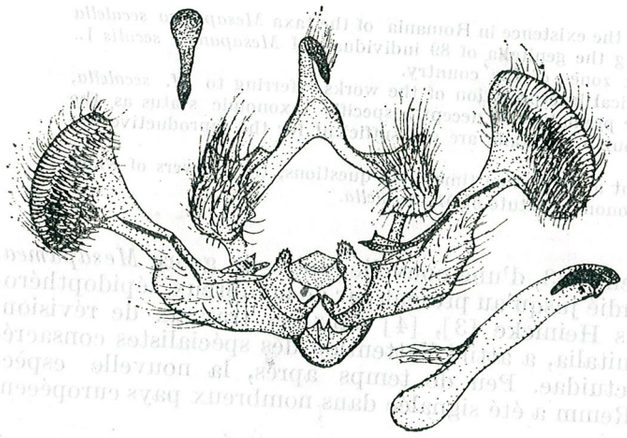

Fig. 1. — L'armature génitale ♂ chez *Mesapamea secalis* L.

En ce qui concerne les genitalia, nous considérons que les petites différences constatées n'assurent un isolement mécanique adéquat nécessaire à l'interfertilité. Pour accepter ou infirmer « l'espèce » de Remm, il ne reste qu'à attendre les résultats des recherches concernant l'isolement reproductif (écologique, éthologique, biochimique).

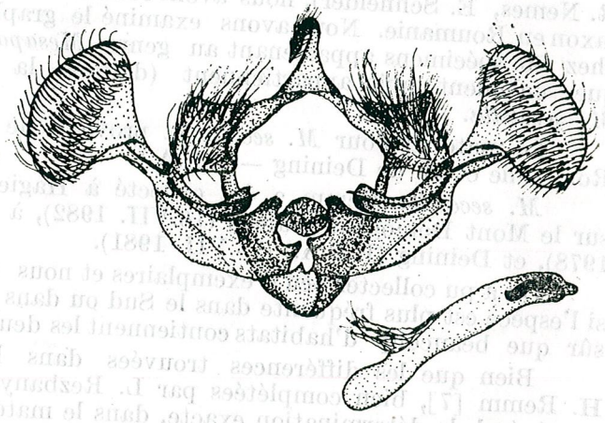

Fig. 2. — L'armature génitale ♂ chez *Mesapamea secalella* Remm.

En considérant les deux taxa comme espèces distinctes, le recouvrement de leurs aires de distribution en Europe met en discussion le problème de la spéciation. La grande ressemblance des deux espèces nous oblige

à considérer qu'elles se sont formées dans les conditions d'habitat très semblables. Or, de telles conditions ne peuvent apparaître dans des aires de distributions géographique séparées par des barrières physico-géographiques. Ainsi, nous devons invoquer comme modalité probable de la spéciation la spéciation sympatrique [6].

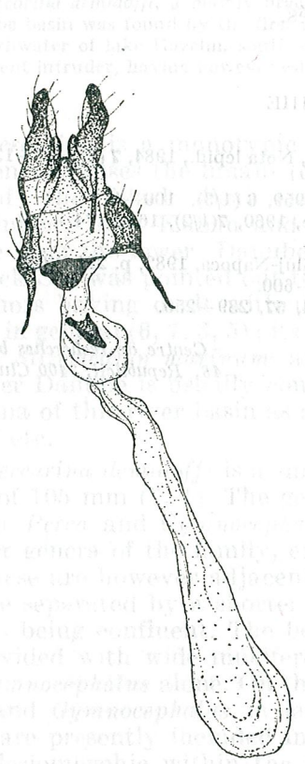

Fig. 3. —L'armature génitale ♀ chez *M. secalis* L.

Fig. 4. — L'armature génitale ♀ chez *M. secalella* Remm.

Un aspect important des deux « espèces », c'est le polymorphisme intraspécifique. On sait que le polymorphisme intraspécifique avantage l'espèce dont il s'agit, en permettant une meilleure intégration et utilisation de l'environnement. Mais dans le cas des deux « espèces », le polymorphisme intraspécifique très large est identique : toutes les formes décrites pour *M. secalis* L. ont été signalées aussi chez *M. secalella* R. Vu que les deux espèces vivent dans le même habitat, nous nous demandons ,naturellement, quels sont les avantages du polymorphisme identique.

Peut-être il serait plus correct de considérer la paire *secalis—secalella* comme une superespèce où l'isolement reproductif (pas encore démontré) se base sur des mécanismes subtils, qui n'impliquent pas nécessairement, l'isolement géographique.

Pour conclure, nous précisons que pour le moment, nous admettons, jusqu'à la preuve contraire, que *M. secalella* n'est pas bona species. Nous attendons, comme preuve de la héterospecificité, la constatation — sur le terrain — de l'isolement reproductif. Pour l'instant nous mentionnons notre l'observation, à savoir l'existence des formes intermédiaires entre les *secalis* et *secalella*, ce qui constituerait un argument pour soutenir notre thèse—la conspécificité des deux entités.

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FIRST RECORD OF *PERCARINA DEMIDOFFI* NORDMANN, 1840 FROM ROMANIA AND FROM THE DANUBE RIVER BASIN *(PISCES, PERCIDAE)*

VASILE OȚEL and PETRU BĂNĂRESCU

Percarina demidoffi, a mainly brackish-water fish not yet recorded in the Danube basin was found by the first author in great quantities in the almost pure freshwater of lake Razelm, south of the Danube Delta, where it is probably a recent intruder, having however established a permenent population.

Percarina is a monotypic genus of Percidae, the known range of which encompasses the limans (estuaries) of the rivers from the northern slope of the Black Sea, east of the Danube: Dniestr, southern Bug, Dniepr, Don, Kuban and the slightly-brackish Sea of Azov; its absence from the lower Danube and from adjacent freshe ned waters of the Black Sea was pointed out first by Antipa (2) and later confirmed by all authors having dealt with the fish fauna of Romania (8, 4) and of Europe in general (6, 7, 3, 5); its absence, as well as that of *Gymnocephalus acerina*, *Stizostedion marinum*, and *Rutilus frisii frisii* from the basin of the lower Danube is usually considered the main negative feature of the fish fauna of this river basin as against that of the more eastern rivers: Dniestr etc.

Percarina demidoffi is a small-sized fish, reaching a maximum total length of 105 mm (7, 1). The genus is to a certain measure intermediate between *Perca* and *Gymnocephalus* (= *Acerina*); it has, like Perca and all other genera of the family, except *Gymnocephalus*, two distinct dorsal fins; these are however adjacent in *Percarina*, while in the other genera they are separated by a shorter or longer distance in *Gymnocephalus* the two fins being confluent. The bones of the head, mainly the preopercle, are provided with wide muciferous cavities, this character being shared with *Gymnocephalus* alone. On the other hand, *Percarina* differs from both *Perca* and *Gymnocephalus* in lacking the predorsal boney. These three genera are presently included in a distinct tribe, Percini, considered the most plesiomorphic within the family Percidae (9, 10); because of the absence of the predorsal bone, *Percarina* is considered not as intermediate between the two other genera, but as representing the apomorphic sister of the pair *Perca-Gymnocephalus* (10).

The family Percidae is ascribed to the primary-freshwater division (12, 13), having reached its present distribution exclusively by continental routes; however, two of its species, *Percarina demidoffi* and *Stizostedion marinum* (ranging from the liman of the Dniestr to the northern freshened part of the Caspian Sea) are fully adapted to brackish water, being only rarely found in pure fresh water.

The first author was quite surprised when finding, in the late autumn of 1984, numerous specimens of *Percarina demidoffi* in the shallow lake Razelm, a part of the lagunar Razelm-Sinoe complex that lies south

of the Danube Delta, being connected through channels both with the southernmost arm of the Danube River (Sfîntu Gheorghe) and with the Black Sea. The salinity of the various components of the lagunar complex underwent strong variations during the last decades and years; the salinity of lake Razelm proper (Razelmul Mare), where *Percarina* was collected, decreased from 4.5 g NaCl/l in 1950—1952 to less than 0.5 g NaCl/1 in 1955—1956 (15); the salinity was only 0.12 — 0.18 g NaCl/l during the months of October and November 1984 when *Percarina* was collected, i.e. the lake was practically freshwater. The species is known to live mainly in slightly brackish water; it is even said to be restricted to such water (7), although it has also been recorded in pure freshwater in the southern Bug-Dniepr liman (1).

Two subspecies of *P. demidoffi* are accepted at present (6, 7 10): *P.d. demidoffi* from the limans of the Dniestr and of the southern Bug — Dniepr and *P. d. maeotica* from the Sea of Azov and the confluences of the Don and the Kuban rivers. The main difference between then concerns the squamation: the prepectoral region is naked in *demidoffi* and scaled in *maeotica*. Berg (7) mentions a further difference: eye diameter equal to preorbital length and 3.6—3.7 times in head length in *demidoffi*, smaller than preorbital and 3.7—4.8 times in head length in *maeotica*. Actually the size of the eye is subject to allometric variation, having little if any toxonomic significance. In the lake Razelm specimens the pre-pectoral region is naked; hence, they belong to the nominate subspecies *P. demidoffi demidoffi*.

Percarina demidoffi was collected from several stations both in the western and eastern areas of lake Razelm.

The problem raised is whether the species is an old inhabitant of the lake, hence being autochthonous in the Danube basin, or a recent intruder. Several authors, starting with Antipa (2) studied the fish fauna of this lake without finding the species; the most thorough investigations were conducted by R. Teodorescu-Leonte et al. in 1950—952 and 1955—1956 (15); the second author of this paper participated in the collecting trips from 1955 and 1956, without a single *Percarina* specimen and quite few young *Gymnocephalus cernuus* were collected. Intensive collecting of fishes in the lake was also undertaken by T. Nalbant between 1973 and 1983, again without finding any *Percarina*.

These facts suggest a recent penetration of *Percarina* in lake Razelm, either directly through the brackish waters of the Black Sea (the salinity of which decreases strongly by high-water) or from the Dniester through the Danube Delta (through recently built channels or with fry of valuable fish, carried from the liman of the Dniester into the lakes of the northern part of the Danube Delta.

Two other fish species, not known previously in the basin, of the Lower Danube have been recorded in lake Razelm, both in 1955 : *Rutilus rutilus heckeli* (a few specimens : 14), which probably belongs actually to *R. pigus*, and *R. frisii frisii* (a single specimen : 11). None of these were found again later in the lake; they were occasional intruders, not having established themselves in the lake. *Percarina* is probably a recent intruder, too; but it has been found in great numbers and it can be assumed that it has become a permanent inhabitant of the lake.

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XESTIA (AMATHES) C-NIGRUM L. *(LEPIDOPTERA-NOCTUIDAE)*: FIELD RESPONSE TO SYNTHETIC PHEROMONAL COMPOUNDS AND INFLUENCE OF TRAP DESIGN AND TRAP MAINTENANCE ON CAPTURE OF MALES

GH. STAN, I. COROIU, N. TOMESCU*, A. ONIȘOR**, CODRUȚA ROMAN **, I. OPREAN** and HILKE CIUPE**

In the field testing carried out in 1983—1984, Z—7—Tetradecenyl acetate (1 mg) had the highest attractactance for the males of *Xestia (Amathes) c-nigrum* L. as compared with E—7—Tetradecenyl acetate, or different mixtures of these two compounds. Z-5-Tetradecenyl acetate in mixture with Z—7—Tetradecenyl acetate, had a synergic effect. In 1984, the variants with the three pheromonal compounds were also attractive for the males of *Agrotis segetum* L. Increasing the level of E—7—Tetradecenyl acetate, in mixture with Z—7—Tetradecenyl acetate, determined a diminution in attractivity for both species. Z—11—Hexadecenyl acetate mixed with Z—7— Tetradecenyl acetate had no attractive influence for the males of *X.c-nigrum*. In 1983 testing, the specificity of the variants was 99 %. The period of response to the sex pheromone and the mating period were recorded during the second half of the night. Lower temperatures determined an early starting of sexual behavior in both sex. Three types of traps were tested, and the Montedison trap caught the largest number of males. The catch was influenced by the quality of the sticky surface and other factors. The duration of optimum attractivity of the baits was about 31 days. Z—7—Tetradecenyl acestate can be useful for detection, estimation and monitoring of the pest population.

Pheromone traps are frequently used for sampling and monitoring populations of economically important lepidopterous insects. The spotted cutworm, *Xestia (Amathes) c-nigrum* L., is a known pest on a large number of crops and noncrop plants (8), (9), (13), (14). The density of populations reaches high values, especially during the second flight (9), (13), (14). There also occur large variations from one generation to another, or from one year to another, due to less studied factors. The lack of host specificity, often determines large damages (8). Because it can be of major economic importance, it would be useful to have an attractant for the adults that could be used to determine population densities, predict infestations in a monitoring program and for control. In laboratory we studied the calling behavior and the influence of some factors upon it (20), (21). Z—7—Tetradecenyl acetate was identified as being a major component of the sexual pheromone (2), (21), (22). In field testings, the males were attracted by Z—7—Tetradecenyl acetate in most areas (2), (11), (19), (23) and in few cases by E—7—Tetradecenyl acetate (16). The crude sex pheromone extract had a high attractivity in laboratory (11), (20), but in field testings its attractivity was lower than that of the synthetic sex pheromone (19).

In the present paper, we report experimental results on the attractivity and specificity of the different variants with synthetic sex phero-

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mone compounds, in field experiments. The males caught were studied in relation with trap design and field maintenance of traps. Some aspects concerning the period of male response to sex pheromone and the mating period are presented as well.

MATERIALS AND METHODS

The sex pheromone compounds (Z7—14 : Ac, E7—14 : Ac. Z11—16 : Ac and Z5—14 : Ac) were synthetized in the Chemical Institute of Cluj-Napoca (Laboratory of natural products), by the acetilenic way (3), (4), (5), (12). The purity of the compounds was 95% and was determined GC on conventional columns (Carbowax 20 M, OV—17; 2 m×2 mm), and the isomeric purity was observed on capilary columns (Carbowax 20 M; 50 m×2 mm). The compounds were dissolved in methylen chloride. Separate sets of Penicillin-bottle rubber stoppers were charged with the sex pheromone compounds in different variants and ratios (Table 1). In the experiments we used sticky traps, made of "honey-comb-like" polyethylene (Focşani Plastic Mass Works), and glue made of polyizobutylene. All traps were fitted ca 1 m above the ground.

Table 1

Variants with synthetic sex pheromone compounds tested in field for *X. c-nigrum* on three experimental areas* (in 1983—1984).

Variants	Compounds**			
	Z7—14 :Ac	E7—14 : Ac	Z5—14 : Ac	Z11—16 : Ac
A	1000	—	—	—
B	—	1000	—	—
C	500	500	—	—
D	1000	—	—	10
E	1000	—	—	100
F	990	10	—	—
G	950	50	—	—
H	990	—	10	—

* the variants tested every year and the testing periods are presented in text;

** the dose is given in micrograme/bait; Z7—14 : Ac = Z—7— Tetradecenyl acetate; E7—14 : Ac = E—7 — Tetradecenyl acetate; Z5-14 : Ac = Z—5—Tetradecenyl acetate; Z11—16 : Ac = Z—11—Hexadecenyl acetate.

Researches were conducted in three areas neighbouring Cluj- Napoca city : the Horicultural Farm Someşeni (E—NE, 12 km away), "Înfrăţirea" Farm Cluj-Napoca (E—SE, 6 km away) and the Experimental Farm Floreşti (W—NW, 13 km away). All the areas were cultivated with vegetables, mostly carrot, parsley, tomato, onion and cabbage. The surface of each lot was larger than 50 ha.

The testing of the synthetic pheromonal compounds was done in all three areas, during both flights, in 1983 and 1984, using Montedison

traps with an effective trapping surface of 960 cm² (30 × 32cm). In testing experiments, two methods were used. In the first method, two blocks/lot of traps separated by 1000 m were established, and each block contained 3 or 4 traps/variant baited randomly. The traps were displayed in rows, at about 1 m above the ground and were separated by ca 50 m. The traps were checked every 2—3 days, males were collected and traps were rotated to the next trapping location after each examination. A rotation was considered one replication. In the second method, 2 traps/variant, at a distance of ca 100 m, were used. The traps with different variant were separated by 250—300 m. Captured males were collected daily and each trap with its bait was rotated each day from station to station, in the same manner. A daily rotation was considered one replication, as well. In all traps the soiled sticky surfaces were cleaned. Durind the second flight, a sticky surface of each trap was changed every 4 days.

The flight circadian period of males was studied by visual observations, every 15 minutes, during the whole 6 nights. The observations were done under different climatic conditions. For the study of the mating period, adults of the first generation, grown up in laboratory on artificial diet (19), were used. They were put in cages of two sizes (2×2×2 m and 30×30×30 cm), with walls of wire net. The observations were carried out in the same manner as for the males' response to sex pheromone. Alongside these observations we registered : temperature, wind speed, light intensity and rainfall. For temperature we considered the mean night temperature (°C), from 11 p.m. to 5.30 a.m.

The influence of the trap design on the number of captured males was studied on three types of traps : Montedison, Pherocon 1C and "mushroom" (7). For each type we used six traps with a trapping surface of 960 cm², 625 cm², and 600 cm², respectively, all of them baited with Z7—14: Ac. The traps were arranged alternatively on three rows ca 100 m distanced. Every 2—3 days the traps were checked, captured males were collected, and traps rotated after 4—6 days.

The influence of trap maintenance, for the catching of males had in view two aspects : duration of optimum attractivity of the pheromonal bait in field, and quality of the sticky surface of the trap. Montedison traps were arranged alternatively, in rows, at ca 100 m distance between rows, and ca 75 m between traps. For each tratment eight traps baited with Z7—14 : Ac were used. The traps were inspected every 2 days for each testing period, males were collected and traps were rotated. For the first aspect we carried out all experiments in 1983. Eight traps were baited with Z7—14 : Ac and unchanged for 50 days, and also, eight baited traps, were changed at every eight days. The optimum attractivity of the pheromonal bait was estimated in relation with the number of captured males. The second aspect was experimented in 1983—1984.

The catching of males was studied taking into account : new trap (new lid and new sticky surface), soiled trap with the sticky surface cleaned at different intervals and soiled trap with uncleaned surface. The catch of males was estimated depending on the density of populations.

The data were statistically calculated : significance coefficient ("t" test), attractivity, specificity.

RESULTS AND DISCUSSION

In our tests, for both flights, at least 6300, in 1983, and 4900, in 1984, males of spotted cutworm (*X. c-nigrum*) were captured in the synthetic pheromone baited traps. Field tests with different variants of synthetic pheromonal compounds and combinations, thereof revealed that males of *X. c-nigrum* were attracted in greatest number to Z7—14 :Ac (variant A). In 1983, the traps with D and E variants, also caught a large

Table 2

Males of *X,c-nigrum* caught in baited traps with different variants of synthetic sex pheromone compounds, tested in field (second flight, 1983).

Variant	Mean number of males /trap/night			
	Someşeni		Cluj-Napoca	
	26 July — 9 Aug.*	10— 16 Aug. **	26 July — 9 Aug. *	10— 16 Aug. **
A	10.43 a***	8.29 a	3.43 a	7.72 a
D	5.47 b	8.0 ab	4.50 b	6.15 b
E	7.40 ab	7.43 b	3.0 a	6.15 b

* 6 traps/variant; 14 nights; 6 replicates.
** 2 traps/variant; 6 nights; 6 replicates.
*** Means followed by the same letter are not significantly different at the 5% level according to "t" test.

number of males (Table 2). For both flights, the number of capturated males was large in all the three variants (Fig.1) and the differences were not significant.

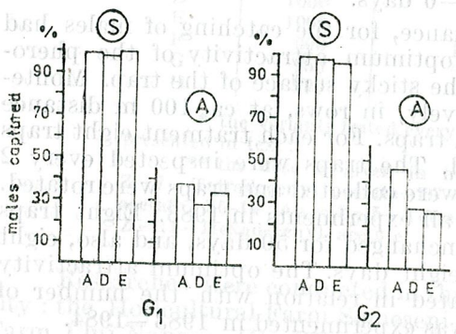

Fig. 1. — Attractivity (A) and specificity (S) of the variants with synthetic sex pheromone compounds tested in flield for *X. c-nigrum* at the first flight (G_1) : May— July, 1983, and the second flight (G_2) : Aug. — Oct., 1983.

In our experiments, Z11—16 : Ac, in mixture with Z7—14 : Ac, did not influence the attractivity of the latter, therefore, had no co-attractant effect. However, in field, a relatively large number of *X. c-nigrum* males were caught in traps with Z—11—16 : Ac (10), (23). In 1984, Z7—14 : Ac (variant A) was also more attractive than the others. During

the first flight, there were significant differences between variants, B and C being practically non-attractive (Table 3). For the second flight, the greatest number of males were attracted to Z7—14 : Ac and traps baited with E7—14 : Ac or a mixture of Z7—14 : Ac with E7—14 : Ac (1 : 1), caught significantly fewer males of *X.c-nigrum* as compared to the other variants. E7—14 : Ac was practically unattractive (Table 4). These data confirm the results previously obtained by us (19), (23), or other authors (2), (11). The attractivity of the mixture (Z) + (E) varied with

Table 3

X.c-nigrum males caught in baited traps with 5 variants of synthetic sex pheromone compounds tested in field in first flight, in two experimental areas 1984

Variant	Mean number of males /trap/night*	
	Someşeni	Cluj-Napoca
A	0.74 a**	1.54 a
B	0	0.02 b
C	0	0.10 c
F	0.28 b	0.21 c
G	0.10 b	0.27 c

* 8 traps/variant; 18 nights; 7 replicates.
** Means followed by the same letter are not significantly different at the 5% level according to "t"test.

Table 4

Males of *X.c-nigrum* and *A. segetum* caught in baited traps with synthetic sex pheromone compounds in second flight* at Someşeni, Aug. 18—30, 1984

Vari-ant	Number of males /trap/night($\bar{x} \pm SE$)**	
	Xestia c-nigrum	Agrotis segetum
A	23.6±3.7 a***	6.1 a
B	0	0.6 b
C	4.7±2.0 b	1.6 c
F	18.0±3.6 a	9.2 a
G	14.8±3.7 a	5.9 a
H	20.8±3.2 a	6.2 a

* 2 traps/ variant; 12 nights; 12 replicates.
** (mean ± standard error).
*** Means followed by the same letter are not significantly different at the 5% level according to "t" test.

the ratio of the two compounds. Increasing the level of E7—14 : Ac in the mixture (1%, 5% and 50%) reduced catches. The separate testing of the variants A and H confirmed that Z5—14 : Ac has a synergic effect (22), increasing the attractivity of Z7—14 : Ac, whereas the captures in trap with variant H were not significantly different from captures with variant A (Table 5). In experiments with variants A and H, 1098 males were caught in the traps baited with Z7—14 : Ac alone, and 1263 males in the traps with the mixture of Z7—14 : Ac and Z5—14 : Ac (99 : 1).

The specificity of all variants tested by us was greater than 99% in 1983 and 97% in 1984 (in the first flight). A very small number of males of a few other species* were caught in traps baited with variants for *X. c-nigrum*. There were caught males belonging to *Caradrina clavipalpis* Scop., *Hoplodrina blanda* Den. et Schiff. *H. alsines* Brahm.,

* We thank prof. L. Rakósy for the determination of species.

Mythimna albipuncta Den. et Schiff. *M. unipuncta* Haworth, *M. turca* L., *Oligia strigilis* L., *Agrotis segetum* L. and *A. ypsilon* L. During the second flight, in 1984, males of *Agrotis segetum* were captured in a large number, in traps baited with synthetic pheromonal compounds mixed in different variants, tested for *X.c- nigrum*, although they were not caught in the same experiments carried out in 1982—1983, and neither in light traps. Field testing evidenced the attractivity of some variants, with synthetic pheromonal compounds for the males of *A. segetum* (1), but the attractivity of Z7—14 : Ac, and of the mixture between Z— and E7 — 14 : Ac were not recorded. The greatest number of males were attracted by (Z) : (E) in a ratio of 99 : 1 (variant F ; Table 4). Thus, the attractivity was greater than that of the variant H, in which Z7—14 : Ac was mixed with Z5—14 : Ac. Increasing the level of E7—14 : Ac in mixture with Z7—14 : Ac reduced the captures for both *X. c-nigrum* and *A. segetum*, and E7—14 : Ac tested by itself had a little attractivity for the latter (Fig. 2).

Table 5

Males of *X.c-nigrum* caught in traps baited with Z7—14 : Ac and a mixture of Z7—14 : Ac and Z5—14 : Ac (99 : 1)

Variant	Number of males /trap/ night ($\bar{x} \pm SE$)*	
	Someşeni **	Floreşti***
A	15.1±3.0 a	24.5±1.6b
H	18.1±2.3 a	25.2±1.8b

* (mean ± standard error); Means followed by the same letter are not significantly different at the 5% level according to "t" test.
** 8 traps/variant; 7 nights — Aug. 31 — Sep. 7, 1984; 3 replicates.
*** 6 traps/variant; 5 nights — Sep. 1—6, 1984; 2 replicates.

In *X.c-nigrum* males, the circadian period of the response to the sexual pheromone, under natural conditions, and the mating circadian period were recorded during the second half of the night (Fig. 3). The behavioral responses were much influenced by temperature. Results of pheromone traps, showed that at low temperatures moths flew earlier, and the flight period was shorter than at higher temperatures. This aspect was recorded in other species, also (17), (18). There is evident that the behavioral responses depend on the fluctuations of the temperature during the flight, and on the temperature before the flight period (6), (24). This fact is also confirmed by our researches concerning the flight behavior, during a long period (1980—1984), in relation to other factors such as : light intensity, atmospheric pressure, relative humidity, rainfall, speed and direction

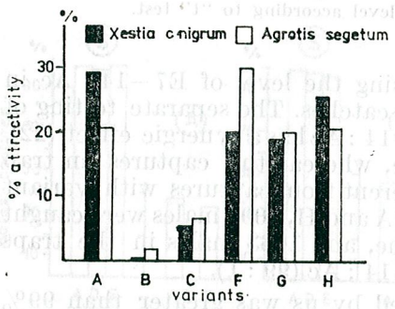

Fig. 2. — Field attraction of *X.c-nigrum* and *A. segetum* males to the variants with synthetic sex pheromone compounds. Someşeni, Aug. 10—30, 1984.

of wind, moon phases (Stan et al., unpublished data). We also observed, that cloudy or clear nights did not influence the start of the "calling", the flight period and mating. The data confirm part of the results we obtained in labratory (21). These facts suggest that the flight behavior of *X. c-nigrum* males is mediated by internal factors and influenced by external ones, especially by temperature.

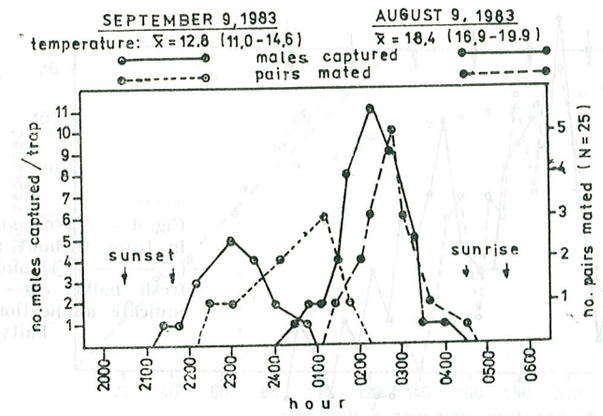

Fig. 3. — Periodicity of male response to synthetic sex pheromone and periodicity of mating activity in field, for *X. c-nigrum*.

The use of pheromone traps in monitoring pest insects showed that th· results depended also on trap design, trap placement, maintenance in field and trap density (15). In our testing for *X.c-nigrum*, the Montedison trap caught a larger number of males than Pherocon 1C and "mushroom" (Table 6), but the differences were in relation with the density of populations and correlated with the size of the adhesive catch surface. The Montedison traps had a larger catching surface. In the first flight, when the density was low, the captures with Montedison traps were not significantly different.

Table 6

The catch of *X.c-nigrum* males in 3 kinds of sticky traps baited with 1 mg Z7—14 : Ac, Someşeni, 1983

Kind of pheromone trap	Mean no. males/trap*	
	Aug. 2—12	Sept. 7—17
Montedison	48.5 a	128.7 a
Pherocon 1C	44.6 a	107.3 ab
"mushroom"	42.8 a	89.7 b

* Means followed by the same letter are not significantly different at the 5% level according to "t" test.

The period of the optimum attractivity of the bait with Z7—14 : Ac was ca 31 days (Fig. 4). In fact, the attractivity of the bait continued over this period, but in traps with changed baits the catch diminished after 31 days since the beginning of the experiment.

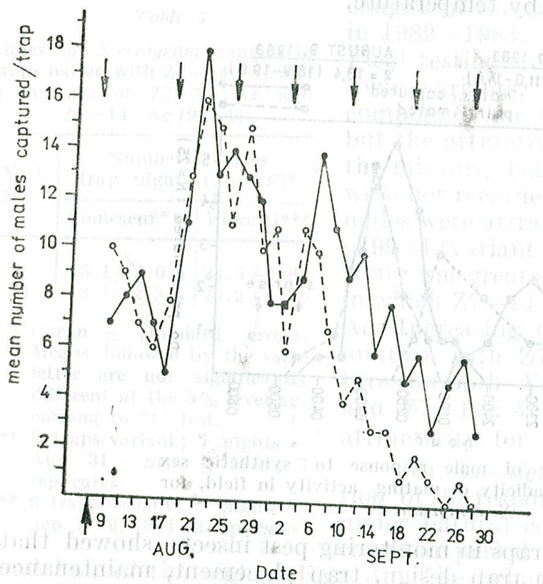

Fig. 4. — *X.c-nigrum* males caught in traps without replaced baits (○———○) and traps with fresh baits (●—●). Arrows indicate applications of fresh baits.

Depending on the conditions of the catch surface, the number of *X.c-nigrum* males caught in traps varied. In general, there were no differences between new traps and those whose sticky surface was cleaned every 2—4 days, but there were differences in respect with soiled traps with uncleaned sticky surface (Table 7). The density of *X.c-nigrum* populations influenced the results. During 24 days (July 27—Aug. 20, 1983), in a low density population, there were small differences among

Table 7

The catch of *X.c.-nigrum* males in Montedison traps under different conditions, Someşeni, Aug. 14—28, 1984

Trap condition	Mean no. males captured/traps	Difference (%) from catches in new traps
New trap	145.5	
Soiled trap, with adhesive cleaned every 2 days	139.2	— 4.30
Soiled trap, with adhesive cleaned every 4 days	137.7	— 5.40
Soiled trap with adhesive not cleaned	69.7	—47.0

captures with soiled traps with sticky surface cleaned at every 7 days for a period of 14 days. In this case we calculated the total cumulative number of males and observed that the catch increased by the same ratio up to 40—45 males, after which, on the following days, the catch diminished in soiled traps with cleaned sticky surface, although the surface was not completely covered. During the same interval of time, in

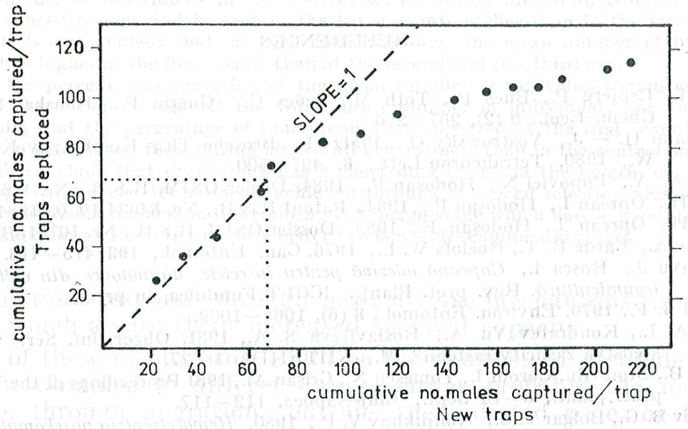

Fig. 5. — The catch of *X. c-nigrum* males in new Montedison traps and replaced traps (soiled trap with adhesive cleaned every 2 days). Someşeni, Sep. 3—17, 1983.

soiled traps with uncleaned surface, no more males were captured after 14 days, although the sticky surface was not covered with moths. Probably, the dead insects captured represent a repellent olfactory factor for the live insects.

In a high density population, during 14 days (Sept. 3—17, 1983), in soiled traps with the sticky surface cleaned every two days, the number of captured males diminished greatly after 8 days, as compared to the new traps. The catch increased by the same ratio up to a cumulative number of 70—80 males, after which the number of males caught in traps with cleaned sticky surface diminished (Fig. 5). The results show that the loss in trapping efficiency is determined by the damage of the sticky surface. If we compare the number of males captured in soiled traps with uncleaned glue to the new traps, we notice that the catches increased by the same ratio up to a cumulative number of 50—55 males, during 4—5 days, after which the soiled traps caught no more males. This fact was brought about by the total covering of the sticky surface, and it seems to be necessary to clean the surface at shorter intervals than for other species [15]. On the other hand, we consider that the catch is influenced by the size of the catching surface, size of the males, density of populations, quality and quantity of glue and climatic factors.

We used pheromonal traps for detection and estimation of adult populations of *X.c-nigrum* for several years running in the same territory

of the areal. We obtained data concerning the ecology and behavior of the species, correlated with the climatic and biotic factors and the characteristics of the agroecosystems (unpublished data). We think that Z7—14 : Ac can be useful for sampling and monitoring of *X.c-nigrum* populations, of course, considering all the factors which influence the captures with pheromone traps.

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INFLUENCE OF DISULFOTONE ON THE LENGTH OF THE DEVELOPMENTAL CYCLE IN *DROSOPHILA MELANOGASTER*

N. COMAN and MANUELA DORDEA

The influence of disulfotone (concentrations of 1/900000, 1/600000 and 1/300000) on the length of the developmental cycle in *Drosophila melanogaster* was studied. The number of individuals in the control set is, during almost all generations, at all concentrations and in each of the three counts higher than in the treated flies. It is also evident that, at each concentrations, the mean number of individuals is higher at the first count, than at the second and the third one. From the percent representation of the mean number of individuals from each of the three counts, against the total mean number of individuals per vials, it results that the percentage of individuals that appeared at the first count is higher in the treated flies, during almost all generations, than in the control ones. We can conclude that disulfotone has a shortening effect on the length of the developmental cycle. Its action is achieved through enhanced selection pressure, those individuals succeeding in attaining the imago stage which had a hereditary load that allowed them to overpass faster the larval developmental stages.

Populations respond through most diverse mechanisms to selective pressures, which assure them increased survival capacity.

One of these mechanisms could be the avoiding of pesticide action, that can be obtained in different ways: either probably the most frequent one, through migration outside the infested zone, or through the favouring of individuals which possess enzymes with metabolizing or annihilating effect of the pesticide.

Disulfotone, the pesticide with which the experiment was performed, is an organo-phosphoric insecticide (0,0-dietyl-S-2-etyl- mercaptoetyl-diosulphate). The pesticide is utilized in agriculture as a systemic insecticide for fighting against a great number of injurious insects. It is very toxic for homeothermic animals, acting as a neurotoxic by blocking of cholinesterase. This effect can be diminished by the administration of atropine (6).

The utilization of disulfotone up to the present led to positive results in the protection of sugar beet (24), wheat, barley (3), rice (7), tobacco (8), pea (21) crops, although sometimes phytotoxic effects (5). Its remanence of 4—10 weeks (2,17) might have negative effects on herbivorous animals and on man (5). Not being selective enough, disulfotone might destroy beneficial insects, too (8). On account of its great toxicity to mammals, it can be used also as a raticide (20).

Its toxic properties appear as dependant on sex. For instance, females of rats are, on average, five times more sensitive than males (22).

The multitude of the disulfotone effects known up to date implies a profound knowledge of its modalities of action. Of great interest is the study of sublethal doses on non-target species. In a previous paper (10) we presented aspects regarding the mode of action of disulfotone on a population of *Drosophila melanogaster* and the degree of its recovering

after the cessation of pesticide administration. The results showed that after treatment the population density decreased, the effect being more pronounced as the disulfotone concentration increased. After ceasing the pesticide administration the population density tends to reach the level of the control. The higher the concentration of disulfotone the slower the recovery.

The present experiment was carried out in an attempt to clear the effect of disulfotone on the length of the developmental cycle, since a lot of studies revealed that populations exposed to higher selection pressures develop different mechanisms that lead to an enhanced degree of survival from one successive generation to another.

Dawood et al. (12—14) revealed that viability in *Drosophila melanogaster* depends on its density and on homo- or heterozygosity, the heterozygotes being more advantaged during competition, on account of their faster development. Dyer et al. (15, 16) concluded that after treatment with low doses of radiation, the competitive capacity of *Drosophila melanogaster* rises through the shortening of the larval development, concomitantly with the extending of the adult longevity. Coman and Wallace (11) showed that the effect of selection was more pregnant during the first six generations, afterwards the pressure lowers, so that the number of progeny rises.

The possibility of inducing resistance to pesticide in *Diptera* populations (4,18) and especially in *Drosophilidae* (9,23) indicated also the possibility of appearance of the mechanisms leading to the increasing of the degree of survival.

MATERIALS AND METHODS

Disulfotone, firstly dissolved in alcohol was included in the "White medium" with semoline, having a temperature of 40°C, so that the concentration of pesticide attained 1/900000 for the first set, 1/600000 for the second and 1/300000 for the third set of experiments, the fourth, without disulfotone being the control one. For each set, ten culture vials were used. In each of the forty vials five pairs of homozygote *Drosophila melanogaster*, wild strain from Riverside, California, were placed. After 48 hours, the parents were discarded. The progeny was counted on the tenth, twelfth and fourteenth day after the placing in the vials.

From the progeny resulted on the tenth day, parents were chosen and placed in vials to obtain the next generation. The procedure was repeated during six generations. Beginning with the seventh generation, disulfotone was removed from the medium, the other conditions remaining constant. The entire experiment was carried out at 25° C.

RESULTS AND DISCUSSIONS

The mean number of individuals from the ten vials used for each treated set, as compared to the mean number of the control set is plotted in Figs 1, 2, 3. The mean number of individuals from the three counts

was represented on the ordinate, and the number of generations, on the abscissa. As can be seen, the number of control individuals is, during all generations, at all concentrations and in each of the three counts, higher than from the treated set. It is also evident that, to each concentration, the mean number of individuals is higher at the first count, than at the second and the third one.

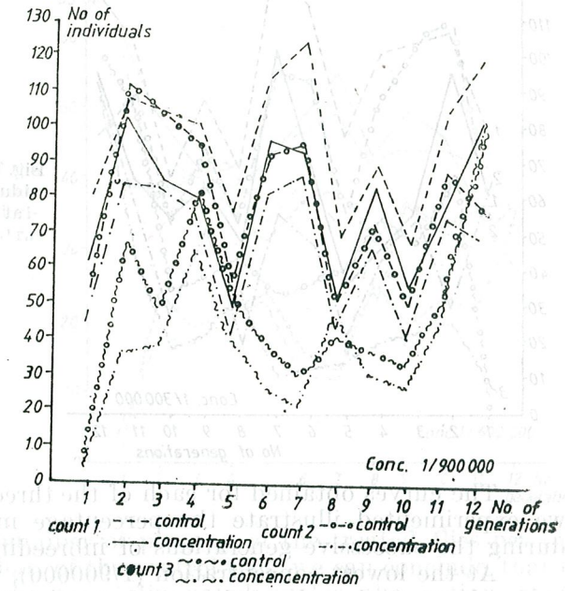

Fig. 1. — The mean number of individuals in each of the three counts at a concentration of 1/900000.

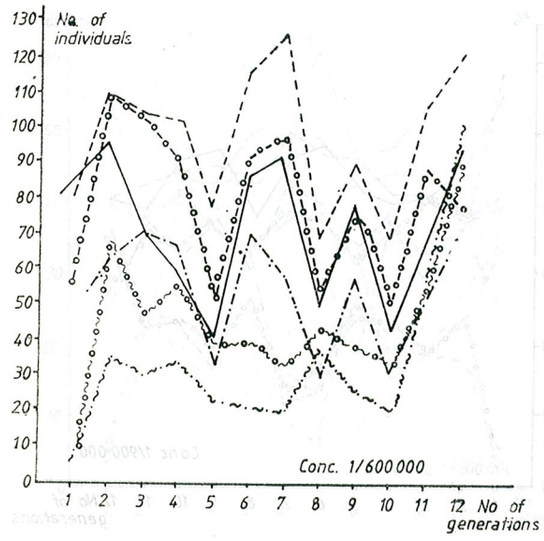

Fig. 2.—The mean number of individuals in each of the three counts at a concentration of 1/600000.

In order to emphasize a possible modifying effect of disulfotone on the length of the developmental cycle, we estimated the percent of the mean number of individuals from each of the three counts, against the total mean number of individuals per vials.

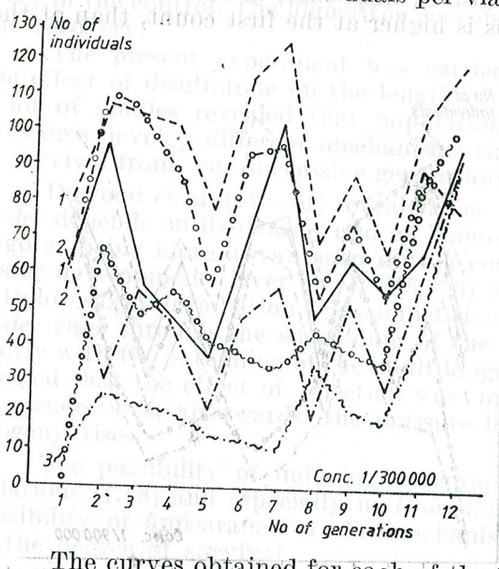

Fig. 3. — The mean number of individuals in each of the three counts at a concentration of 1/300000.

The curves obtained for each of the three concentrations with which we experimented illustrate the percentage mentioned above (ordinate) during the successive generations of inbreeding (abscissa) (Figs 4, 5, 6).

At the lowest concentration (1/900000), no significant modification in developmental cycle of the treated population (Fig. 4), as referred to

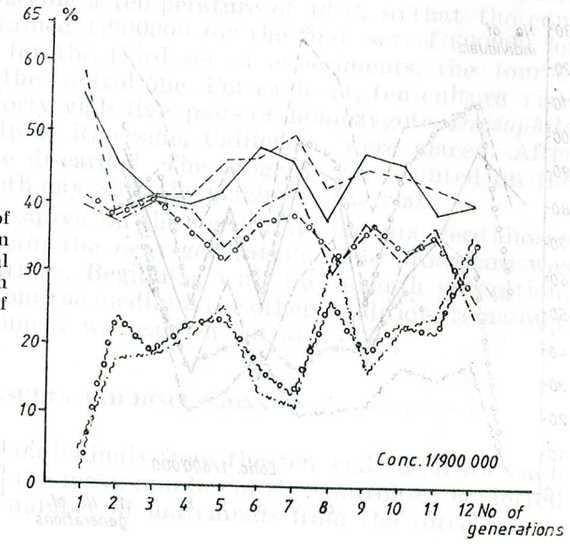

Fig. 4. — The percent value of the number of individuals in each count, against the total number of individuals of each generation (concentration of 1/900000).

the control, was noticed. At higher concentrations, (Fig. 5 and especially Fig. 6) the percentage of individuals appeared at the first count is higher in the treated flies, almost in all generations, than in the control ones. It means that disulfotone has a shortening effect on the length of the developmental cycle.

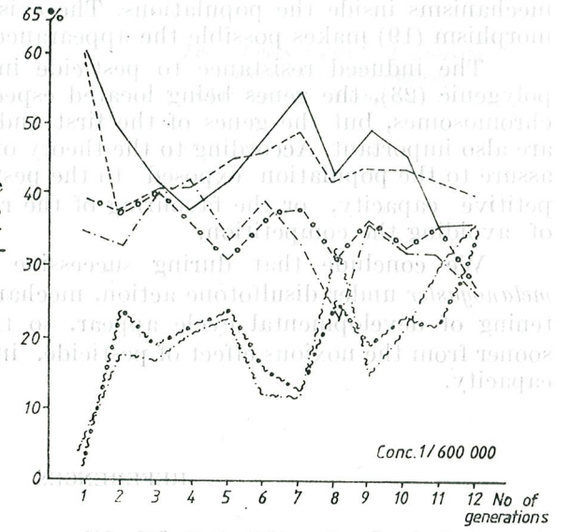

Fig. 5. — The percent value of the number of individuals in each count, against the total number of individuals of each generation (concentration of 1/600000).

Knowing that the number of progeny of the treated flies per total (Figs 1—3) is less than that of the control flies, we can conclude that the more sensitive individuals were eliminated under the action of the

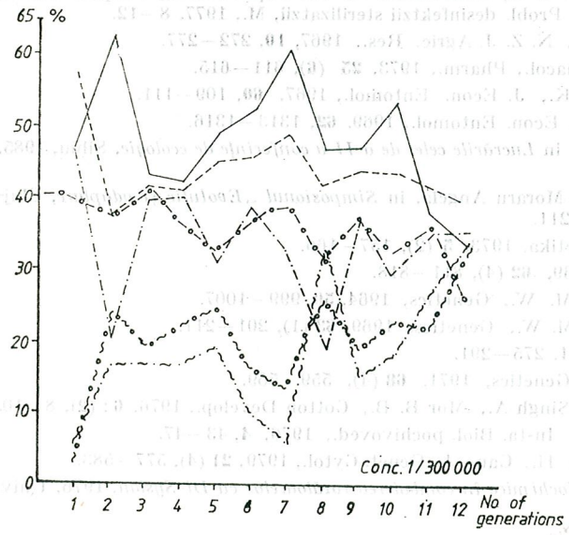

Fig. 6. — The percent value of the number of individuals in each count, against the total number of individuals of each generation (concentration of 1/300000).

pesticide, surviving only those with preadaptations which allowed them a faster development.

Under high selection pressure (concentration of 1/300000), those individuals succeeded in attaining the imago stage which had a hereditary load that permitted them to overpass faster the larval developmental stages. Our results support Wallace's point of view (25) of soft selection mechanisms inside the populations. The existence of a population polymorphism (19) makes possible the appearance of such mechanisms.

The induced resistance to pesticide in *Drosophila melanogaster* is polygenic (23), the genes being located especially on the second pair of chromosomes, but the genes of the first and third pair of chromosomes are also important. According to the theory of Ayala (1) such mechanisms assure to the population exposed to the pesticide, either a positive competitive capacity, or the favouring of the resistant individuals, capable of avoiding the competition.

We conclude that during successive generations of *Drosophila melanogaster* under disulfotone action, mechanisms that induce the shortening of developmental cycle appear, so that the populations escape sooner from the noxious effect of pesticide, increasing thus their survival capacity.

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ÉTUDES ÉCOLOGIQUES SUR LES *ORIBATES* (*ACARINA : ORIBATEI*) D'UN PÂTURAGE DE *LOLIUM PERENNE* ET *POA BULBOSA*

MAGDA CĂLUGĂR et N. VASILIU

The authors analyse the seasonal dynamics of the Orbatid Mites in a secondary mesoxerophyllous meadow. It was found that during the investigated year thesemites have not had a seasonal migration on the vertical. It was also remarked that some species have maximum populations of adults in the winter period and others in spring and beginning of summer.

Ce travail est consacré à l'analyse de la dynamique saisonnière de la communauté *d'Oribates* d'un pâturage de la vallée du Miletin (alt. 140 m) du bassin Prut (département d'Iași).

CARACTÉRISTIQUES DU BIOTOPE*

Le biotope étudié est une formation secondaire de steppe mésoxérophyl le, employée comme pâturage.

Le climat tempéré continental à nuances excessives a la moyenne de température annuelle de 8,9° C, les précipitations atmosphériques annuelles de 456 mm et les vents dominants du nord-ouest et nord (tableau 1).

Le sol est un tchernoziom, faiblement acide, riche en humus.

La végétation est caractérisée par une formation de *Lolium perenne* et *Poa bulbosa* avec de petites îles de *Botriochloa ischaemum* et *Artemisia austriaca*.

MATÉRIEL ET MÉTHODES

On a prélevé, mensuellement, au cours de l'année 1972, des quadrats de sol herbeux de 400 cm². Chaque quadrat a été ensuite divisé verticalement en trois parties : la couche superficielle herbeuse ; la couche de sol de 5—10 cm de profondeur ; la couche de sol de 10—20 cm de profondeur.

L'extraction de la mésofaune a été realisée au laboratoire dans des entonnoirs de Berlese — Tullgrenn. Ainsi on a obtenu 39,994 individus d'*Oribates*.

La structure de la communauté d'*Oribates* a été appreciée à l'aide des indices suivants : constance ; densité relative (Balogh, 1958 ; Dajoz, 1971) et l'indice de diversité Shannon — Wiener (Dajet, 1976 ; Lions, 1975, 1977).

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* Nous remercions les dr. Davidesco G., dr. Ștefan N. et dr. Chifu Th. pour les données du sol, du climat et de la végétation.

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Tableau 1

Valeurs des facteurs climatiques de l'année 1972*

Facteurs climatique	J	F	M	A	M	J	J	A	S	O	N	D
Précipitations atmosphériques (quantités mensuelles en mm)	13,4	9,7	13,8	51,7	50,3	91,5	64,2	162,3	74,4	98,3	41,9	6,5
Nombre de jours avec la couche de neige au sol	17	2	6	—	—	—	—	—	—	—	3	8
Humidité relative de l'air (moyennes mensuelles — %)	84	85	74	72	74	70	78	83	82	88	88	94
Température de l'air (moyennes mensuelles — °C)	—7,0	—1,6	3,4	12,7	16,2	20,3	21,5	19,8	13,9	7,4	5,6	—0,2
Température de la surface du sol (moyennes mensuelles — °C)	—6,8	1,4	3,8	14,5	19,2	24,9	24,6	22,1	15,2	7,5	4,4	—0,6
Nombre de jours avec les températures de l'air négatives	29	22	21	1	—	—	—	—	1	4	16	21

* D'après dr. G. Davidesco

Fig. 1. — Analyse synécologique en dynamique mensuelle de la communauté d'Oribates.

RÉSULTATS

Dans l'intervalle étudié, on a identifié 45 espèces ayant des particularités en ce qui concerne la distribution dans le temps et l'espace (fig. 1).

On a constaté que la plupart des espèces — adultes et immatures — peuplent la couche herbeuse du sol et c'est seulement accidentellement, qu'ils parviennent dans les couches profondes (fig. 2). Ce sont seulement *Epilohmannia cylindrica, Perlohmannia dissimilis* et *Ceratozetella thienemanni* qui ont eu le comportement des espèces édaphobiontes permanentes. C'est pourquoi nous avons présenté les résultats d'une manière globale et non différenciée sur couches.

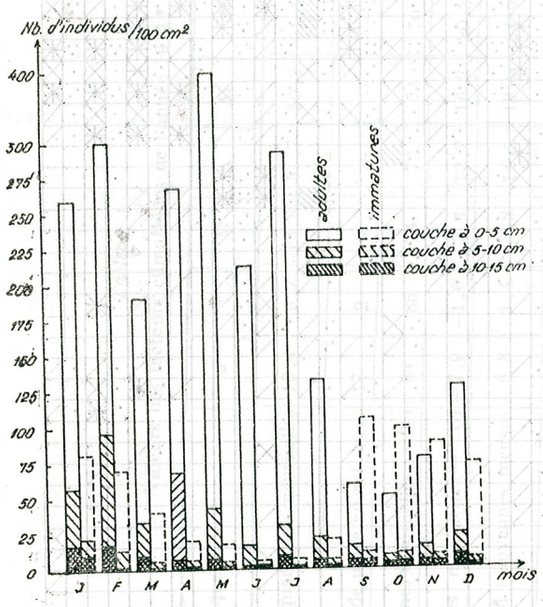

Fig. 2. — Distribution verticale et les fluctuations mensuelles de la densité numérique de la communauté d'Oribates.

Les adultes ont eu, en général, deux maximums de densité (au mois de février et au mois de mai) et un minimum (dans l'intervalle septembre — octobre), qui a coïncidé à l'époque, où les immatures ont été les plus nombreux.

L'analyse cénologique (fig. 1) met en évidence le fait qu'un pourcentage important de ces valeurs globales (une moyenne de 70,03% du total) revient aux espèces dominantes (une moyenne de 20,00% du total des espèces). Le nombre des espèces à valeurs de dominantes a été plus élevé dans les intervalles mars — avril et octobre — novembre. C'est au mois de juillet que leur nombre a été le plus bas — quatre espèces ayant presque 70% du total des individus.

Au cours de l'année (fig. 3), le groupe dominant n'a pas été constitué par les mêmes espèces. En hiver, les espèces dominantes sont *Peloptulus phaenotus, Ceratozetella thienemanni, Epilohmannia cylindrica, Lieb-*

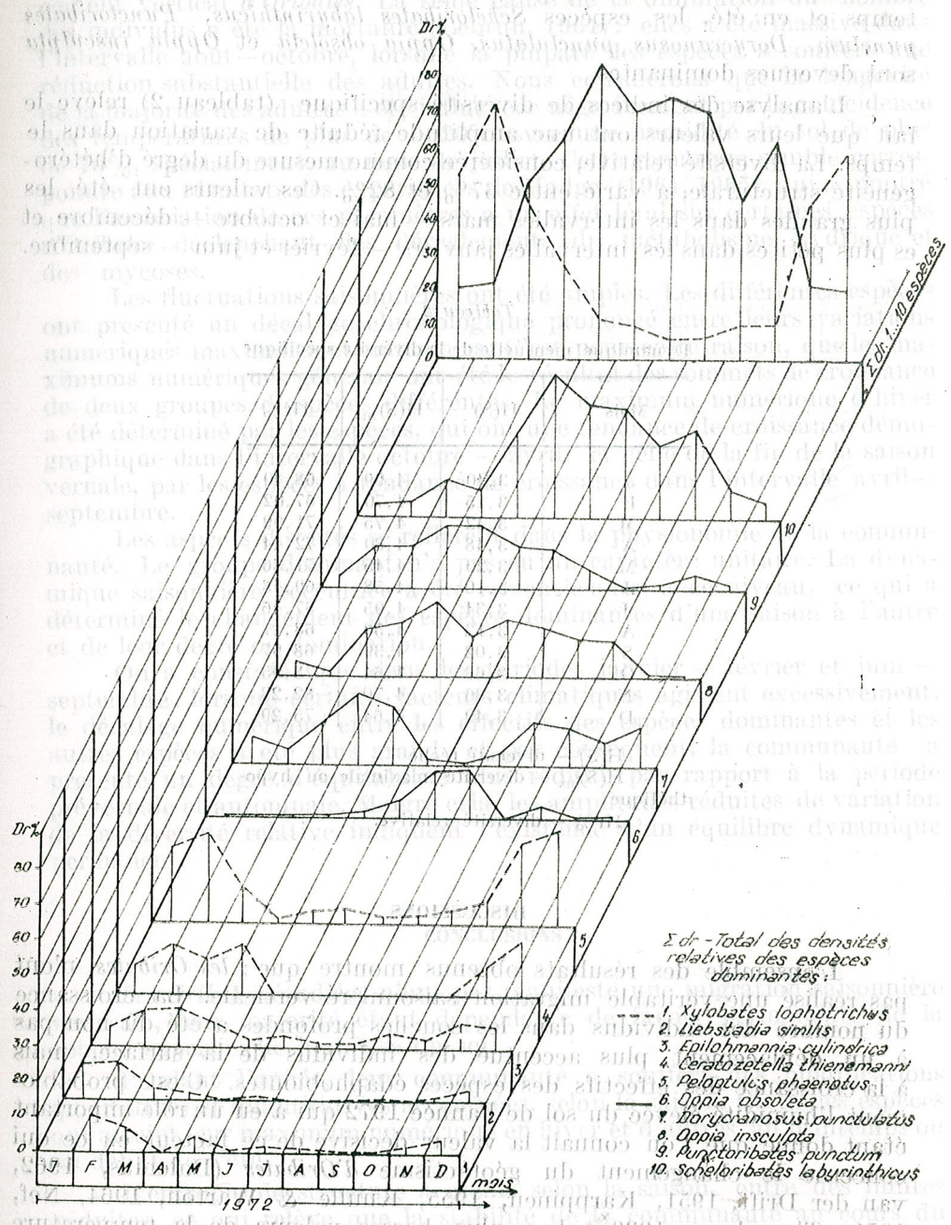

Fig. 3. — Fluctuations mensuelles des espèces d'*Oribates dominantes*.

stadia similis et *Xylobates lophotrichus*, dont les adultes ont ensuite diminué graduellement avec l'élévation de la température et ont presenté un minimum de la densité dans l'intervalle mai — septembre. Au printemps et en été, les espèces *Scheloribates labyrinthicus*, *Punctoribates punctum*, *Dorycranosus punctulatus*, *Oppia obsoleta* et *Oppia insculpta* sont devenues dominantes.

L'analyse des indices de diversité specifique (tableau 2) relève le fait que leurs valeurs ont une amplitude réduite de variation dans le temps. La diversité relative, considérée comme mesure du degré d'hétérogénéité structurale, a varié entre 67% et 82%. Ces valeurs ont été les plus grandes dans les intervalles mars — mai et octobre — décembre et es plus petites dans les intervalles janviers — février et juin — septembre.

Tableau 2

Dynamique mensuelle de la diversité spécifique

Mois	H(S)	$H(S)_{mx}$	Hr%
J	3,40	4,99	68,40
F	3,15	4,70	67,02
M	3,42	4,75	71,99
A	3,48	4,80	72,44
M	3,20	4,45	71,86
J	3,10	4,58	69,48
J	3,34	4,95	67,46
A	3,12	4,58	68,11
S	3,02	4,39	68,97
O	3,66	4,64	78,95
N	3,86	4,70	82,28
D	3,49	4,64	75,29

H(S) — diversité réelle

$H(S)_{mx}$ — diversité maximale ou hypothétique

Hr% — diversité relative.

DISCUSSIONS

L'ensemble des résultats obtenus montre que : *les Oribates* n'ont pas réalisé une véritable migration saisonnière verticale. La croissance du nombre des individus dans les couches profondes a été dû non pas à un déplacement plus accentué des individus de la surface, mais à la croissance des effectifs des espèces édaphobiontes. C'est probablement l'humidité élevée du sol de l'année 1972 qui a eu un rôle important étant donné que l'on connaît la valeur décisive de ce facteur en ce qui concerne le changement du géotactisme *d'Oribates* (Dalenius, 1962, van der Drift, 1951, Karppinen, 1955, Knülle & Warton, 1964, Nef, 1971, Thamdrup, 1939). Les plus basses valeurs de la température négative du sol enregistrées en janvier (la valeur minimale absolue de

—22,7° C le 27 janvier) et les plus élevées valeurs de la température positive enregistrées en juin et en juillet (la valeur maximale absolue de + 58,5° C le 7 juin), n'ont pas déterminé une intensification du déplacement vertical *d'Oribates*. La seule cause de la diminution du nombre des individus a été la mortalité (Lebrun, 1964) : elles a été massive dans l'intervalle août—octobre, lorsque la plupart des espèces a souffert une réduction substantielle des adultes. Nous considérons que la longévité de la majorité des adultes a été influencée négativement par la coïncidence des températures de plus de + 20°C avec une humidité du sol de plus de 70%, spécialement, au mois de juillet. Cette remarque semble correspondre aux résultats des expériences de Madge (1964, 1965), qui a montré que l'association de ces paramètres a un effet létal sur certaines espèces *d'Oribates* déclenchant des dérèglements du métabolisme hydrique et des mycoses.

Les fluctuations saisonnières ont été amples. Les différentes espèces ont présenté un décalage chronologique prononcé entre leurs variations numériques maximales et minimales. C'est pour cette raison, que les maximums numériques généraux ont été le résultat des sommets de croissance de deux groupes d'espèces différentes. Le maximum numérique d'hiver a été déterminé par les espèces, qui ont une tendance de croissance démographique dans l'intervalle octobre — avril, et celle de la fin de la saison vernale, par les espèces à tendance de croissance dans l'intervalle avril—septembre.

Les aspects discutés se reflètent dans la physionomie de la communauté. Le groupe dominant n'a pas eu un caractère unitaire. La dynamique saisonnière accentuée a été ressentie aussi à ce niveau, ce qui a déterminé le changement des espèces dominantes d'une saison à l'autre et de leur degré de domination,

On a constaté que dans les périodes janvier — février et juin — septembre, lorsque certains facteurs climatiques agissent excessivement, le décalage numérique entre les effectifs des espèces dominantes et les autres espèces a été plus grand et par conséquent, la communauté a presenté un degré d'équitabilité plus réduite par rapport à la période prévernale et automnale. Malgré cela, les amplitudes réduites de variation de la diversité relative indiquent l'existence d'un équilibre dynamique permanent.

CONCLUSIONS

Les Oribates étudiées n'ont pas manifesté une migration saisonnière verticale, leur majorité étant dépendente de façon permanente de la couche superficielle herbeuse du sol.

Pendant l'année, leur communauté a souffert des modifications qualitatives et quantitatives accentuées, selon la saison. Certaines espèces ont atteint leur maximum numérique en hiver et d'autres, au printemps ou au début de l'été.

L'entropie de structure a oscillé selon la saison, entre des limites réduites, ce qui relève que la stabilité de la communauté au cours du temps, se réalise par les mécanismes de réglage de chaque population.

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PHOSPHATASES IN *CRASSOSTREA ANGULATA* (Lmk) OF THE SPANISH SOUTH ATLANTIC COAST

M. L. GONZALEZ DE CANALES GARCIA and M. P. MARTIN DEL RIO

The enzymatic activity of phosphomonoesterases in different anatomical parts of the marine mollusc *Crassostrea angulata* (Lmk) has been studied seasonally. This activity was studied in samples collected in the Cádiz (Spain). To determine alkaline phosphatase activity the method described by Klein et al. (1970) was used. To determine total acid phosphatase activity Babson's method (1960) was used.

Generally speaking, high activity has been observed in the digestive system (59.10 mcg of phenolphthalein and 6.90 Babson units). Activity is lower in the branchiae (12.26 and 0.8) and hardly seen in the labial palpi and adductor muscle.

The presence of phosphomonoesterase in different invertebrates having been demonstrated (4), (15), (1), we undertook the study of their presence and distribution in *Crassostrea angulata* (Lmk), species commonly found along sur coast and considered a gastronomic delicacy. Our objetive was to obtain a pattern as a control in cases of natural illness, with controlled feeding and experimental pollution studies.

Alkaline phosphatase (E.C.3.1.3.1) is an enzyme, the physiological role of which is still under debate (9), (3). However, acid phosphatase has been assigned a role in both intra- and extracellular digestive processes. Both enzymes modify their activity in pathological processes.

MATERIAL AND METHODS

The specimens of *Crassostrea angulata* used in our work came from the natural sand bank of Sánlucar de Barrameda (Cádiz). The mean values of the physico-chemical parameters of the water from which the live spacimens came were the following: oxygen = 6.025 mg/l; nitrites = 0.472 mg atN/l; sulfides = none; pH = 7.60; salinity = 36.353‰, temperature = 20.3°C. The age of the specimens ranged from 14 to 16 months.

A total of 302 animals were used, of which 159 were females and 143 were males. The mean weight, without the shell, was of 5.60 ± 0.24 g.

Fresh gonads were studied both macro- and microscopically. In macroscopic study, the volume and consistency of the gonad was assessed, as was the resistance offered by the gametes to extraction when pressed with a glass rod. Sex, size and aspect of the ovocytes in females, and sperm motility in males, were determined microscopically. For this, a small piece of gonad was crushed between the slide and cover glass with a drop of sea water (13). The individual specimens were classified on the basis of gonad maturation, into stages 1,2,3 and 4, defined as repose, gametogenesis, maturity and spawning, respectively (Table 1).

The "in vivo" study of the enzymatic activity of the phosphomonoesterases was carried out in both sexes in different parts of the anatomy. The animals were sacrificed by detaching their valves. The following parts were obtained from each animal by dissection : labial palpi, mantle, adductor muscle, branchiae and digestive system (mouth, esophagus, stomach, crystalline style, intestine and gastric diverticules) which also includes the gonad.

Table 1

Stages of the gonad throughout the year

Stage	Nos. of specimens: Females	Nos. of specimens: Males	State of gonad	Months
1	38	30	Sexual repose	December January February
2	35	39	Gametogenesis	March April May
3	54	46	Maturation and spawning	June July August
4	32	28	Spawning and sexual repose	September October November

To determine enzymatic activities, the same structures from 4 specimens were homogenized together, and mixed with twice-distilled water in the ratio 1 :10. The mixture thus obtained was kept for approximately 15 h at 4°C. The samples were then centrifuged at 4000 r.p.m. for 5 min. The supernatant was diluted again with twice-distilled water, till a ratio between 1 :75 and 1 :150 of tissue : water was obtained.

To determine alkaline phosphatase activity, the colorimetric method was used (11). This consists in the addition of phenolphthalein phosphatase buffered with Tris to the samples, which react to the phosphatases by releasing phenolphthalein, measured at 550 mμ, the amount released being proportional to the enzymatic activity. To determine total acid phosphatase activity, the method described by Babson's (2) was used. A Babson unit of acid phosphatase is defined as the amount of enzyme that will release 1.0 mg of alpha-naphthol in one hour at 37°C, measuring at 530 mμ.

Incubation was normally carried out at 37°C for 30 min at pH 5.6 for acid phosphatase and 9.2 for alkaline phosphatase.

Activity was measured in a Perkin-Elmer spectrophotometer, model 550 SE. Statistical analysis was carried out with the non-paired Student's t test. Statistically significant differences were considered $p < 0.005$ or less.

RESULTS

With the aim of determining the influence of incubation time on the substrates, a series of assays were performed with the conditions specified previously, with incubation times of 10, 20, 30, 40 and 60 min. Incubation times and the release of phenolphthalein and alpha-naphthol are seen to have lineal correlation. The alkaline and acid phosphatase activities, expressed in mcg of phenolphthalein and Babson units in 100 ml of extract of serum respectively, found in the different structures are set out in Table 2.

Table 2

Stages of maturation and alkaline and acid phosphatase activity found in the different anatomical parts of *Crassostrea angulata*. Mean ± SE. Phosphomonoesterase units/100 ml serum. Alk. phosph. mcg phenolph. = Alkaline phosphatase in micrograms of phenolphthalein. Acid phosph. = Acid phosphatase in Babson units. Data were obtained from the figures found for each month of that stage.

Anatomical portions	Stage of maturation	Alk. phosph. mcg phenolph.: males	Alk. phosph. mcg phenolph.: females	Acid phosph. Babson units: males	Acid phosph. Babson units: females
Labial palpi	1–4	1.09±0.84	1.23±0.95	0.20±0.053	0.21±0.06
		P < 0.10		P < 0.10	
	1	38.70±0.40	39.00±0.20	0.60±0.070	0.60±0.068
	2	37.80±0.70	38.80±0.57	0.60±0.059	0.62±0.048
Mantle	3	36.80±0.56	37.20±0.42	0.70±0.023	0.68±0.039
	4	37.90±0.29	38.01±0.31	0.65±0.032	0.66±0.050
Adductor muscle	1–4	1.05±0.64	1.10±9.52	0.20±0.048	0.20±0.032
	1	11.90±0.35	11.20±0.47	0.20±0.061	0.20±0.027
	2	12.01±0.71	11.00±0.46	0.40±0.078	0.32±0.060
Branchiae	3	12.26±0.57	11.60±0.54	0.60±0.034	0.40±0.048
	4	11.01±0.46	11.40±0.78	0.80±0.023	0.32±0.050
	1	58.01±0.28	57.08±0.23	6.60±0.040	6.80±0.029
	2	58.90±0.63	58.90±0.56	6.80±0.032	6.80±0.033
Digestive system	3	57.30±0.49	59.10±0.40	6.80±0.030	6.90±0.040
	4	57.50±0.39	58.04±0.61	6.80±0.040	6.90±0.035

Enzymatic activities are very low, below 10 mcg of phenolphthalein and 5 Babson units (considered activity threshold values), in the labial palpi and the adductor muscle. No differences related to the stage of maturation are found in either structure. Low alkaline activity was found in the branchiae, being greater in the males (12.26) during the maturation and spawning period, than in females (11.60) during the same stage.

The highest activity values were found in the digestive system, followed by the mantle, for both enzymes. The highest values in the digestive system correspond to the female during May, June, July and August, 59.10 for alkaline activity and 6.90 for acid activity. Phosphomonoesterase activities in the digestive system of the males peak in March and April, with values of 58.90 and 6.80 for alkaline and acid activities respectively.

As for the mantle, highest activity in females correspond to the months of December, January and February (39.00), this value being similar to that of the males for alkaline activity (38.70). Acid activity was not positively manifested.

When the mean annual value of activity is compared statistically, highly significant differences are found in each anatomical ($p < 0.001$ in Student's group comparison). However, when comparing the mean values of each structure in males and females, these differences are not significant.

DISCUSSION

According to our experimental work, samples from different tissues from *Crassostrea angulata* show phosphomonoesterase activity when using buffered phenolphthalein phosphatase (alkaline activity) and sodium alpha-naphthol phosphatase (total acid activity) as substrates. These data are in agreement with those found in the *American oyster* (6) and in other bivalves (16), in that hydrolytic activity in mollusca is mainly found in the digestive system and mantle.

In *Crassostrea angulata*, food ingestion is associated with a demonstrable rise in intestinal alkaline phosphatase coinciding with the warmer months of the year, during which time there is intense metabolic activity. This rise suggests that increased synthesis of the enzyme is envolved, although it has not been possible to associate this rise with any particular nutrient. This increased activity during maturation and spawning could be attributed to the presence of the gonad in our samples of the digestive system, yet in previous works (10), low alkaline activity in mature gametes of both males and females of this species was observed, leading us to believing that specialized absorptive cells account for the invariable high alkalina activity.

As in (8), (5), we relate the high activity found in the mantle to the role of phosphatase enzymes in the shell-formation processes, which are cyclical and independent of the stage of maturation. The influence of sex on phosphomonoesterase activity in marine species has already been described (12), (14).

However, this has not been studied in the bivalves. In our results, no differences are observed between the sexes.

The technique used, designed for mammals, may be considered innappropiate for our study, as incubation temperatures are higher than those of the normal molluscan habitat. However, previous and simultaneous experiments were carried out at temperatures of 20, 25, 30, 35, 40, 45 and 50°C. Maximal enzymatic activity in this species was found at 35—40°C for alkaline phosphatase, and 40—45°C for total acid phosphatase. To our knowledge, temperatures of maximal phosphomoesterase activity in marine invertebrates are not described in the literature, although for other marine species temperatures of 35—40°C for alkaline cativity, and 37—42°C for total acid activity are described (7).

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YOLK PLATELETS DEGRADATION AND ACID PHOSPHATASE ACTIVITY IN THE FIRST DEVELOPING STAGES OF HAPLOID AND DIPLOID EMBRYOS OF *RANA RIDIBUNDA*

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The electron microscopic study shows that yolk platelets consist of a crystalline central core surrounded by a superficial layer of granular material. Cytochemical observations in both haploid and diploid embryos suggest that platelets degradation is associated with the presence of acid phosphatase at their level. During the first stages of embryonic development, the process of degradation of platelets was polarised in some cells. The process begins in young blastulae and ensures material for membranes biogenesis during development.
The biochemical study indicates that a marked activation of acid phosphatase has occurred after fertilization, in both haploid and diploid embryos. The activation was progressive up to the gastrula stage. In the following stages (neurula and tadpole), the enzyme presented a lower activity in haploid embryos, which was correlated with the retardation of the breakdown of yolk platelets in haploid cells.

Yolk platelets are the most abundant component in the cytoplasm of amphibian eggs and of embryonic cells. The structure of yolk platelets has been investigated in several amphibian species (6, 7, 9). Many authors have pointed out that in amphibian and fish embryos there was a correlation between the utilization of platelets and embryonic development (8, 18, 20, 21). The participation of yolk platelets in development rest on morphological changes in platelets and the presence of some enzyme at their level. It was suggested that acid phosphatase plays an important role in yolk degradation and utilization during the embryonic development of amphibia (1, 10, 12, 16), but the biochemical evidences of enzyme participation in this process are very slender. However, a 3-fold increase in acid phosphatase activity was observed during fertilization of the teleost eggs and pronucleus formation (11).

Comparative studies on the haploid and diploid amphibian embryos have indicated many morphological and biochemical differences (2, 3, 4, 14). In general, cellular differentiation is retarded in haploids and the degree of retardation is reflected in the retention of large numbers of lipids and platelets in cells.

In this paper we investigated the structural changes of yolk platelets during the first stages of embryonic development of haploid and diploid embryos in association with acid phosphatase activity.

MATERIALS AND METHODS

Animals. The experiments were carried out on Rana ridibunda frogs obtained from the lakes around Bucharest. The frog's ovulation

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was experimentally achieved by administration of human gonadotrophin and total proteic extract from the hypophysis of frogs. Fertilization of the mature oocytes was artificially achieved with a sperm suspension made in Nyu-Twyiti solution. For production of haploids, the sperm suspension was irradiated before fertilization into UV with a HBC 200 source. Fertilized eggs were reared in tap water at about 18—20°. The following embryonic stages have been studied : mature eggs, two-cells stage, blastula, gastrula, neurula and eclosed tadpole. The embryonic forms were removed from the fertilization envelope and jelly coats.

Electron microscopic procedure. Embryonic forms of haploid and diploid embryos were fixed in 3% glutaraldehyde containing 0.25 M sucrose, buffered to pH 7.4 with 0.1 M cacodylate, at 4°C for 2 h. The embryonic forms were washed with 0.1 M cacodylate buffer pH 7.4, followed by fixation for 3 h in cold 1% osmium tetroxide buffered with cacodylate (pH 7.4). The tissues were washed and dehydrated after the procedure described by Kalt and Tandler (5). The tissues were embedded in Epon 812. Thin sections were cut on an LKB ultramicrotome, were stained with lead citrate and uranyl acetate and were examined under a Philips 201 electron microscope.

For ultrastructural localization of acid phosphatase, the embryonic. forms were prefixed in 1% paraformaldehyde buffered with tris-acetate 0.1 M, pH 7.4 containing 0.25 M sucrose, for 30 min. The embryonic forms were washed several times with the same buffer without formaldehyde and were incubated in an Incubation medium containing beta glycerophosphate as a substrate, lead acetate 1 mM, sucrose 0.25 M in 50 mM acetate buffer pH 5.0. Incubation was carried out at 37°C for 60 min. After incubation, the tissues were washed with cacodylate buffer and were postfixed in 1% OsO_4 prepared in 50 mM cacodylate buffer, for 2 h. The tissues were processed by the usual procedure for dehydration and embedding for electron microscop .

Phosphatase assay. The acid phosphatase activity was determined employing *p*-nitrophenyl phosphate as substrate in 50 mM acetate buffer pH 5.0 in a final volume of 2 ml. The mixture was preincubated for 5 min at 37°C and the reaction was started by the addition of a suitable volume of enzyme preparation. After 10 min, the reaction was stopped by addition of 1 ml of 1 N NaOH, and the amount of *p*-nitrophenol released was measured at 410 nm. The acid phosphatase was expressed in specific activity which was defined as that amount of enzyme which released 1 μ mole of *p*-nitrophenol per min per mg protein.

For each stage, total proteic extracts were obtained by homogenization of embryonic forms in 0.1 M sodium acetate buffer pH 4.5 containing 5 mM mercaptoethanol, 5% glycerol, 1 mM EDTA and 0.05%. Triton X—100 in a Potter homogenizer (20 embryonic forms per ml). The extracts were centrifuged at 10.000 × g for 15 min and the supernatants were used for enzymatic determinations.

The concentration of protein in the total proteic extracts was determined by the method of Lowry et al. (13), using bovine serum albumin as standard.

RESULTS

Ultrastructure. Yolk platelets appear as very numerous ovoid structures in eggs and embryonic cells, heterogeneously distributed in the cytoplasm. The basic structure of the yolk platelets is principally the same in mature oocytes and in embryonic cells. Each yolk platelet presents a central body surrounded by a less opaque granular layer. Electron microscopy images show that yolk platelets have at periphery a limiting surrounding membrane (Pl. 1A). Under high magnification, the central core presents a highly ordered fine structure similar to a crystalline lattice (Pl. 1 B). The granular layer has a medium density, without an internal structure. In this superficial layer of yolk platelets two structural zones may be distinguished : a relatively thin layer of dense material and a thin layer of a less dense material, in the vicinity of the limiting membrane (Pl. 1 B). Electron microscopic observations on haploid embryos did not show significant differences in the morphology of the yolk platelets during the first stages of embryonic development.

Cytochemical and biochemical study. Cytochemical identification of acid phosphatase demonstrates that the enzyme is closely related to yolk degradation. In cells from all the stages examined, in both haploid and diploid embryos, the enzyme was located mainly at the level of yolk platelets which are in the process of degrading ((Pl. 2 A—C). The association of acid phosphatase with these structures was observed only on some platelets in a cell. During the early stages of development, only few cells have yolk platelets which present a positive reaction for acid phosphatase, suggesting that the process of degradation is polarized or modulated by some intracellular signals, which control the utilization of yolk components.

The cytochemical analysis pointed out that the process of degradation of platelets is complex and heterogeneous. Some platelets are subdivided into smaller fragments and the precipitate of lead phosphate is seen on the membranous structures which surround these yolk fragments (Pl.2 A). These membranous structures lead, in a further stage, to numerous vesicles which show a heterogeneous content. It may be noted that the fragmentation of the crystal structure was due to acid phosphatase activity. A variable number of vesicles and vacuoles which revealed a positive reaction for acid phosphatase are seen in the neighbourhood of some platelets (Pl. 2 B). Occasionally, yolk platelets contain a positive reaction product for acid phosphatase within them (Pl. 2 C). The laminar structures surrounding a yolk platelet in the course of degrading suggest the participation of these structures in the formation of the membranous systems during development (Pl. 2 A arrow). In haploid cells, the picture of the platelets degradation and acid phosphatase localization did not show significant differences when compared with diploid cells.

The acid phosphatase activity was determined in extracts of mature eggs and in the first stages of embryonic development of haploid and diploid embryos. As shown in Fig. 1, the specific activity of acid phosphatase increases rapidly after fertilization in both haploid and diploid embryos. In diploid embryos, acid phosphatase activity was 2.5-fold higher

in blastula stage and 4.4-fold higher in gastrula stage, in comparison with the level of the enzyme in mature unfertilized eggs. The enzyme presented a decrease in activity in neurula stage, followed by a marked increase in activity in tadpole stage (5.5-fold). In haploid embryos, the changes in

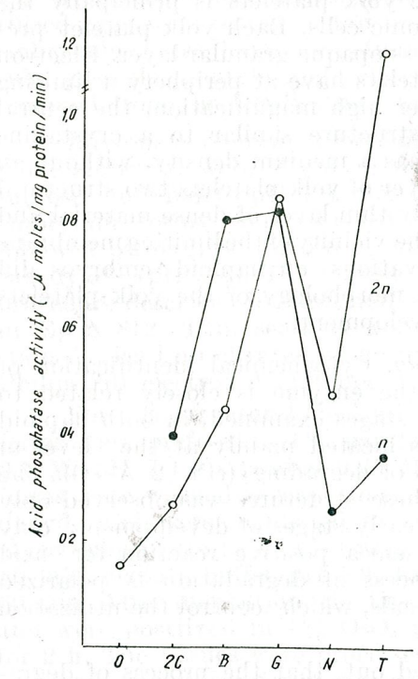

Fig. 1. — Changes in the activity of acid phosphatase in the first developing stages of haploid and diploid embryos. ○, unfertilized egg; 2 C, two-cell stage; B, blastula stage; G, gastrula stage; N, neurula stage; T, tadpole stage.

acid phosphatase activity after fertilization presented some particularities : the enzyme appeared higher in 2 cells and blastula stages in comparison with the same stages of diploid embryos, but presented lower activity in neurula and tadpole stages. In haploid cells of tadpole, acid phosphatase activity is 3.4-fold lower in comparison with the level of the enzyme in the similar stage of diploid embryos.

DISCUSSION

The morphological observations of the yolk platelets in mature eggs and embryonic cells of frog show that these structures have a ultra-structural organisation similar in all species of amphibia (6,7, 9, 17). Concerning the number of yolk platelets and the process of their breakdown no apparent differences between haploid and diploid cells in the early stages of embryonic development were recorded. Within the range of stages studied, the haploid cells presented some morphological changes in tadpole stage, in which the majority of haploid cells possess a larger number of platelets and yolk bodies.

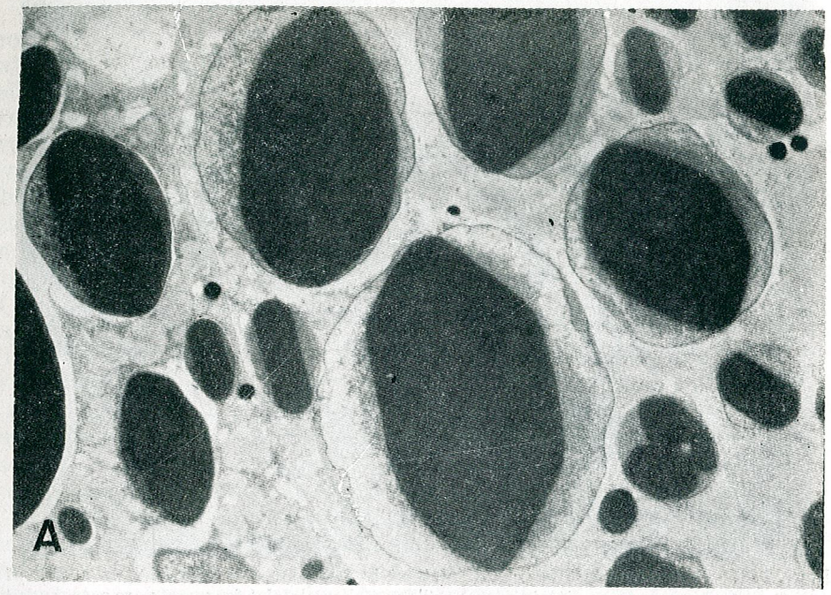

Plate 1 A. — Electron micrograph of yolk platelets from a cell of a two-cell stage. The cells have numerous typical platelets of variable size in the cyroplasm. Note the presence of a surrounding membrane at the periphery. × 3500.

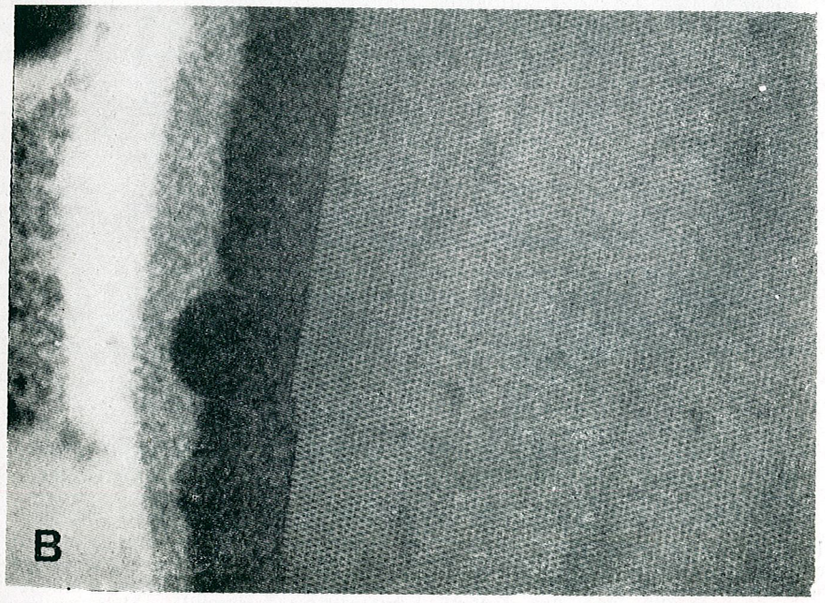

Plate 1 B. — High magnification electron micrograph showing the fine crystalline lattice and the superficial layers of a yolk platelet. The limiting membrane was not preserved. × 18700.

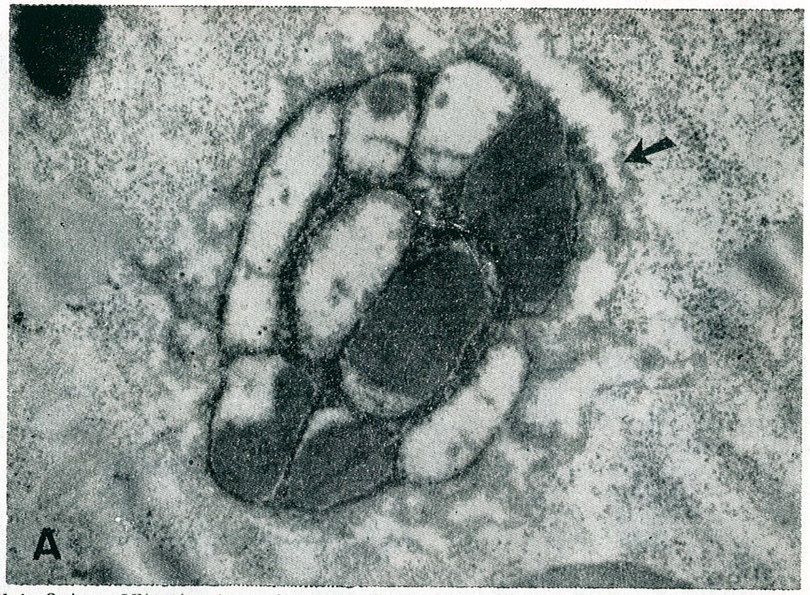

Plate 2 A. — Ultrastructure of a yolk platelet in the course of degrading in a cell of young gastrula stage from diploid embryo. The smaller platelet fragments are surrounded by a limiting membrane, which shows an intense reaction product for acid phosphatase. Note also the presence of laminar structures surrounding the platelet (arrow). ×7800.

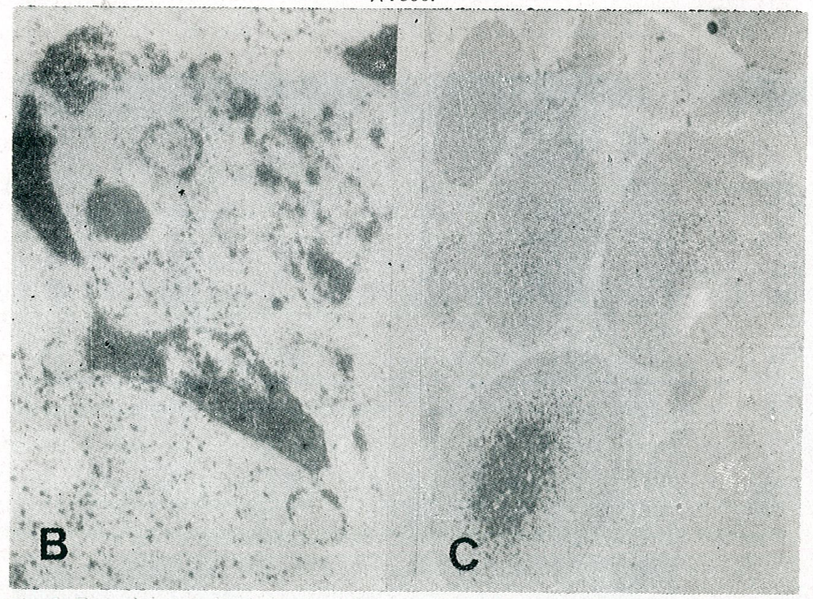

Plate 2 B. — Advanced stage in the degradation of a yolk platelet in a cell from the neurula stage of a haploid embryo. Unstained preparation. Note the presence of many vesicles heterogeneous in size. ×23500.

Plate 2 C. — Electron micrograph of yolk platelets from a neurula stage cell of diploid embryo. The reaction product of acid phosphatase is visible inside the platelet. Unstained preparation. ×4600

Although morphological and histochemical studies pointed out that most yolk platelets persist for a long time in many embryonic cells (after hatching), the process of their degradation was observed to begin in some cells in young blastula stage (10). Our morphological and cytochemical observations indicate also that in both types of cells, the yolk platelets breakdown can be detected very early during embryonic development. Cytochemical observations show that the reaction product of the acid phosphatase activity appears, in both types of embryos, on certain platelets which are in the process of degradation. The enzyme was identified at the peripheries and within the crystalline body of the platelets, suggesting the possibility of a double origin of the acid phosphatase. The process of platelets degradation is very complex. The platelets subjected to degradation appear to pass through several intermediate morphological stages with a heterogeneous and polymorphous aspect. Moreover, the existence of a selective degradation of platelets in a given cell during the early stages of development, suggests that the process of breakdown is dependent on some intracellular signals. The presence of adenylate cyclase in the surrounding membrane of yolk platelets could represent one of the factors involved in the control of the breakdown of yolk platelets (19).

Our biochemical results demonstrate that after fertilization, an increase in acid phosphatase activity occurs, in both haploid and diploid embryos of frog. In both types of embryos, the specific activity of acid phosphatase was progressive from unfertilized eggs to young gastrula stage. After gastrulation, the activity of the enzyme decreases in both types of embryos (in neurula stage). The greatest discrepancy between haploid and diploid embryos was observed in tadpole stage. In diploid tadpole, a marked activation of acid phoshatase activity was observed. These results suggest that the retardation in the disappearance of the yolk platelets in the cells of haploid embryos is related to the low activity of acid phosphatase in the later stages of embryonic development.

The changes in the level of acid phosphatase activity after fertilization and during the first stages of embryonic development are difficult to explain. The activation may have many possible causes and may be related to specific events in different stages of development. The activation of acid phosphatase in fish egg homogenate during fertilization (11), was correlated to the inactivation of a maturing promoting factor. There is no doubt that phosphoproteins are major characteristic components of the yolk platelets and their dephosphorylation is required to render the yolk soluble. It was suggested that, in frog ovary, acid phosphatase may act as an acid phosphoprotein phosphatase (15). It is obvious that there is a close correlation between the process of yolk platelets degradation and phosphatase activity, in the control of dephosphorylation of yolk proteins.

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L'ÉTUDE DES CHROMOSOMES MITOTIQUES DE DEUX POPULATIONS DE *SALMO GAIRDNERI* RICH

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Two populations of *Salmo gairdneri* were investigated as regards the number and the type of chromosomes — one from Potoci (Neamţ county), the other from Prejmer (Braşov county).
In both populations, in the mitotic metaphases investigated. the number of chromosomes varied between 60 and 61.
A first important difference between the two populations was noticed as to the type of chromosomes : 18 acrocentrics at Prejmer and only 17 at Potoci. At the same time, the unpaired chromosomes had a median centromer at the population from Prejmer and an acrocentric one at the population from Potoci. At the Prejmer population one of the chromosomes with a median centromer was split and from the cells with $2n = 60$ resulted cells with $2n = 61$.At the Potoci population one of the chromosomes was lost and each of the other two chromosomes lost one arm and because of this the cells with $2n = 60$ produced cells with $2n = 61$.

De nombreuses investigations cytogénétiques effectuées sur diverses espèces de la famille Salmonidae ont relevé l'existence de la variabilité du nombre de chromosomes. Le nombre diploïde de chromosomes varie entre 54 et 60 chez *Salmo salar* (Prokofieva, 1934 ; Svärdson, 1945 ; Boothroyd, 1959 ; Nygrin & coll., 1968 ; Roberts, 1970 — cités par Gold & coll., 1975), et entre 58 et 65 chez *Salmo gairdneri* (Ohno & coll., 1965 ; Raicu et Taisescu, 1977 ; Taisescu, 1979). Une étude récente (Hartley et Horne, 1982) sur une population de *Salmo gairdneri* d'une ferme d'Ecosse a mis en évidence la présence des individus dont le nombre des chromosomes variait entre 59 et 63.

La nature exacte de la variabilité du nombre des chromosomes pour la famille Salmonidae n'est pas encore elucidée. Roberts (1968, 1970) et Ohno & coll. (1965) considèrent qu'il s'agit des translocations robertsoniennes. Svärdson (1945) et Boothroyd (1959) suggèrent que le nombre variable de chromosomes pour *Salmo salar* ne serait dû pas tant aux translocations robertsoniennes qu'à un phénomène d'aneuploïdie qui n'a pas été suivi de réponses phénotypiques. A l'aide de tout ce que les auteurs sus-mentionnés soutiennent, sont présentées des données d'ordre cytoloqique et biochimique (Davisson & coll., 1972, cités par Gold, 1975) obtenues des trisomiques de *Salvelinus fontinalis*, normaux du point de vue du phénotype. Hartley et Horne (1982) offerent l'explicatoni suivante. Dans les premières phases embryonnaires, ou dans le zygote, certains bras des chromosomes s'engagent dans la dissociation et la fusion. Bien des zygotes de truite sont hétérozygotes pour de nombreuses translocations robertsoniennes. Il est certain que le problème de la variabilité du nombre de chromosomes reste ouvert, les investigations ultérieures ayant pour but d'apporter de nouveaux complèments et clarifications.

MATÉRIEL ET MÉTHODE

Dans l'étude présente ont été sujets d'investigations deux populations de *Salmo gairdneri*, l'une provenant de la ferme expérimentale de Potoci appartenant à la Station de recherches « Stejarul » du département de Neamț, l'autre provenant de la ferme de truites de Prejmer.

Les exemplaires analysés ont été collectés en février 1982 (la population de Potoci) et en juin 1982 (la population de Prejmer). La méthode utilisée pour la préparation des chromosomes a été présentée en détail dans les ouvrages antérieurs : utilisation de la colchicine comme agent statmocinétique, traitement avec une solution hypotone, fixations successives et dessications à l'air. Les chromosomes mitotiques ont été analysés dans environ 100 cellules provenant de 20 exemplaires. Bref, les investigations ont eu en vue : d'établir s'il y a une variabilité numérique ou bien dans la structure des chromosomes ; et si l'on pourrait faire un parallèle entre cette variabilité et l'isolement géographique et reproductif existant entre les deux populations.

RÉSULTATS ET DISCUSSIONS

Pour les deux populations, dans les métaphases mitotiques sélectionées, le nombre des chromosomes a varié entre 60 et 61.

À la population de Potoci, pour les métaphases ayant 2n = 61 les dimensions moyennes des chromosomes ont varié entre 3,93 (la I^{ère} paire) et 1,22 microns (la XXX^{e} paire), le groupe haploïde totalisant 80,89 microns pour un NF = 107. Pour les métaphases ayant 2n = 60 chromosomes, la longueur moyenne a varié entre 4,30 (la I^{ère} paire) et 1,51 microns (la XXX^{e} paire), la longueur du groupe haploïde totalisant 87,99 microns pour un NF = 104.

Pour la population de Prejmer les métaphases ayant 2n = 61 totalisaient 66,72 microns pour un groupe haploïde, les dimensions moyennes des chromosomes variant entre 3,67 et 1,16 microns, pour un NF = 104. Pour la métaphase ayant 2n = 60, la longueur des chromosomes variait entre 2,52 microns (la I^{ère} paire) et 0,81 microns (la XXX^{e} paire), le groupe haploïde totalisant 58,88 microns, le NF étant le même (104).

Pour la même population, celle de Potoci, la longueur totale du groupe haploïde est plus grande à la métaphase ayant 2n = 60, qu'à la métaphase ayant 2n = 61, bien que nous étions tentés à découvrir une situation inverse (voir les tableaux). Difficilement à expliquer sont les différences entre les chromosomes sous l'aspect des autres paramètres — index centromérique et rapport des bras, déterminés par la position du centromère.

Pour la population de Prejmer, aux métaphases ayant 2n = 61, 4 paires de chromosomes sont métacentriques (I, VI, X, XIII), 18 paires sont médianes et 17 chromosomes sont acrocentriques. A la métaphase

ayant 2n = 60, 2 paires ont les chromosomes métacentriques (VI et XX), 19 paires ont les chromosomes médians, 1 paire a les chromosomes sous-médians (XII) et 16 chromosomes sont acrocentriques.

Tableau 1

Les caractéristiques des chromosomes mitotiques de *Salmo gairdneri* Rich. (population de Potoci) dans des métaphases 2n = 61

La paire de chromosomes	La longueur totale	Le bras long	Le bras court	Le rapport des bras	Index centromérique	La longueur relative	Le type chromosomial
I	3,93	2,06	1,87	1,09	47,66	4,86	m
II	3,69	2,00	1,69	1,17	45,85	4,55	m
III	3,48	1,87	1,61	1,16	46,19	4,30	m
IV	3,44	1,75	1,69	1,03	49,11	4,25	m
V	3,40	1,71	1,69	1,01	49,70	4,20	m
VI	3,40	1,71	1,69	1,01	49,70	4,20	m
VII	3,26	1,63	1,63	1,00	50,00	4,03	M
VIII	3,18	1,63	1,55	1,02	48,71	3,92	m
IX	3,18	1,61	1,57	1,02	49,35	3,92	m
X	3,18	2,02	1,16	1,73	36,53	3,92	sm
XI	3,18	2,02	1,16	1,73	36,53	3,92	sm
XII	3,14	1,91	1,22	1,56	38,96	3,87	m
XIII	3,10	1,95	1,14	1,71	36,84	3,82	sm
XIV	2,85	1,63	1,22	1,33	42,85	3,52	m
XV	2,81	1,42	1,38	1,02	49,27	3,47	m
XVI	2,69	1,34	1,34	1,00	50,00	3,32	M
XVII	2,65	1,42	1,22	1,16	46,15	3,27	m
XVIII	2,65	1,42	1,22	1,16	46,15	3,27	m
XIX	2,61	1,30	1,30	1,00	50,00	3,22	M
XX	2,57	1,30	1,26	1,03	49,20	3,17	m
XXI	2,12	—	—	—	—	2,91	a
XXII	2,08	—	—	—	—	2,56	a
XXIII	2,04	1,06	0,98	1,08	48,00	2,51	m
XXIV	2,00	1,10	0,90	1,22	44,89	2,46	m
XXV	1,71	—	—	—	—	2,11	a
XXVI	1,63	—	—	—	—	2,01	a
XXVII	1,59	—	—	—	—	1,96	a
XXVIII	1,59	1,26	0,33	3,87	20,51	1,96	st
XXIX	1,34	—	—	—	—	1,66	a
XXX	1,22	—	—	—	—	1,51	a
XXXI	1,18	—	—	—	—	1,46	a
	80,89						

A la population de Potoci ayant les métaphases 2n = 60 on constate 20 paires à chromosomes médians, 2 paires à chromosomes soustélocentriques et 16 chromosomes acrocentriques. Pour les métaphases à 2n = 61 on a 3 paires de chromosomes métacentriques (VII, XVI, XIX), 16 paires de chromosomes médians, 3 paires sousmédianes, une paire soustélocentrique et 15 chromosomes acrocentriques.

Si on prenait donc en considération les métaphases à 2n = 61, entre les deux populations on constate une première dissemblance (que nous considérions suffisamment importante) concernant le nombre d'acrocentriques : 17 pour celle de Prejmer et 15 seulement pour celle de Potoci. Un autre aspect particulièrement important est constitué par le type

de chromosome impair : tandis qu'à la population de Prejmer il est médian (r = 1,06 ; i = 48,38) ; pour la population de Potoci il est acrocentrique. En tant que longueur, dans le premier cas le chromosome impair se situe après la XXI^{e} paire, tandis que dans le deuxième, il se situe en dernière place. Du point de vue des métaphases à 2n = 60, on constate que les deux ont chacune 16 chromosomes acrocentriques.

Tableau 2

Les caractéristiques des chromosomes mitotiques de *Salmo gairdneri* Rich. (population de Potoci) dans des métaphases 2n = 60

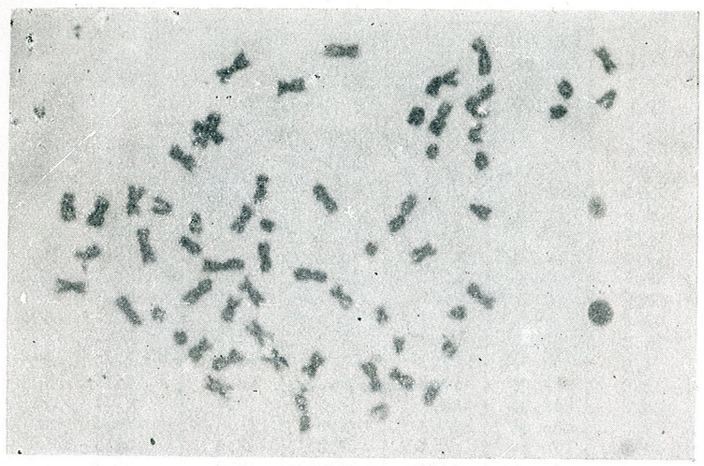
La paire de chromosomes	La longueur totale	Le bras long	Le bras court	Le rapport des bras	Index centromérique	La longueur relative	Le type chromosomial
I	4,34	2,40	1,93	1,24	44,60	4,93	m
II	4,08	2,48	1,59	1,56	39,00	4,63	m
III	4,04	2,16	1,87	1,15	46,46	4,58	m
IV	4,02	2,32	1,67	1,40	41,62	4,56	m
V	3,97	2,00	1,95	1,01	49,74	4,51	m
VI	3,89	2,06	1,83	1,12	47,12	4,42	m
VII	3,67	2,08	1,59	1,30	43,33	4,16	m
VIII	3,63	2,08	1,55	1,34	42,39	4,12	m
IX	3,59	2,00	1,59	1,25	44,31	4,07	m
X	3,59	2,00	1,59	1,25	44,31	4,07	m
XI	3,46	1,83	1,63	1,12	47,05	3,93	m
XII	3,40	1,71	1,69	1,01	49,70	3,86	m
XIII	3,34	1,79	1,55	1,15	46,34	3,79	m
XIV	3,30	1,67	1,63	1,03	49,38	3,75	m
XV	3,26	2,00	1,26	1,58	38,75	3,70	m
XVI	3,10	1,57	1,53	1,02	49,34	3,52	m
XVII	3,06	1,79	1,26	1,41	41,33	3,47	m
XVIII	2,53	1,30	1,22	1,06	48,38	2,87	m
XIX	2,48	1,26	1,22	1,03	49,18	2,82	m
XX	2,44	1,26	1,18	1,06	48,33	2,77	m
XXI	2,44	—	—	—	—	2,77	a.
XXII	2,28	2,00	0,28	7,00	12,50	2,59	st
XXIII	2,22	—	—	—	—	2,52	a
XXIV	2,00	1,18	0,81	1,45	40,81	2,26	st
XXV	1,79	—	—	—	—	2,03	a
XXVI	1,71	—	—	—	—	1,94	a
XXVII	1,63	—	—	—	—	1,85	a
XXVIII	1,63	—	—	—	—	1,85	a
XXIX	1,59	—	—	—	—	1,80	a
XXX	1,51	—	—	—	—	1,71	a

Assurément, durant ce commentaire. on pourrait faire des références à propos des différences existantes entre les autres chromosomes, mais nous considérons qu'elles sont moins significatives, du fait que dans le cadre du même individu on pourrait constater des différences suivantes entre les métaphases.

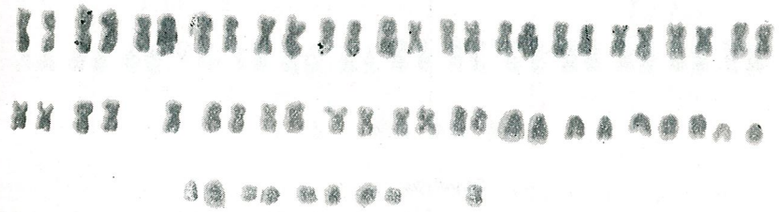
La situation des acrocentriques et du NF est définitoire et elle relève les différences suivantes entre les deux populations.

Nous apprécions qu'à la population de Prejmer un des chromosomes médians des cellules à 2n = 60 a subi une fission, ce qui a mené

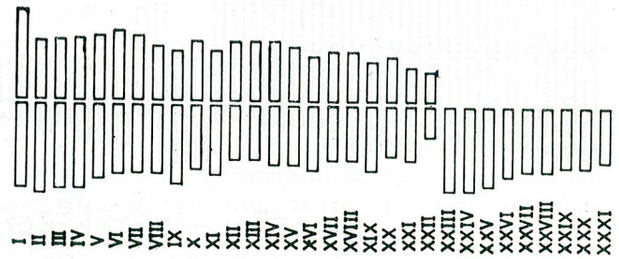
SALMO GAIRDNERI RICH
(population Prejmer)



Metaphase (2n = 61)



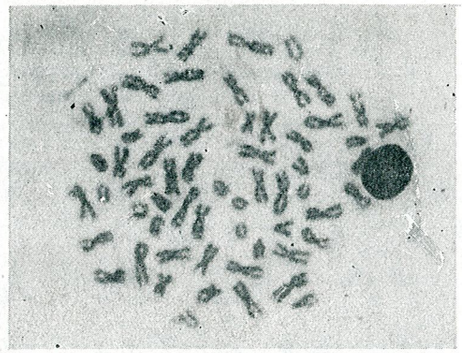
Caryotype



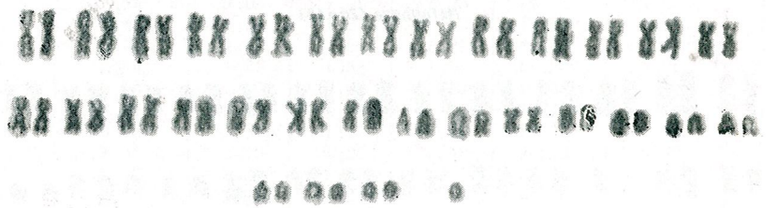
Idiogramme

Fig. 1

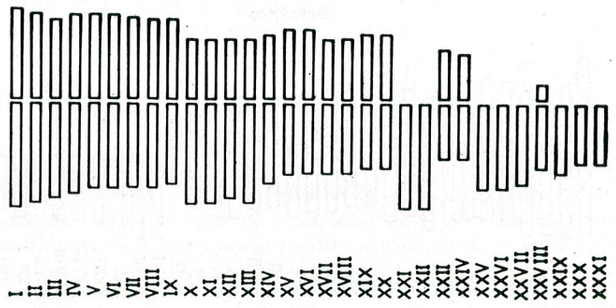
SALMO GAIRDNERI RICH
(population Potoci)



Metaphase (2n=61)



Caryotype



Idiogramme

Fig. 2

Tableau 3

Les caractéristiques des chromosomes mitotiques de *Salmo gairdneri* Rich. (population Prejmer) dans des métaphases 2n = 60

La paire de chromosomes	La longueur totale	Le bras long	Le bras court	Le raport des bras	Index centro-mérique	La longueur relative	Le type chro-mosomial
I	2,52	1,28	1,24	1,03	49,19	4,59	m
II	2,45	1,34	1,11	1,21	44,89	4,43	m
III	2,44	1,24	1,20	1,03	49,16	4,44	m
IV	2,40	1,22	1,18	1,03	49,15	4,37	m
V	2,40	1,22	1,18	1,03	49,15	4,37	m
VI	2,36	1,18	1,18	1,00	50,00	4,30	m
VII	2,36	1,20	1,16	1,03	49,13	4,30	M
VIII	2,36	1,22	1,14	1,07	48,27	4,30	m
IX	2,32	1,24	1,08	1,10	46,48	4,22	m
X	2,32	1,20	1,12	1,07	48,24	4,22	m
XI	2,28	1,20	1,08	1,11	47,31	4,15	m
XII	2,25	1,42	0,83	1,71	37,25	4,19	sm
XIII	2,24	1,20	1,04	1,15	46,36	4,08	m
XIV	2,14	1,08	1,06	1,01	49,50	3,89	m
XV	2,09	1,19	0,91	1,28	45,60	3,80	m
XVI	1,93	1,10	0,83	1,32	43,23	3,51	m
XVII	1,74	0,95	0,79	1,19	46,00	3,17	m
XVIII	1,72	0,87	0,85	1,02	49,44	3,13	m
XIX	1,72	0,87	0,85	1,02	49,44	3,13	m
XX	1,62	0,81	0,81	1,00	50,00	2,95	M
XXI	1,60	0,83	0,77	1,08	48,07	2,91	m
XXII	1,60	0,81	0,79	1,15	46,57	2,91	m
XXIII	1,22	—	—	—	—	2,22	a
XXIV	1,18	—	—	—	—	2,15	a
XXV	1,10	—	—	—	—	2,00	a
XXVI	1,04	—	—	—	—	1,89	a
XXVII	0,93	—	—	—	—	1,69	a
XXVIII	0,91	—	—	—	—	1,65	a
XXIX	0,83	—	—	—	—	1,51	a
XXX	0,81	—	—	—	—	1,47	a
	54,88						

à l'apparition d'un chromosome de plus (2n = 61), en même temps qu'un médian est resté sans paire et que le nombre des acrocentriques s'agrandit de deux (18 au lieu de 16).

Le fait que le NF = 104 pour les deux cas porte un argument de plus à notre hypothèse.

Pour ce qui est de la population de Potoci, la situation est bien plus difficile à élucider; premièrement — pour le cas particulier que nous avons mis en discussion — nous ne savons pas encore quel sens donner au processus : de 2n = 60 vers 2n = 61 ou bien inversement? Dans la littérature de spécialité on considère que la tendance serait vers la réduction du nombre de chromosomes. Le fait que le nombre fondamental (NF) des bras décroit (dans notre cas 107 à 104) nous détermine à être du même avis.

Nous avons observé que lorsqu'il y a 2n = 61, il existe 44 chromosomes du groupe des médians (6 métacentriques, 32 médians et 6 sousmédians), tandis qu'à 2n = 60 il y a 40 chromosomes seulement (tous médians). Mais, en même temps, de 15 chromosomes acrocentriques on a atteint 16 et de 2 soustélocentrique on a atteint 4. Evaluée donc de la

sorte, la situation se présente ainsi : de 46 chromosomes à 2 bras on a atteint 44 et de 15 à 1 bras on a atteint 16. Or, en admettant que 2 chromosomes ont passé en sousterminaux par la perte d'une portion d'un

Tableau 4

Les caractéristiques des chromosomes mitotiques de *Salmo gairdneri* Rich. (population Prejmer) dans des métaphases 2n = 61

La paire de chromosomes	La longueur totale	Le bras long	Le bras court	Le rapport des bras	Index centrométrique	La longueur relative	Le type chromosomial
I	3,67	1,83	1,83	1,00	50,00	5,53	M
II	3,16	1,87	1,24	1,31	39,35	4,76	m
III	2,95	1,69	1,26	1,33	42,75	4,46	m
IV	2,89	1,65	1,24	1,32	42,95	4,36	m
V	2,83	1,51	1,34	1,12	47,48	4,27	m
VI	2,69	1,36	1,34	1,00	50,00	4,06	M
VII	2,67	1,36	1,30	1,04	48,85	4,02	m
VIII	2,63	1,46	1,16	1,26	44,18	3,96	m
IX	2,59	1,59	1,00	1,59	38,58	3,90	m
X	2,53	1,26	1,26	1,00	50,00	3,81	M
XI	2,46	1,46	1,00	1,46	40,49	3,72	m
XII	2,44	1,24	1,20	1,03	49,16	3,69	m
XIII	2,44	1,24	1,20	1,00	50,00	3,69	M
XIV	2,40	1,26	1,14	1,10	47,45	3,62	m
XV	2,34	1,22	1,12	1,09	47,82	3,53	m
XVI	2,24	1,30	0,93	1,39	41,81	3,38	m
XVII	2,10	1,12	0,97	1,14	45,60	3,16	m
XVIII	2,06	1,08	0,97	1,10	47,52	3,10	m
XIX	2,06	1,24	0,81	1,52	36,36	3,10	m
XX	1,93	0,97	0,95	1,02	49,47	2,92	m
XXI	1,83	1,14	0,69	1,64	37,77	2,76	m
XXII	1,26	0,65	0,61	1,06	48,38	1,90	m
XXIII	1,69	—	—	—	—	2,55	a
XXIV	1,69	—	—	—	—	2,55	a
XXV	1,55	—	—	—	—	2,33	a
XXVI	1,40	—	—	—	—	2,12	a
XXVII	1,30	—	—	—	—	1,96	a
XXVIII	1,28	—	—	—	—	1,93	a
XXIX	1,26	—	—	—	—	1,90	a
XXX	1,22	—	—	—	—	1,84	a
XXXI	1,16	—	—	—	—	1,75	a
	66,72						

bras, tandis qu'un autre chromosome est devenu acrocentrique par la perte intégrale d'un bras, tout ça signifie qu'un chromosome s'est résorbé, a disparu entièrement. Pratiquement il y a eu perte de 3 bras ; c'est en fait, la situation analysée par nous.

Des études ultérieures, surtout dans la descendance de certains individus, pourront nous apporter des éléments supplémentaires relativement au rôle de l'habitat dans ce processus. Pour l'instant, il est hasardeux de parler de directions divergentes d'évolution de l'action différenciée de la selection naturelle.

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Station de recherches « Stejaru »
Piatra Neamț, Alexandru cel Bun 6

ON POTENTIATION OF GIROSTAN IOB ANTITUMOR ACTIVITY BY CHEMICALLY MODIFIED NYSTATIN (NsMC)

P. ROTINBERG, SMARANDA KELEMEN and AL. SAUCIUC*

The in vivo potentiation of the antitumor activity of Girostan IOB alkylating agent on rats bearing solid or ascitic Guérin T—8 tumors was obtained by its therapeutic association with NsMC.
The potentiation effect induced by NsMC is due probably to its membranotropic action of permeabilisation, leading to an increased intracellular concentration of the Girostan IOB.

One way of increasing the efficiency of human antineoplastic chemotherapy is provided by identification of some substances which intensify the cancerostatic action of the pharmacological agents for clinical use (10), (22), (26).

Implication of the membrane characteristic modifications in the initiation and the development of the malignant process justifies the importance attributed to pharmacological membranotropic agents as cytostatics, on the one hand, and as potentiation drugs for the cancerostatic compounds, on the other hand. Among these the polyene antibiotics represent the objective of some complex investigations (26).

In previous papers, the cytostatic and antitumoral activity of some forms of NsMC, developed at the Center for Antibiotic Research Iași, as well as its possible mechanisms of action have been evidenced (2), (3), (4), (5), (17), (18), (19), (20).

In the present investigation the results of in vivo testing of a combined therapy with Girostan IOB and NsMC on rat bearing ascitic or solid Guérin T—8 tumors are discussed.

MATERIAL AND METHODS

White Wistar female rats weighing 150 g bearing Guérin T—8 lymphotropic epithelioma of subcutaneous solid type or intraperitoneal ascitic form (23) have been used.

The treatment started 24 hours after the tumor transplants and lasted for 16 days in the case of solid tumor or until the death of the last control animal for the ascitic tumor.

The daily intraperitoneal treatment was applied either alone by administration both of the NsMC, in a dose of 500 mg/kg.b.w. and of the Girostan IOB, at a dose of 1 mg/kg.b.w., or by combined simultaneous injection of the two drugs in the same doses.

The estimation of the antitumor activity was based on the follow-up of the mean tumor weight at sacrifice in the case of solid tumor or of the mean survival time in the case of ascitic tumor in the treated groups, comparatively to controls.

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The evaluation of the antitumor action was made by the determination of the mean tumor regression for solid tumor, by the increase of the mean survival time in the case of ascitic tumor, and by calculation of the statistic significance using Student's "t" test and the T/C value (where T = mean tumor weight or mean survival time for the treated group and C = mean tumor weight or mean survival time for the control group).

RESULTS

Table 1 presents the results obtained in the testing of the antitumoral activity of Girostan IOB and NsMC administered either alone or associated to the rats bearing solid Guérin T-*8* tumor.

In comparison to the control group it is observed that NsMC administration induced a nonsignificant 12.8% tumor regression, correlated with a 0.87 T/C value.

Table 1

Antitumoral activity of the associated therapy with Girostan IOB (1mg/Kg.b.w./i.p./daily) and NsMC ((500 mg/Kg.b.w./i.p./ daily) on solid Guérin T—8 tumor. Figures in parentheses indicate the number of animals

Group/ Treatment	Mean tumoral weight (g)	% tumor regression	Statistic significance	T/C value
CONTROL	7.8±1.2(14)	—	—	—
NsMC	6.8±1.3(10)	12.8	N.S.	0.87
Girostan IOB	5.8±1.0(10	25.7	N.S.	0.74
Girostan IOB + NsMC	3,7±0.9(10)	52.6	p<0.02	0.47

The treatment with Girostan IOB determined a nonsignificant antitumoral effect; mean tumor regression registered and the T/C value was of 25.7% and 0.74, respectively.

On the contrary, comparatively with the control group and also with those treated only with NsMC or Girostan IOB, in the case of the group treated with Girostan IOB associated with NsMC the mean tumor weight showed a significant decrease ($p < 0.02$). This reveals a tumor regression of 52.6% with a corresponding T/C value of 0.47.

The results registered under the same experimental conditions for rats bearing ascitic type tumor are included in Table 2.

It is observed that daily i.p. administration of NsMC induced a slight and nonsignificant antitumoral effect, illustrated by a 6.2% mean survival time increase only and a 1.06 T/C value.

The Girostan IOB treatment determined also a nonsignificant increase of the mean survival time of 15.3%, correlated with a T/C value of 1.15.

It can be emphasized, once again, that the combined Girostan IOB and NsMC therapy on the rats bearing ascitic tumor induces a significant cancerostatic activity ($p < 0.01$) in comparison to the control

Table 2

Antitumoral activity of the associated therapy with Girostan IOB (1mg/Kc.b.w./i.p./daily) and NsMc (500mg/Kcb. w./i.p./ daily) on ascitic tumor line. Figures in parentheses indicate the number of animals

Group/ Treatment	Mean survival time (days)	% increase in survival time	Statistic significance	T/C value
CONTROL	17.6±1.2(14)	—	—	—
NsMC	18.7±1.4(10)	6.2	N.S.	1.06
Girostan IOB	2 .3±1.3(10)	15.3	N.S.	1.15
Girostan IOB + NsMC	34.4±4.9(9)	95.4	p<0.01	1.95

group and the animals treated with Girostan IOB or NsMC only. This antitumoral activity is characterized by a survival time increase of 95.4% and a T/C value of 1.95. Moreover, one case of tumor undevelopment was registered.

DISCUSSION

The results of these investigations clearly show that the therapeutic association of Girostan IOB with NsMC induces a significant antitumoral activity on the two tumor lines in comparison to the control group and the rats treated with Girostan IOB or NsMC only. This is confirmed by the tumoral regression values, by the survival time increases and by the T/C values.

In the case of the solid tumoral line, the tumor regression induced by the associated treatment was 4 times higher (with 40%) than that obtained by NsMC administration and twice (with 27%) than that registered after Girostan IOB therapy.

Also, in the case of ascitic tumor line, the increase effect of the mean survival time observed after combined therapy was 15 times higher (with 89%) than that registered on the rats treated only with NsMC and 6 times higher (with 80%) than that induced by Girostan IOB treatment.

The increased antitumoral activity induced by therapeutic association of that two drugs did not represent only the sum of their effects because the intensity of the combined treatment effect was 1.3 times higher (with 14%) than the sum of the effects induced by the separate administration of the agents to the rats with solid tumor and 4.5 times higher (with 74%) than those observed on rats with ascitic tumor.

Therefore, it can be appreciated that the augmented effectiveness of the combined therapy is the result of a potentiation of the antitumoral activity of Girostan IOB by NsMC. This potentiation effect is more accentuated in the case of ascitic tumor line.

It is known that the cytotoxicity of the polyene antibiotics is based on their interaction with membrane sterols of the prokaryotic and eukaryotic cells. The formed molecular antibiotic-sterol complexes modify

the structure and properties of the cell membranes leading to their permeabilisation for intracellular components (1), (7), (8),(9), (12), (13), (14), (15), (24), (25).

Among others, previous studies on the action mechanisms of NsMC — a cytotoxic and antitumoral preparate (2), (3), (4), (5), (17), (18), (19), (20) — revealed that the chemical modification of nystatin did not change its property to interact with membrane sterols leading to a membrane permeabilisation. Moreover, an interaction not only with the membrane lipid components but with the membrane proteins too was evidentiated, leading to a complexation with and/or a solubilization of certain membrane components (4), (5).

These are in agreement with the data reported for polyene antibiotics by others (11), (16).

The potentiation action of the antitumoral activity of the Girostan IOB alkylating cytostatic, is probably due to the intracellular concentration increase of the drug, as a result of the membranotropic action of NsMC. Thus, the massive intracellular penetration of Girostan IOB is facilitated by NsMC which increases the membrane permeability.

This possible mode of action of the NsMC is suggested by the intensification of the molecular effects of actinomycin D and puromycin on the polysomal profiles, probably in relation to their intracellular concentration increase as a consequence of membrane permeabilisation induced by concomitant action of the NsMC (6).

The potentiation of antitumoral activity of Girostan IOB — a cytostatic used in human antineoplastic chemotherapy — by NsMC, reflects probably an effect similar to that described for amphotericin B, polyene antibiotic which potentiates the antitumoral activity of nitrosourea (21), of 5 fluorouracil (26), of alkylating agents thiophosphamide and cyclophosphane (27).

The results obtained by us in this investigation ask for preclinical and clinical testing of some associated treatments with NsMC and other cytostatics for the potentiation of their antitumoral activity.

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EXPERIMENTAL HETEROTRANSPLANTATION OF "KB" CARCINOMATOUS CELLS INTO THE EYEBALL OF RABBITS (ASSAY OF THE RELATIVE AMOUNT OF NUCLEAR DNA)*

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The purpose of this study was to find a possible correlation between cholesterol-rich diet, tumor transplantation and the relative amount of nuclear DNA in the eye of rabbits. For tumor transplantation a suspension of carcinomatous cells strain "KB" was inoculated into the left eyeball of rabbits (group I, II). The experiments were carried out on two groups of animals : group I received 1 g cholesterol daily during 3 months, while group II, with tumors, but without cholesterol, served as control. The relative DNA content from Feulgen-stained nuclei was measured cytophotometrically by the two-wavelength method. The results show that cholesterol feeding produces a decrease in the nuclear DNA content in tumoral cells as compared with the tumoral cells without cholesterol. In both groups, the mean amount of DNA/nucleus was increased as compared to normal cell.

The purpose of this study is to detect a possible correlation between a cholesterol-rich diet, the tumor transplanted and the relative amount of nuclear DNA in the eye of rabbits. It is a sequel to the experiments carried out in 1975—1976 and 1978—1979 by Schuller et al. (5—8).

MATERIAL AND METHODS

The experiments were carried out on 9 adult male rabbits, weighing 1800—2000 g, Rex. species. Six animals (group I) received daily, for 3 months, 1 g cholesterol mixed up with corn flour, otherwise the animals were kept on a usual diet. At the beginning of the experiments, a suspension of carcinomatous cells, strain "KB" was inoculated into the anterior chamber (0.1 ml) and vitreous body (0.3 ml) of the left eyeball in local anesthesia with 2% cocaine. Group II, made up of 3 ani mals, was not fed on cholesterol, but was inoculated with carcinomatous cells strain "KB" : 0.1 ml into the anterior chamber and 0.3 ml into the vitreous body of the left eyeball. After 3 months, the animals were killed by cardial puncture and the eyeballs were fixed in 4% formaldehyde. The left eyeballs of the 9 animals were processed for cytophotometry, by Feulgen's reaction.

The two-wavelength method (1—4) with a reduced degree of error was used.

First, the two working-wavelengths were established. This was carried out by increasing the absorption spectrum of 10 nuclei each, of

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Table 1

Assay of relative amount of nuclear DNA/in arbitrary units/ in "KB" carcinoma, heterotransplanted into the eyeball of rabbits fed with cholesterol

GROUP 1

Frequency classes of DNA (a.u.)	Normal cells + cholesterol								Tumoral cells + cholesterol							
	Slide 1		Slide 2		Slide 5		Total		Slide 1		Slide 2		Slide 5		Total	
	No. of nuclei	Freq. %	No. of nuclei	Freq. %	No. of nuclei	Freq. %	No. of nuclei	Freq. %	No. of nuclei	Freq. %	No. of nuclei	Freq. %	No. of nuclei	Freq. %	No. of nuclei	Freq. %
6— 8.9	1	1	3	2.7	—	—	4	1.3	2	1.8	—	—	2	1.9	4	1.2
9—11.9	5	5	10	9.0	6	5.6	21	6.6	1	0.9	12	11.2	6	5.6	19	5.9
12—14.9	22	22	19	17.3	23	21.5	64	20.2	11	10.0	21	19.6	9	8.4	41	12.7
15—17.9	38	38	29	26.4	28	26.2	95	30.0	18	16.4	28	26.2	24	22.4	70	21.6
18—20.9	26	26	31	28.2	31	29.0	88	27.8	24	21.8	26	24.3	33	30.8	83	25.6
21—23.9	6	6	11	10.0	15	14.0	32	10.0	26	23.6	12	11.2	17	15.9	55	17.0
24—26.9	1	1	6	5.5	3	2.8	10	3.2	12	11.0	5	4.7	8	7.5	25	7.7
27—29.9	1	1	1	0.9	1	0.9	3	0.9	3	2.7	3	2.8	5	4.7	11	3.4
30—32.9	—	—	—	—	—	—	—	—	8	7.3	—	—	3	2.8	11	3.4
33—35.9	—	—	—	—	—	—	—	—	3	2.7	—	—	—	—	3	0.9
36—38.9	—		—	—	—	—	—	—	2	1.8	—	—	—	—	2	0.6
39—41.9																
42—44.9																
45—47.9																
48—50.9																
51—53.9																
54—56.9																
57—59.9																
Total nuclei	100		110		107		317		110		107		107		324	
Mean DNA	17.4±3.37		17.6±4.28		17.5±3.85		17.5±3.84		19.3±5.00		19.4±4.36		19.3±4.91		19.4±5.39	

the cells considered as normal (non-tumoral), by 10 to 40 nm. The obtained (transmission) values were transformed in the corresponding extinction, by a table adapted to the apparatus used (Leitz Microscope-Photometer MPE). The two working-wavelengths were established in such a manner that the extinction of the first wavelength be double as compared to the second one, in our case $A_1 = 550$ nm and $A_2 = 500$ nm.

For each slide (1,2,5 of group I and 7, 9, 7B of group II), 100 — 110 nuclei from the cells of the normal population and 105—110 nuclei of the tumoral cell — population were asseyed, at the two working-wavelengths. With the aid of Mendelsohn's tables (2) and the calculation which takes into account the area analysed photometrically (depending on the ocular and objective used in our measurements), we established the relative amount of DNA for each nucleus assayed.

The mean value was calculated statistically for each slide, as well as the mean for all normal and tumoral cells, both for the variants treated and not treated with cholesterol.

The results are presented in tables and graphs. Table 1 shows the results for the variants with cholesterol (slides 1, 2 and 5 of group I). The table comprises the distribution of the number of nuclei, assayed

GROUP I. GROUP II.

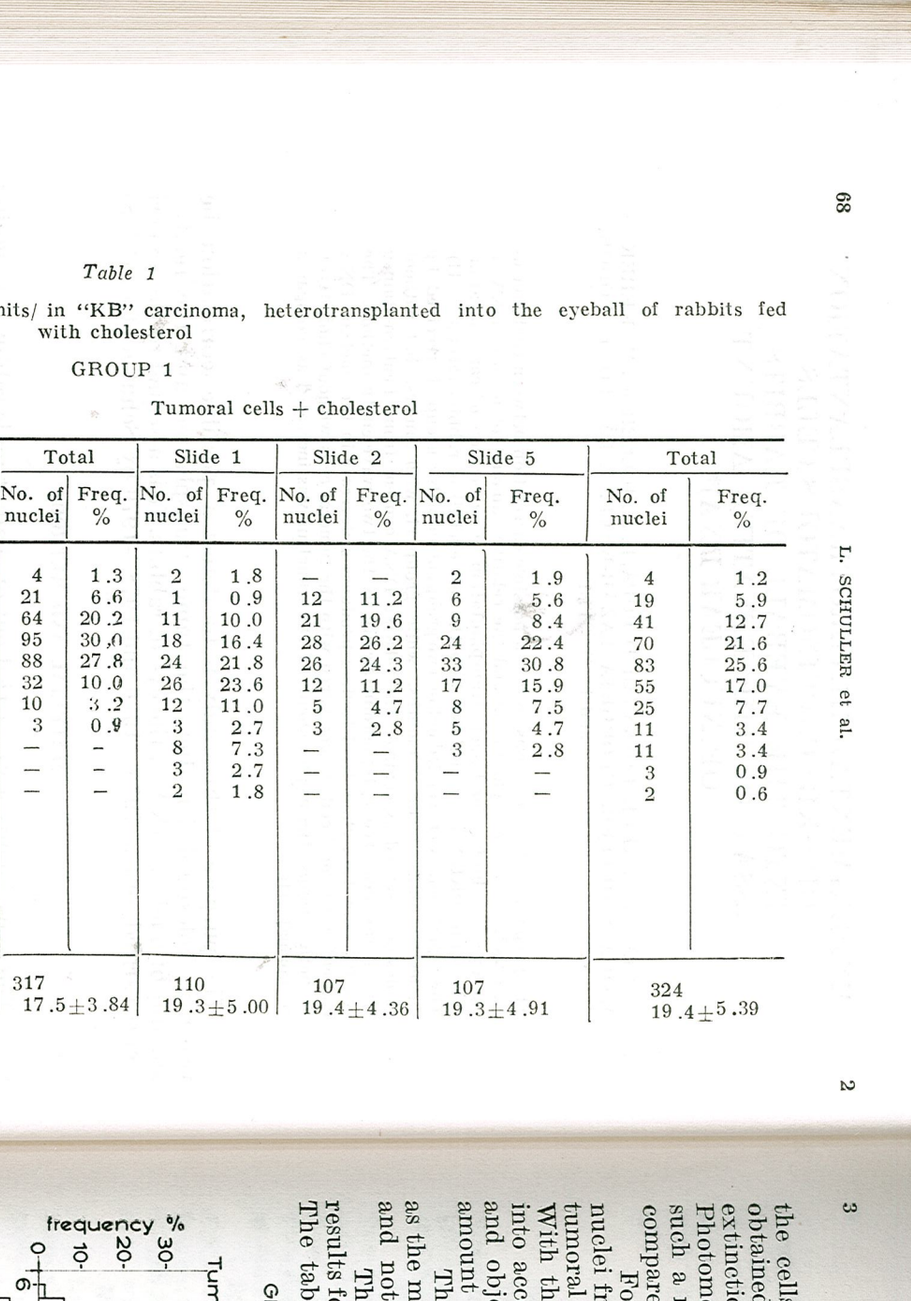

Fig. 1. — Frequency histograms of the relative nuclear DNA content in normal and tumoral eyeball-cells of rabbits fed on cholesterol (group I), as compared to control animals without cholesterol (group II).

for frequency classes of nuclear DNA in arbitrary units (a.u.), as compared to the frequency distributions of normal and tumoral cells (treated with cholesterol), the relative frequency (%) of the nuclei corresponding to the DNA classes, separately for each slide, as the total amount for the three slides assayed. The table comprises also the mean amount of DNA/ nucleus and the standard deviation for each slide in part and for their

Table 2

Control group without cholesterol

GROUP II

Tumoral cells without cholesterol

Frequency classes of DNA (a.u.)	Slide 7.		Slide 9		Slide 7 B		Total	
	No. of nuclei	Freq. %	No. of nuclei	Freq. %	No. of nuclei	Freq. %	No. of nuclei	Freq. %
Normal cells without cholesterol								
6— 8.9	—	—	—	—	—	—	1	1.0
9—11.9	4	3.8	4	3.8	1	1.0	6	5.7
12—14.9	20	19.0	28	26.9	19	18.1	35	33.3
15—17.9	38	36.2	25	24.0	35	33.3	24	22.9
18—20.9	28	26.7	25	24.0	24	22.9	18	17.1
21—23.9	6	5.7	15	14.4	18	17.1	1	1.0
24—26.9	4	3.8	7	6.7	1	1.0	1	—
27—29.9	3	2.9	—	—	1	—	—	—
30—32.9	2	1.9	—	—	—	—	—	—
33—35.9	—	—	—	—	—	—	—	—
36—38.9	—	—	—	—	—	—	—	—
39—41.9	—	—	—	—	—	—	—	—
42—44.9	—	—	—	—	—	—	—	—
45—47.9	—	—	—	—	—	—	—	—
48—50.9	—	—	—	—	—	—	—	—
51—53.9	—	—	—	—	—	—	—	—
54—56.9	—	—	—	—	—	—	—	—
57—59.9	—	—	—	—	—	—	—	—
Total nuclei	105		104		105		317	
Mean DNA	17.6±4.28		17.5±3.91		17.3±3.72		17.6±3.93	
Tumoral cells without cholesterol								
6— 8.9	1	0.3	—	—	—	—	1	0.3
9—11.9	14	4.5	3	2.8	—	—	3	1.0
12—14.9	67	21.3	5	4.7	—	—	5	1.6
15—17.9	98	31.2	20	18.7	1	4.8	26	8.2
18—20.9	77	24.5	23	21.5	4	13.3	41	12.9
21—23.9	39	12.4	21	19.6	10	21.9	54	17.0
24—26.9	12	3.8	11	10.3	11	21.0	44	13.8
27—29.9	4	1.3	6	5.6	8	16.2	31	9.8
30—32.9	2	0.6	5	4.7	15	9.5	30	9.5
33—35.9	—	—	4	3.7	12	6.7	23	7.3
36—38.9	—	—	4	3.7	10	4.8	19	6.0
39—41.9	—	—	2	1.9	12	1.9	16	5.0
42—44.9	—	—	—	—	3	—	3	0.9
45—47.9	—	—	1	0.9	9	—	10	3.2
48—50.9	—	—	1	0.9	5	—	6	1.9
51—53.9	—	—	—	—	3	—	3	0.9
54—56.9	—	—	—	—	2	—	2	0.6
57—59.9	—	—	—	—	—	—	—	—
Total nuclei	314		107		105		317	
Mean DNA	17.6±3.93		17.35±3.72		34.7±9.27		27,8±9,14	

mean. The mean relative frequency for the three assayed slides is represented graphically in Fig. 1. We mention that we considered as normal the cells beyond the carcinoma, without considering the cells with pyknotic nuclei. Table 2 shows the data of the variants which were not treated with cholesterol (slides 7, 9 and 7B of group II). The mean relative frequency for these three slides is represented in Fig. 1 B.

RESULTS AND DISCUSSION

The following conclusions can be drawn from the results :

The mean amount of DNA/nucleus is higher in tumoral cells than in normal ones. In the variant without cholesterol the mean amount of DNA/nucleus in the tumoral cells is higher by 58% than the mean amount of DNA/nucleus of normal cells.

Results with slides from animals without cholesterol (*Table 2. Fig. 1 B*). The frequency distribution of the amount of DNA/nucleus in normal cells is of a gaussian type, the maximal frequency being the frequency class of 15 — 17.9 DNA a.u., with extreme limits of 6 — 33 DNA a.u. This distribution is characterised by a low standard deviation (of ± 3.93) around the mean of 17.6 DNA a.u., the variation coefficient being 22.3%.

In tumoral cells the DNA values are more dispersed and higher DNA values occur. As it appears from Table 2, the maximal frequency lies between 21 — 24 DNA a.u., the limits of variation are between 6 and 57 DNA a.u., the standard deviation is 9.14 around the mean of 27.8 DNA a.u., while the variation coefficient is 33.3%.

Results with slides from animals with cholesterol (Table 1 and Fig. 1 A). It is to be noted that normal cells from the cholesterol-fed animals and normal cells originating from animals without cholesterol are similar in point of their DNA content and of other features.

In tumoral cells the mean amount of DNA has its maximal frequency in the range of 18 — 20.9 DNA a.u., being with only one class superior to normal cells. The mean amount of DNA increased by only 11% against normal cells and the variation limits, although greater than in normal cells, rank between 6 — 39 DNA a.u. They are lower then the variant without cholesterol (6 — 57 DNA), the standard deviation is of ± 5.39 around the mean of 19.4 DNA a.u. and the variation coefficient is 28%.

In group II the mean amount of DNA/nucleus in the tumoral cells is higher by 43.5% than the mean amounts of DNA/nucleus of the tumoral cells from group I.

CONCLUSIONS

In animals from group I, inoculated with "KB" tumoral cells and fed on cholesterol, the decrease of the mean amount of DNA/nucleus may be observed, in contrast to the animals of group II, also inoculated with "KB" tumoral cells, but without a cholesterol diet. The difference (43.5%) is significant. The dispersion of the values in tumoral cells (with

cholesterol) is lower than in the tumoral cells of animals which received no cholesterol. In both groups, the mean amount of DNA/nucleus is increased as compared to normal cells.

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ETAPPEN DER ZOOBENTHOS-ENTWICKLUNG IN DER DONAU, IM GEBIET DES "EISERNEN TORS", ABSCHNITT MRACONIA

VIRGINIA POPESCU-MARINESCU

The paper presents the evolution of zoobentos in the Danube and "Iron Gate" area — section Mraconia and the mouth of the river Mraconia, both before and after river damming.

Vorliegende Daten beziehen sich auf die Entwicklung des Zoobenthos im Gebiet des "Eisernen Tors", Abschnitt Mraconia, in den Etappen 1958, 1966—1968 vor der Aufstauung der Donau und 1972—1973, 1981—1982 nach Entstehung des Stausees. Bedeutsam ist, dass in allen Etappen der Untersuchungen im Raum Mraconia-Mündung die Differenzierung zweier grundverschiedener Abschnitte in Erscheinung tritt u.zw. a) der alte Stromlauf der Donau, heute ein Teil des Sees, b) die gewesene Mündung des Mraconia-Flusses, die jetzige Bucht.

In der Etappe 1958 bestand in der Donau im Abschnitt der Mraconia (ein Teil des Kleinen Kasanpasses), auf felsigem Untergrund mit 50—70 m tiefen Senken und bei einer Strömungsgeschwindigkeit von 3—5 km/Stunde eine lithorheophile Biozönose, an der folgende Organismengruppen beteiligt waren: Gammariden (46—65%), Corophiiden (15—53%), Trichopteren (0,03—14%), Gastropoden (0,11—3%), Triklanden (1%), Isopoden (1%), Chironomiden (0,01—1%), Polychaeten (0,04 %). Von den wichtigsten Arten zählen wir auf: *Chaetogammarus tenellus behningi*, *Dikerogrammarus -haemobaphes fluviatilis*, *D. villosus bispinosus*, *Pontogammarus obesus*, *Corophium curvispinum*, *C. robustum*, *C. maeoticum*, *Hyrdopsyche ornatula*, *H. contubernalis*, *H. guttata*, *Neureclipsis bimaculata*, *Theodoxus transversalis*, *T. danubialis*, *Lithoglyphus naticoides*, *Polycelis nigra*, *P. cornuta*, *Palaeodendrocoelum romanodanubialis*, *Rheotanytarsus exiguus*, *Hypania invalida*, *Jaera sarsi*, *Simulium columbaczense*. Besonders betont sei die Anwesenheit und Abundanz der reliktären pontisch-kaspischen Elemente in der Zusammensetzung der Benthosfauna dieser Zone, sowie die Bedeutung dieser Zoozönosen als Ernährungsgrundlage für die wirtschaftlich wertvollen Fische, wie z.B.: Sterlet, Waxdick, Nase und Quappe (3).

Ebenfalls im Mraconia-Abschnitt der Donau, aber im Jahre 1968, bestand am Ufer, bei geringer Tiefe (1—2 m) und schwacher Strömung, auf steinigem oder sandig-steinigem Untergrund eine lithorheophile Biozönose, welche hauptsächlich folgende Organismengruppen -oft mosaikartig- enthielt: Corphiiden (5—51%), Gammariden (6—39%), Schnecken (3—64%), Coelenteraten (3—25%), Chironomiden (1—18%), Trichopteren (1—6%), Isopoden (3—5%), Oligochaeten (1—4%), Ephemeropteren (0,4—1%). Zahlendichte und Biomasse der Zoobenthos ist in Tabelle 1 widergegeben. Ausser den 1958 vermerkten Arten kommen 1968 noch hinzu: *Otoplana antipai*, *Limnodrilus hoffmeisteri*, *Tubifex tubifex*,

Nais behningi, *Ilyodrilus coccineus*, *Phreoryctes gordioides*, *Dreissena polymorgha* (1).

Hinsichtlich des Zustandes an der Mraconia-Mündung in den Jahren 1966—1967 (1) ist zu bemerken, dass dieser Nebenfluss der Donau auf seinem ganzen Lauf einen gebirgigen Charakterhat. Auf dem vornehmlich steinigen und stellenweise sandig-schlammigen Untergrund, bei ziemlich starker Strömung und stetiger Wasserführung, bestanden die benthonischen Zoozönosen vor allem aus—zu allen Jahreszeiten dominierenden—Simuliiden-Larven (50—80%), gefolgt von Chironomiden (20—44%), Oligochaeten (5—39%), Trichopteren (1—23%), Plecopteren (0,2—8%), Mollusken (0,3—7%), Ephemeropteren (0,1—3%).. Zahlendichte und Biomasse des Zoobenthos ist in Tabelle 1 widergegeben.

Tabelle 1

Zahlenmässige Dichte und Biomasse des Zoobenthos der Donau im Abschnitt Mraconia und bei der Einmündung des Flusses Mraconia, in den Jahren 1966—1968 (Mittelwerte)

Organismen-Gruppe	Donau		Mündung des Flusses Mraconia			
	1968		1966		1967	
	Ex./m²	g/m²	Ex./m²	g/m²	Ex./m²	g/m²
Coelenterata	707	0,035	—	—	—	—
Turbellaria	6	0,009	—	—	—	—
Nematoda	53	0,001	—	—	5	—
Polychaeta	3	0,003	—	—	—	—
Oligochaeta	100	0,009	1158	0,052	1033	0,158
Hirudinea	5	0,003	—	—	—	—
Tardigrada	—	—	44	0,001	—	—
Gastropoda	387	2,055	28	0,275	27	0,323
Copepoda	137	0,003	—	—	—	—
Cladocera	18	0,003	—	—	—	—
Isopoda	82	0,010	—	—	—	—
Gammaridae	487	1,218	—	—	—	—
Corophiidae	171	0,154	—	—	—	—
Hidracarina	5	0,001	17	0,009	133	0,015
Ephemeroptera	21	0,025	27	0,212	5	0,005
Plecoptera	—	—	39	0,069	10	0,007
Odonata	—	—	—	—	4	0,007
Coleoptera	3	0,003	22	0,007	—	—
Trichoptera	171	1,813	130	3,032	260	2,675
Chironomidae	521	0,042	1317	0,553	2807	0,345
Simuliidae	—	—	tausende Exemplare		tausende Exemplare	
Diptera varia	—	—	189	0,207	247	0,512
GESAMTWERT	2877	5,387	2971	4,417	4531	4,047

Zu den wichtigsten Arten zählen : *Wilhelmia balcanica*, *W. salopiensis*, *danubialis*, *W. iveshentzovi*, *Eusimulium aureum*, *Cnephia tredecimata*, *Friesia condici*, *Rheotanytarsus exiguus*, *Tanytarsus mancus*, *Micropsectra praecox*, *Orthocladius saxicola*, *O. bathophilus*, *Cricotopus algarum*, *Diamesa l. carpatica*, *Rhyacophila nubila*, *Polycentropus* sp., *Psychomya pusilla*, *Hydropsyche pellucidula*, *Cheumatopsyche lepida*, *Stactobia* sp., *Hydroptila* sp., *Leptocerus* sp., *Baetis venustulus*, *B. rhodani*, *Caenis macrura*, *Ecdyonurus*

fluminum, *Ephemerella ignita*, *Capnia nigra*, *Leuctra fusca*, *Perla burmeisteriana*, *Taenioptheryx schoenemundi*, *Nais communis*, *N. elinguis*, *N. pardalis*, *N. bretscheri*, *Enchytraeus albidus*, *Limnodrilus hoffmeisteri*.

Die Schaffung des "Eisernes Tor"-Stausees im Jahre 1970 hatte bedeutende Veränderungen der Biotopbedingungen zur Folge. So bewirkte das Absinken der Strömungsgeschwindigkeit auf 0,36 m/s eine Zunahme der Sedimente, was die Natur der Untergrund-Fazies änderte; die schlammige Fazies wurde sowohl im Zentrum des Sees, als auch in der Mraconia-Bucht dominierend. Der Übergang von einem Fluss-Typ des Biotops zu einem See-Typ findet seinen Niederschlag in der Ausbildung der benthonischen Zoozönosen.

Tabelle 2

Zahlenmässige Dichte und Biomasse des Zoobenthos aus dem Stausee „Eisernes Tor", im Abschnitt Mraconia, in den Jahren 1972—1973 (Mittelwerte)

Organismen-gruppe	1972		1973	
	Ex./m²	g/m²	Ex./m²	g/m²
Coelenterata	297	0,275	277	0,270
Turbellaria	33	0,039	41	0,057
Nematoda	20	—	647	0,009
Polychaeta	1560	1,363	1992	1,876
Oligochaeta	1029	0,534	1776	0,970
Lamellibranchia	7	0,091	498	6,495
Gastropoda	193	16,235	185	15,124
Copepoda	300	0,004	1361	0,018
Cladocera	20	—	238	0,007
Ostracoda	—	—	—	—
Isopoda	186	0,056	100	0,033
Gammaridae	640	0,365	11794	6,700
Corophiidae	1176	2,352	7773	16,252
Hidracarina	—	—	—	—
Collembola	—	—	—	—
Ephemeroptera	—	—	—	—
Odonata	40	0,035	—	—
Coleoptera	7	0,009	—	—
Trichoptera	13	0,013	—	—
Chironomidae	233	0,699	8	0,003
Diptera varia	—	—	—	—
GESAMTWERT	5754	22,070	26690	47,814

Demzufolge dominierten im Mraconia-Abschnitt (2), (4) — im See—im Zeitraum 1972—1973, auf vorwiegend schlammige mGrund in der Benthos-Fauna im ersten Jahr die Polychaeten (10—35%), die Oligochaeten (9—25%), die Corophiiden (12—23%) und die Gammariden (1—23%), im zweiten Jahr die Gammariden (44—46%), Corophiiden (29—44%), Polychaeten (8—39%), Oligochaeten (7—10%) und Lamellibranchien (2—5%). Zahlendichte und Biomasse der Zoobenthos ist in Tabelle 2 wiedergegeben. Die Artenzahl sank durch das Ausbleiben der streng rheophilen, aber infolge der Anpassung der verbliebenen an die neuen Bedingungen nahm die Individuenzahl stark zu. *Hypania invalida*

Tabelle 3

Zahlenmässige Dichte und Biomasse des Zoobenthos aus der Mraconia-Bucht, in den Jahren 1972—1973, (Mittelwerte)

Osganismengruppe	1972				1973			
	Ufer		Mitte		Ufer		Mitte	
	Ex./m²	g/m²	Ex./m²	g/m²	Ex./m²	g/m²	Ex./m²	g/m²
Nematoda	93	—	17		—	—	—	—
Polychaeta	—	—	3	0,012	11	0,054	83	0,190
Oligochaeta	683	0,819	506	0,607	1024	1,232	1942	2,464
Lamellibranchia	—	—	—	—	210	1,134	6	0,023
Gastropoda	—	—	—	—	—	—	11	0,430
Copepoda	3073	0,153	1683	0,084	321	0,016	244	0,012
Cladocera	257	0,038	160	0,024	122	0,018	338	0,032
Ostracoda	160	0,003	27	0,001	149	0,003	167	0,003
Gammaridae	—	—	—	—	11	0,028	—	—
Corophiidae	—	—	—	—	33	0,033	6	0,008
Hidracarina	40	0,004	33	0,003	—	—	33	0,003
Collembola	27	0,002	—	—	—	—	—	—
Ephemeroptera	20	0,029	—	—	39	0,047	—	—
Trichoptera	—	—	—	—	50	0,027	—	—
Chironomidae	4063	3,349	1493	1,239	5284	4,565	493	1,640
Diptera varia	13		20		17	0,027	—	—
GESAMTWERT	8429	4,397	3942	1,970	7271	7,184	3323	4,805

Tabelle 4

Zahlenmässige Dichte und Biomasse des Zoobenthos aus dem Stausee "Eisernes Tor", Abschnitt Mraconia, in den Jahren 1981—1982 (Mittelwerte)

Organismengruppe	1981				1982			
	Ufer		Mitte		Ufer		Mitte	
	Ex./m²	g/m²	Ex./m²	g/m²	Ex./m²	g/m²	Ex./m²	g/m²
Coelenterata	17	0,001	—	—	—	—	—	—
Turbellaria	17	0,065	272	0,569	13	0,064	293	0,879
Nematoda	10.100	0,030	816	0,002	38	—	26	—
Polychaeta	16.698	20,339	4.675	5,676	12.406	11,726	3889	3,978
Oligochaeta	83.529	55,257	38.947	6,049	55.704	91,566	20.846	81,256
Hirudinea	391	21,990	17	2,555	382	14,061	1.606	20,247
Lamellibranchia	48.439	3392,435	2.210	8,308	49.228	2671,239	45.696	2659,236
Gastropoda	153	7,371	51	5,747	306	15,788	4.322	149,060
Isopoda	—	—	—	—	13	0,019	—	—
Gammaridae	1.479	1,038	1.394	10,623	778	8,528	306	1,415
Corophiidae	5.850	2,122	106.256	49,385	4.679	1,191	5.444	4,303
Mysidacea	17	0,003	—	—	—	—	—	—
Collembola	17	0,005	—	—	—	—	—	—
Chironomidae	17	0,012	—	—	13	0,015	64	0,089
Diptera varia	17	0,005	34	0,017	—	—	—	—
GESAMTWERT	166.741	3500,673	154.672	88,931	123.560	2814,197	82.492	2920,463

z.B., eine pelophile Form, die vor der Aufstauung unter 5 Ex./m² zählte, gelangte nach her zu Tausenden Ex/m². Hohe Werte erzielten auch *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, *Branchiura sowerbyi*, *Corophium curvispinum*. *C. robustum*, *C. maeoticum*, *Dikerogammarus haemobaphes fluviatilis*, *D. villosus bispinosus*, *Chaetogammarus tenellus behningi*, *Dreissena polymorpha*, *Lithoglyphus naticoides* und *Palaeodendrocoelum romanodanubialis* waren häufig und *Otoplana antipai* bloss anwesend.

In der Mraconia-Bucht war in der Jahren 1972—1973 (2), (4) die Benthos-Fauna eintöniger als im See; hier dominierten im ersten Jahr die Chironomiden (27—91%), und die Oligochaeten (8—47%), wozu sich 1973 die Lamellibranchien (0,2—3%) gesellten. Zahlendichte und Biomasse der Zoobenthos ist in Tabelle 3 widergegeben. Als Arten seien ausser denjenigen vom See (mit Ausnahme der Amphipoden) die Naididen erwähnt, die auch früher, vor der Aufstauung, von der Flussmündung bekannt waren, und auch die Trichopteren *Hydropsyche ornatula* und *Neureclipsis bimaculata*. Eine starke Entwicklung erfuhren eine Reihe von Chironomiden wie z.B.*Chironomus bathophilus*, *C. plumosus*, *Limnochironomus nervosus*, *Procladius Skuze*, *Polypedilum nubeculosum*, *Tanytarsus lauterborn* . Hervorzuheben wäre auch das Verschwinden ganzer Organismengruppen u.zw. der streng rheophilen wie z.B. der Simuliiden und Plecopteren. Bemerkenswert ist auch die Häufigkeit von *Lithoglyphus naticoides*.

1981—1982 — also über 10 Jahre nach der Aufstauung beim Eisernen Tor, stellten wir fest, dass im See (Marconia-Abschnitt) eine gewisse Stabilisierung der Bestandteile der Benthos-Zoozönosen stattgefunden hat. Die dominanten Gruppen waren stets die Oligochaeten (25—94%), die Lamellibranchien (2—55%), die Polychaeten (3—16%) und Corophiiden (1—9%). Die massenhaft vorkommenden Arten in der Mehrzahl der dominierenden Gruppen waren dieselben wie in den Jahren 1972—1973, die Lamellibranchien *Sphaerium riviculum* und *S. corneum* haben jedoch allmählich *Dreissena polymorpha* als Dominante verdrängt. Zalendichte und Biomasse der Zoobenthos ist in Tabelle 4 widergegeben. Bemerkenswerterweise ist die Artenmannigfaltigkeit in der Benthos-Zoozönose vom Ufergebiet des Sees grösser als im Zentraum, wie auch die Stabilität.

Im Zeitraum 1981—1982 waren im Bereich der Mraconia-Bucht in der Benthosfauna stets die Oligochaeten (17—60%), Chironomiden (2—54%) und Lamellibranchien (1—12%) dominant. Zalendichte und Biomasse der Zoobenthos ist in Tabelle 5 widergegeben. Wie im See, so ist auch im Golf im Ufergebiet eine grössere Vielfalt der Arten festzustellen als im Zentrum, jedoch waren in beiden Zonen der Bucht die Gammariden, Corophiiden, Gastropoden und Polychaeten anwesend. Zu bemerken ist das Ausscheiden der Ephemeropteren aus den Benthos-Zoozönosen. Massenhaft vorkommend waren: *Limnodrilus hoffmeisteri*, *Chironomus plumosus*, *Polypedilum nubeculosum*, *Procladius Skuze*, *Dreissena polymorpha*, *Sphaerium riviculum*, *S. corneum*.

Abschliessend dürfen wir feststellen, dass im Gebiet des Eisernen Tors einschliesslich Mraconia-Abschnitt die Aufstauung der Donau zur Umwandlung des schnellfliessenden Biotops in ein langsamfliessendes geführt hat. Dies verursachte tiefgreifende Strukturänderungen der Ben-

Tabelle 5

Zahlenmässige Dichte und Biomasse des Zoobenthos aus der Mraconia-Bucht, in den Jahren 1981—1982 (Mittelwerte)

Organismengruppe	1981				1982					
	Ufer		Mitte		Ufer		Mitte			
	Ex./m²	g/m²	Ex./m²	g/m²	Ex./m²	g/m²	Ex./m²	g/m²		
Coelenterata	—	—	—	—	119	0,002	—	—		
Nematoda	1.887	0,049	1.615	0,049	51	0,001	306	0,012		
Polychaeta	1.293	0,692	—	—	17	0,020	17	0,170		
Oligochaeta	12.329	26,668	5.901	33,910	6.205	7,242	4.403	27,809		
Hirudinea	—	—	—	—	306	0,595	51	0,510		
Lamellibranchia	2.024	810,441	459	2,882	391	73,083	612	9,401		
Gastropoda	85	5,141	119	3,090	1.003	18,780	153	5,428		
Copepoda	1.785	0,218	187	0,010	102	0,002	255	0,005		
Cladocera	50	0,026	—	—	—	—	—	—		
Ostracoda	952	0,299	150	0,025	—	—	—	—		
Isopoda	—	—	—	—	—	—	—	—		
Gammaridae	255	0,459	17	0,008	—	—	—	—		
Corophiidae	2.041	1,059	442	0,068	51	0,077	—	—		
Hidracarina	17	0,015	—	—	—	—	—	—		
Odonata	17	1,795	—	—	—	—	—	—		
Chironomidae	986	0,761	4.643	2.088	1.258	2,779	986	2,945		
Insecta varia	17	0,102	34	0,026	—	—	—	—		
GESAMTWERT	23.738	847,725	13.567	42,156	9.503	102,581	6.783	46,280		

thos-Zoozönosen, deren Artenzahl stark zurückging, vor allem durch das Ausscheiden der stenotop rheophilen (lithorheophilen) Arten und deren Verdrängung durch eurytope stagnophile (zu den pelophilen gehörige) Arten, die eine sehr starke Entfaltung erfuhren.

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DIE ETAPPENWEISE ENTWICKLUNG DES ZOOBENTHOS IN DER DONAU, IM BEREICH DES "EISERNEN TORS", CERNA-ABSCHNITT

ELENA PRUNESCU-ARION

The paper is a brief evaluation of the zoobentos evolution of the zoobentos evolution in the Iron Gate area, the river waterway (contained within the lake limits) and the terminal portion of the river Cerna (today Cerna gulf).

Vorliegende Arbeit bietet einen Überblick über die Entwicklung des Zoobenthos im Bereich des Eisernen Tors (die alte Schiffahrtsrinne — heutzutage im Stausee gelegen, und der Endabschnitt des Cerna-Flusses, die jetzige Cerna-Bucht) in den Jahren 1958 und 1968 vor der Überflutung, in den Jahren 1972—1973 und 1981—1982 nach der Schaffung des Stausees.

Die vor der Aufstauung der Donau durchgeführten hydrobiologischen Forschungen haben die Existenz einer rheophilen benthonischen Fauna ausgewiesen, für welche charakteristische Umweltbedingungen vorhanden waren. So war in der Schiffahrtsrinne im Cerna-Mündungsgebiet die Benthos-Fauna durch einige Gruppen der Wirbellosen vertreten; 1958 (2) herrschten davon die Chorophiidae (max. 62%) und die Gammaridae (max. 34%) vor, gefolgt von Trikladen (max. 3%) and Trichopteren (max. 1%). Artenmässig dominierten die lithorheophilen Elemente der reliktären pontisch-kaspischen Fauna wie z.B.: *Corophium curvispinum*, *C. robustum*, *Dikerogammarus haemobaphes fluviatilis*, *D. villosus bispinosus*, *Chaetogammarus tenellus behningi*. Weniger fielen ins Gewicht die Trikladen *Polycelis nigra*. *P. cornuta* und *Palaeodendrocoelis romanodanubialis* und von den Trichopteren *Hydropsyche ornatula*, *H. contubernalis*, *H. guttata* und *Neureclipsis bimaculata*. Nach Ablauf von 10 Jahren, 1968 (1) waren die Gammaridae (max. 41%) dominant geblieben; auf diese folgten jedoch die Chironomiden (max. 18%), die Oligochaeten (max. 17%) und die Trichopteren (max. 10%). In den Jahren 1958 und 1968 wurdendie gleichen Gammariden- und Trichopterenarten gefunden; ausserdem erwähnen wir noch die Chironomiden *Tendipes j. l. thummi*, *T. j. l. semireductus*, *Tanytarsus gr. lauterborni* und die Oligochaeten *Tubifex tubifex*, *Limnodrilus hoffmeisteri* und *Nais pardalis*.

Was den Endabschnitt der Cerna anbelangt, so wies dieser 1958 (2) und 1968 (I) sowol schwach sturzbachartige als auch ruhig fliessende Streken auf, wobei in diesen Biotopen jeweils bestimmte Organismengruppen und Arten dominierten.

Es muss aber darauf hingewiesen werden, dass an der Einmündung der Cerna in die Donau die Struktur der Biozönosen durch ein Gemenge von Strom- und Fluss-Spezies gekennzeichnet ist. Demgemäss waren in den mässig-schnell fliessenden Teilstrecken in der Biozönose die Ephemeropteren vertreten durch: *Baëtis pumilus*, *Ecdyonurus insignis*,

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E. venosus, *Ephemerella ignita*, *Baëtis rhodani*, *B. venustulus*, *Ephemera danica*, *Ephemerella (Torleya) major*, *Haproleptoides modesta*, *Caenis macrura*; die Trichopteren durch *Brachycentrus subnubilus*, *Hydropsyche angustipennis*, *H. ornatula*, *H. guttata*, *H. coniubernalis*, *Neuronia rufi-crus*, *Psychomyia pusilla*, *Rhyachophila nubila*; die Plecopteren durch *Isoperla gramatica*, *Leuctra* sp., *Perlodes microcephala*, *Perla burmeis-teriana*, *Taeniopterix* sp. und die Simuliidae durch *Odagmia variegata* und *Wilhelmia balcanica* (3).

Auf den ruhiger fliessenden Strecken, mit von Periphyton und pflanzlichem und schlammigem Detritus bedeckten Steinen, ist das Vorherrschen der Borstenwürmer (max. 37%) und der Zuckmücken (max. 27%) hervorzuheben. Die Oligochaeten waren vertreten durch: *Nais pseudoptusa*, *N. bretscheri*, *N. pardalis*, *Stylaria lacustris*, *Chaetogaster diaphanus*, *Ophidonais reckei*, *Limnodrilus claparèdeanus*, *L. longus*, *Pelo-scolex ferox*, *Rhyacodrilus coccineus*; von den Chironomiden seien erwähnt: *Tendipedini gen. l. monoculata*, *Eukiefferiella longicalcar*, *E. quadri-dentata*, *Tanytarsus gr. lauterborni*, *Orthocladius potamophilus*, *O. gr. saxicola*, *Cricotopus biformis*, *Lauterbornia*, *Tanytarsus labatijrons*, *T. gr. mancus*, *Tendipes f. l. plumosus*, *Polypedilum breviantennatum*, *Limnophies gr. transcaucasius*, *Metriocnemus hygropetricus*, *Cricotopus gr. algarum*, *Dia-mesa campestris*, *Cryptochironomus l. polysetica*, *Polypedilum gr. con-victum* und *Tendipes f. l. semireductus* (3).

Die Veränderung der Umweltbedingungen, besonders der abioti-schen, hat zu tiefgreifenden Änderungen der alten Systeme geführt, was sich logischerweise in entsprechenden Modifizierungen der Benthos-Fauna auswirkte. Wie zu erwarten, sind nach der Aufstauung ganze Organismen-gruppen verschwunden und die Dominanten durch andere ersetzt worden. Demzufolge wurde vor der Cerna-Bucht im Zeitraum 1972—1973 (5) festgestellt, dass die dominanten Ephemeropteren- und Triopteren-Larven durch pelophile Polychaeten (max. 52%), Oligochaeten (max. 40%) gefolgt von vornehmlich stagnophilen Chironomiden, ersetzt worden waren. In dieser Periode dominierten von den Würmern einige Taxa u.zw.: *Hypania invalida* und *Limnodrilus hoffmeisteri*. Von anderen zu der Biozönose gehörigen Organismen seien noch aufgezählt: *Lithoglyphus naticoides*, *Corophium curvispinum*, *Chironomus f.l. plumosus*, *C.f.l. semi-reductus*, *Cricotopus biformis*, *C. silvestris*, *Limnochironomus nervosus*.

Nach der Aufstauung der Donau und der Entstehung des Cernagolfs gehörten in der gewesenen Cerna-Mündung in den ersten Jahren nach der Überflutung, 1972—1973, noch immer die stagnophilen Chironomiden (max. 50%) und Oligochaeten (max. 45%) zu den dominierenden Orga-nismengruppen: dazu kamen im Jahre 1972 noch die Copepoden (max. 32%) hinzu. In den darauf folgenden Jahren nahm der Anteil der Cla-doceren und Copepoden stark ab (5). In allgemeinen können in der Cerna-Bucht zwei deutlich voneinander verschiedene Zonen erkannt werden u.zw.: am Ufer und im Zentrum. Die einzelnen Benthos-Organismen sind in verschiedenen Jahren in der einen oder anderen Zone zahlreicher. In den ersten Jahren nach der Aufstauung zählten zu den dominanten Taxa in der Cerna-Bucht der Borstenwurn *Limnodrilus hoffmeisteri* und die Chironomiden *Cryptochironomus defectus*, *C. pararostratus*, *Limno-chironomus nervosus*, *Polypedilum convictum*, *Eukiefferiella longicalcar*,

Procladius Skuze, *Thienemannimyia lentiginosa*. Es kann also gefolgert werden, dass in den ersten Jahren nach Entstehung des Stausees sowohl in der gewesenen Schiffahrtsrinne als auch im Einmündungsgebiet der Cerna eine Verarmung der Benthosfauna stattgefunden hat, was auf der Vereinheitlichung der Lebensbedingungen beruht, aber nicht zu einer quantitativen Verarmung, sondern zum Ansteigen der Biomasse geführt hat.

Neuere, zwischen 1981 und 1982 durchgeführte ökologische Studien, also lange Jahre nach der Überflutung, haben ergeben, dass der mit der Bildung des Stausees eingeleitete Umwandlungsprozess des Ökosys-tems das Reifestadium erreicht hat. Dies wird durch die strukturelle Stabilisierung der neuen Benthos-Biozönosen bestätigt, in denen die typisch stagnophilen Organismen als dominant auftreten. Wir betonen, dass auch in den letzten Jahren im Cerna-Golf die Oligochaeten (max. 65—72%) dominant geblieben sind, gefolgt von den Polychaeten (max. 33—14%), den Chironomiden-Larven (max. 20—12%) und den Lamelli-branchien (Max. 15—11%). Artenmässig ausgedrückt waren folgende hauptsächlich dominant: *Limnodrilus hoffmeisteri*, *Hypania invalida*, *Dreissena polymorpha*, *Sphaerium* sp., *Chironomus f.l. plumosus*, *Poly-pedilum nubeculosum* und *Procladius Skuze*.

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DAS ZOOPLANKTON DES EISERNES TOR-STAUSEES IM GEBIET DER CERNA-MÜNDUNG, IM ZEITRAUM 1981—1984

VICTOR ZINEVICI und LAURA TEODORESCU

The paper presents the qualitative and quantitative structure of zooplankton, on trophic levels — primary consumers (c_1) and secondary consumers (c_2) — for 4 years. The investigations reveal a disturbance of the ecological balance in the gulf as well as in the stagnant biocenoses. From a quantitative point of view increased values between 1981—1983 were recorded, and in 1984 a tendency of return to the case of 1981, can be explained by the variation in the degree of trophicity.

Durch die Aufstauung der Donau im Jahre 1971 bei Gura Văii-Sip stieg das Wasserniveau im unteren Teil des Stausees um beiläufig 15 Meter; dies führte zur Überflutung der Flussmündungen, welche in Buchten umgewandelt wurden. Die Cerna-Bucht (Donaukilometer 954) entstand im Mündungsgebiet des grössten Nebenflusses der Donau am See, und stellt den grössten der Golfe dar.

Gegenstand dieser Arbeit ist die qualitative und quantitative Struktur des Zooplanktons nach trophischen Niveaus (Primärkonsumenten $= c_1$ und Sekundärkonsumenten $= c_2$) in einem Zeitraum von 4 Jahren. Die Proben wurden allmonatlich, vom Mai bis Oktober, mit dem Patalas-Gerät eingesammelt.

Tabelle 1

Die taxonomische Zusammensetzung des Zooplanktons (%) nach trophischen Niveaus, in der Cerna-Bucht, in den Jahren 1981—1984

Troph. Niveau	Taxon. Gruppe	1981	1982	1983	1984	1981—1984
c_1	*Ciliata*	6,89	13,95	14,29	9,43	10,38
	Testacea	3,44	2,34	14,29	7,55	9,43
	Rotifera	68,98	58,13	50,00	62,26	59,44
	Lamellibranchia	1,72	2,34	2,38	1,89	0,94
	Cladocera	15,53	16,27	11,90	13,21	15,09
	Copepoda	3,44	6,97	7,14	5,66	4,72
	Gesamtzahl	58	43	42	53	106
c_2	*Rotifera*	16,67	14,28	20,00	28,57	30,00
	Cladocera	16,67	14,28	20,00	14,28	10,00
	Copepoda	66,66	71,44	60,00	57,15	60,00
	Gesamtzahl	6	7	5	7	10

Die qualitative Analyse (Tabelle 1) über die gesamte Untersuchungs-Dauer ergibt ein taxonomisches Spektrum, welches gegenüber der Periode 1978—1980 (1) etwas erweitert erscheint. Für die Primärkonsumenten liegt der Schwerpunkt bei den Rädertieren, gefolgt von Cladoceren, Pro-

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tozoen und Copepoden und zuletzt von Lamellibranchia. Bei den Sekundärkonsumenten weisen die Copepoden den höchsten Prozentsatz auf; es folgen Rädertiere und Cladoceren. Die Dynamik über mehrere Jahre der taxonomischen Zusammensetzung zeigt ein viel schmäleres taxonomisches Spektrum auf, als dem Mittel der Untersuchungsperiode entspricht. Der grosse Unterschied zwischen den Jahreswerten der qualitativeu Dynamik des Zooplanktons und dem Durchschnitt der Untersuchungsperiode beweist die Instabilität des ökologischen Gleichgewichts innerhalb des Golfes und ist auf die Artenfolge im Laufe der 4 Jahre zurückzuführen. Diese Instabilität wird auch bestätigt durch die geringe Anzahl konstanter Elemente im Zeitraum 1981—1984; für das Zooplankton c_1 *Bosmina longirostris* und *Diaphanosoma orghidani (Cladocera)*, Nauplien und Copepoditen des I—III Stadiums (*Cyclopida* g.sp.); für das Zooplankton c_2 *Acanthocyclops vernalis (Copepoda)*.

Tabelle 2

Die Dichte (Anzahl/Liter), die numerische Abundanz (%), die Biomasse µg/l feuchte Substanz, und die Abundanz der Biomasse (%) des Zooplanktons c_1

Jahr	Anz./l / µg/l	Abundanz					
		Cil.	Test.	Rot.	Lam.	Clad.	Cop.
1981	56,3 / 504,5	4,26 / 0,03	0,05 / —	19,53 / 2,59	1,60 / 0,12	15,80 / 54,98	58,76 / 42,28
1982	119,1 / 1749,4	12,42 / 0,01	0,08 / —	15,33 / 4,97	2,60 / 0,11	24,31 / 72,69	45,26 / 22,22
1983	130,0 / 3748,0	2,55 / —	0,70 / 0,01	13,57 / 0,21	0,58 / 0,01	51,57 / 93,24	31,03 / 6,53
1984	123,0 / 571,6	25,57 / 0,33	0,31 / 0,01	42,92 / 8,08	0,97 / 0,14	9,99 / 76,11	20,24 / 15,33
$\bar{X}_a$ 1981—1984	107,1 / 1643,4	12,13 / 0,03	0,33 / 0,01	23,28 / 2,35	1,39 / 0,06	27,35 / 83,34	35,52 / 14,21

Die zahlenmässige Dichte (Tabellen 2 und 3) ist in der Periode 1981—1984 grösser als in derjenigen von 1978—1980, ohne aber einem reichhaltigen Zooplanton zu entsprechen. Für das Zooplankton c_1 nimmt die Dichte von 1981 bis 1983 zu, wann auch das Maximum erreicht wird, um dann 1984 eine leichte, unbedeutende Abnahme zu erfahren. Im allgemeinen überwiegen die Copepoden vor den Cladoceren, mit Ausnahme des Jahres 1984, wann die Rädertiere an die erste Stelle rücken und die Copepoden auf die zweite. Das Zooplankton c_2 zeigt ein Austeigen der zahlenmässigen Dichte von 1981 bis 1983, um dann 1984 beträchtlich bis auf ein Minimum zu sinken. Der Mittelwert der numerischen Dichte des Zooplanktons ($c_1 + c_2$) im Zeitraum 1981—1984 ist über dreimal so hoch als in der Periode 1978—1980, was grossenteils in der hydrologischen

Charakteristik begründet ist. In den letzten Jahren hat die schwache Wasserführung der Donau die Aufrechterhaltung eines hohen Pegelstandes im Stausee veranlasst, was die Entwicklung einer für Stagnation typischen Biozönose begünstigt, welche reichhaltiger ist als die rheophile.

Tabelle 3

Die Dichte (Anz./Liter), die numerische Abundanz (%), die Biomasse (µg/l feuchte Substanz) und die Abundanz der Biomasse (%) des Zooplanktons c_2

Jahr	Anz./l / µg/l	Abundanz		
		Rotifera	*Cladocera*	*Copepoda*
1981	16,9 / 92,9	85,87 / 36,14	0,12 / 1,43	14,01 / 62,43
1982	103,9 / 455,0	82,21 / 40,48	0,28 / 7,34	17,51 / 52,18
1983	155,1 / 1161,6	93,28 / 13,49	0,97 / 67,15	5,75 / 19,36
1984	13,3 / 130,2	94,97 / 77,37	0,75 / 7,68	4,28 / 14,95
$\bar{X}_a$ 1931—1984	72,3 / 459,9	88,95 / 25,83	0,66 / 44,83	10,39 / 29,34

Die Biomasse des Zooplanktons (Tabellen 2 und 3) ist ebenfalls grösser als diejenige des Zeitraums 1978—1980. Die Zunahme der Eutrophierung in der Cerna-Bucht auf der Stufe des Zooplanktons zeichnet sich durch Überwiegen der Organismen höherer Körpergrösse *(Cladocera,*

Tabelle 4

Die Produktivität (µg/l/24 h, feuchte Substanz) des Zooplanktons c_1 und c_2

Troph. Niveau	Jahr	µg/l/24 h	*Rot.*	*Lam.*	*Clad.*	*Cop.*
c_1	1981	140,1	1,6	0,1	121,3	17,1
	1982	232,1	9,7	6,5	185,3	30,6
	1983	528,8	3,5	0,1	501,9	23,3
	1984	65,6	12,9	0,1	46,2	6,4
	$\bar{X}_a$ 1981—1984	241,6	6,9	1,7	213,7	19,3
c_2	1981	10,8	8,1		0,1	2,6
	1982	96,6	83,8		5,0	7,8
	1983	150,5	54,3		89,1	7,1
	1984	33,9	32,6		1,0	0,3
	$\bar{X}_a$ 1981—1984	72,9	44,7		23,8	4,4

Cil = Ciliata; Test. = Testacea; Rot. = Rotifera; Lam. = Lamellibranchia; Clad. = Cladocera; Cop. = Copepoda.

Copepoda) aus, was auch grössere Biomasse bedeutet. Beim Zooplankton c_1 nimmt die Biomasse von 1981 bis 1983 zu und erreicht ein Maximum, um dann 1984 ungefähr auf den Wert des ersten Jahres zurückzugehen. Dasselbe gilt auch für das Zooplankton c_2. Es fällt auf, dass unter den Primärkonsumenten die Cladoceren über die ganze Versuchsdauer eine Vorrangstellung einnehmen, gefolgt von den Copepoden und bei den Sekundärkonsumenten, die Copepoden mit Ausnahme des Jahres 1984, wenn sie durch Rotiferen ersetzt werden (kleine Organismen, was geringe Biomasse bedeutet).

Die Werte für die zooplanktonische Produktivität (Tabelle 4) erreichen die Höchstwerte in 1983, sowohl für c_1 als auch für c_2, Die Maxima beruhen auf der erhöhten Produktivität der Cladoceren (c_1) und für c_2 auf der grösseren Produktivität der Rotiferen, mit Ausnahme von 1983. Vergleicht man die Produktivität im Untersuchung-Zeitraum mit derjenigen der Jahre 1978—1980 (2), so sind die ersteren grösser, ohne aber die Einreihung der Cerna-Bucht als Ökosystem mittlerer Produktivität zu beeinträchtigen.

Quantitativ ist eine Zunahme der Werte von 1981 bis 1983 zu verzeichnen, während 1984 ein Zurückgehen auf die Lage von 1981 erfolgt, was seine Erklärung in der Fluktuation des Trophizitätsgrades findet.

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A. A. ECHELLE, I. KORNFIELD (eds) *Evolution of Fish Species Flocks* University of Maine at Orono Press, Orono, 1984, 257 pp.

The book grew up of a symposium organized at the 1983 annual meeting of the American Society of Ichthyologists and Herpetologists at Tallahassee, Florida. It consists of 18 chapters, written by 27 authors most of them Americans, a few from other countries (England, The Netherlands, Uganda, Malawi, South Africa); all but one are ichthyologists, the exception being E. Mayr. Four chapters deal with general problems of species flocks, the others being devoted each to one group of fishes or even to a single aspect of the problem in one fish group. The introductory chapter by E. Mayr (Evolution of Fish Species Flocks : A Commentary) reviews the general problems of species flocks in relation to modes of speciation, evolutionary theories, adaptive radiation, competitive exclusion. In discussing the term "species flocks", Greenwood defines it as a strictly monophyletic taxon; the same family or other higher taxon can encompass several species flocks in the same lake; Ribbink defends the opposite viewpoint, considering that a flock includes all the species of a family, order, etc., endemic to a restricted area.

Seven of the 14 special chapters are devoted to cichlids from the great African lakes, two to the silverside genus *Chirostoma* from Mexico, one to each Lake Lanao cyprinids, the Andean killfish genus *Orestias*, cyprinodonts from a Mexican lagoon, the small species flocks from northern temperate lakes and another to an extinct group, the semionotids from the Mesozoic lakes of North America. One of the seven cichlid chapters (by Greenwood) is rather theoretical; the author points out that the Lake Victoria haplochromine flock (or flocks) 'appear to be an outstanding example of an extant punctuational evolutionary phase' and 'provides little evidence for the effects of natural selection or of species selection in its origin and development'. Liem and Kaufman consider that the polymorphic ciclid *Cichlasoma minckleyi* represents a stage of divergence in speciation', i.e. they adopt the heterodox viewpoint that morphs of polymorphic species may get reproductive isolation through a mechanism of ecological sympatric speciation.

The various chapters of the book consider not only the morphological characters of the species dealt with, but also the biochemical ones (cyprinodonts from Laguna Chichancanab, Lake Victoria cichlids), feeding habits (various African cichlids), sexual selection (African cichlids), breeding habits (again in cichlids). The viewpoints of the various authors differ : some defend the allopatric, others the sympatric speciation model. A single paper makes references to species flocks in other animals than fishes : that by Dominey on the effect of sexual selection and life history on speciation in African cichlids that makes comparison with Hawaian Drosophila; It would have been especially interesting to make thorough comparison above all with other freshwater animals, such as prosobranchiate snails and especially amphipods. The valuable contribution by Echelle and Echelle on the *Chirostoma* species flock from the Mesa Central of Mexico mentions only briefly that two other lineages of fishes (the cyprinodont family Goodeidae and the cyprinid genus *Algansea*) underwent a phenomenon of active speciation in the same area; a comparison at least of the ranges of the numerous *Chirostoma* and goodeid species (all endemics with restricted ranges) would have been very suggestive. When discussing the cyprinids from lake Lanao, Kornfield and Carpenter compare, the three extant (or surviving) *Puntius* species considering that the 14 or 15 others became extinct; no firm position is taken about the opinion, expressed by other ichthyologists, that the presumed extinct species (some of them ascribed to distinct genera) actually are imaginary, based on aberrant specimens or on extremes of a continuous series of variation.

The last chapter, by the two editors of the book "Who's Tending the Flock", concludes that the extinction of all lakes and of their whole fauna (endemic and on endemic species as well) is inevitable.

The book represents the last comprehensive summary of the various problems raised by one of the most fascinating and controversed phenomenon of speciation and evolution,

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