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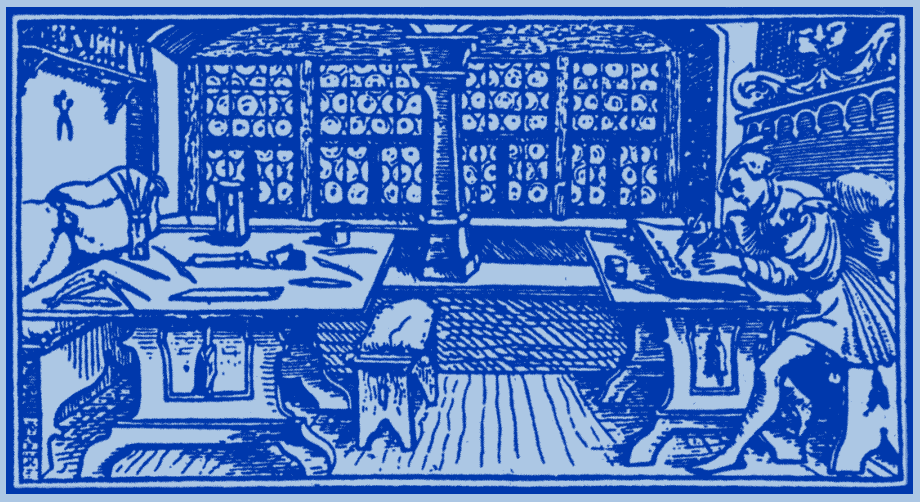

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All authors are responsible for submitting manuscripts in comprehensible US or UK English and ensuring scientific accuracy.

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COMPARATIVE ANALYSIS OF LEAF-BEETLES (COLEOPTERA, CHRYSOMELIDAE) FROM THE SCIENTIFIC RESERVES SCĂRIȚA-BELIOARA, CHEILE TURZII AND CHEILE TURULUI (TRANSYLVANIA COUNTY, ROMANIA)

ALEXANDRU CRIŞAN[1]

SUMMARY. In the three sampled areas we identified 144 species of leaf-beetles, belonging to 11 subfamilies. It represents a rich and varied leaf-beetle fauna, in accord with the biodiversity of the vegetation and the diversity of the ecological conditions offered by these zones. The subfamilies Alticinae, Chrysomelinae and Cryptocephalinae have had a great number of species, while the rest of the represented subfamilies were medium or scarcely represented. Each group occuped the biotop in general accord with its known ecological demands.

KEYWORDS: leaf-beetles, scientific reserves, comparations

Introduction

The Chrysomelidae family, one of the richest in species of the Coleoptera order, contains herbivorous insects both in larvae and adult stages, frequently damaging the plant leaves and, therefore, being named „leaf-beetles".

Their body shape is diverse: cylindrical, oval, hemisphaerical, more or less flattened, small to medium, not exceeding 15 mm in length, with various, intense colours, frequently metallic; more rarely grey or dark coloured species.

Trophic spectrum ranges from monophagous to polyphagous in various species, although most leaf-beetle species are specialised to few plant species. This explains the direct proportion between plant and leaf-beetle biodiversity in a determined area. This high diversity is the reason we explored leaf-beetle biodiversity mostly in botanical reserves or procteted areas.

Scientific information on leaf-beetle group living on Romanian territory are scarce. Several old reports have presented Coleoptera group in the context of general studies in different Romanian areas (Fleck, 1905, Ieniştea, 1968, 1974, Ienistea and Negru, 1975; Konnert-Ionescu, 1963, Marcu, 1927, 1928, 1931, 1933, 1936, 1957; Negru, 1968; Negru and Roşca, 1967; Panin, 1951, Petri, 1912; Roşca, 1973, 1974, 1976, Seidlitz, 1891). Starting with the last decade of the 20 th century,

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some authors have begun researches focusing on leaf-beetle fauna and ecology, in different territories of Romania (Gruev *et al*., 1993, Ilie, 2001; Maican and Serafim, 2001; Szel *et al*.,1995; Nistor *et al*.., 2000; Crişan,1993 a, b, 1994, 1995, Crişan and Teodor, 1994, 1996; Crişan and Bonea, 1995; Crişan and Druguş 2001; Crişan *et al*., 1998, 1999, 2000.). To date, areas of Scarita-Belioara, Cheile Turzii and Cheile Turului, have not been analysed for the composition and ecology of leaf-beetles fauna, our paper being the first to report such data.

Materials and Methods

In Scarita-Belioara Botanical Reserve we have collected and made observations strictly in the scientific reserve perimeter, a relatively small territory of about 7 ha. This area is a lawn with a slight southern inclination, situated at approximate 1250 m altitude, in the southern part of Gilaului Mountains, about 35 km South-West from Cluj-Napoca town. The area is bordered by a bound of about 50 m width having a more strong inclination and then by a very tilted zone going about 300 m downward to Belioara and Posaga valleys. The northern and eastern parts of the lawn are bordered by a foliaceous forest with the domination of beech-trees. The eastern part of of the Botanical Reserve have also some yung pine trees, and the rest of the lawn contains only monocotyledonous and dicotyledonous herbs, abundant in many different species, the reason of the status of Botanical Reserve of the area.

In ”Cheile Turzii”(the Turda’s gorges), an area situated in the south-eastern part of Gilaului Mountains, at about 30 km south of Cluj-Napoca town, we sampled and made observations at 2-3 weeks interval during the active period of the year (May- September) over 4 habitats: the Hajdate valley proximity, the southern exposed slopes, the northern exposed slopes, and the high plateau-land above the gorges. Here we sampled both the herbs, bushes and trees, mentioning sometimes also the vegetal species on which we captured leaf-beetles. Ecological conditions of the 4 sampled habitats are very different. Along the Hasdate valley there is a foliaceous forest with high trees that ensure a humidity favourable to dicotyledonous herbs, covering the land from the slope to the edge of the valley, rarely mixed with species of grasses. All of these are hygrophilous species. On the southern exposed slopes, there is a dominant xerical to mesoxerical herbaceous vegetation, with steppic elements, mixed with some xerical bushes, and bordered in the eastern part by a pine forst mixed with foliaceous trees species. The northern exposed slopes are covered with a foliaceous forest mixed with rare old spurce trees. The herbaceous vegetation is scarcely represented here. The high plateau-land above the gorges is covered by a lawn with rare bushes of different species. Here dominate the species of grasses characteristical to dry subalpine lawns.

In the area ”Cheile Turului” (the Tur’s gorges), at about 25 km South of Cluj-Napoca town, we sampled 3 habitats: the proximity of the Racilor brook, the right side

and the left side of the gorges. The first habitat have mostly wooden vegetation represented by *Salix, Alnus* and *Populus* species, mixed with hyghrophilous herbs. On the right side, there is a foliaceous forest with northern exposition, dominated by *Carpinus* and *Corylus* species, and a bushes area with *Crataegus*, *Rosa* and *Corylus* species. On the left side of the gorges we also sampled a bushes area with southern exposition and a strongly xerophilous lawn with specific grasses and herbs.

In all cases we have made preliminary observations on the types of biotopes of each area establishing its characteristics, then we sampled by sweeping the vegetation with an insect net, and also by directly hand collections of the insects. We also tried to identify and count plant species in the cases of direct collections, in order to register the trophical spectrum of the leaf-beetles species.

Collected insects were put on 78% alcohol and then were kept dry till the identification, made in the laboratory using different litterature (Mohr, 1966; Panin, 1951; Kaszab 1962-1971; Kippenberg and Doberl, 1994; Schaufuss, 1915; Reitter, 1914; Rozner, 1996; Warchalowski, 1993).

Results and discussion

In Table 1 we present the identified leaf-beetle species in the three researched zones, also showing the estimated abundance and the type of the vegetation.

Table 1.
Leaf-beetles (Coleoptera, Chrysomelidae) from the natural reserves Scărița-Belioara, Cheile Turzii and Cheile Turului.

Crt. nr.	Subfamily/ Species	Sc.-Bel.	Ch. Tur-zii	Ch. Tur	h	w
	I. Donaciinae Kirby, 1837					
1	*Plateumaris (Juliusiana) consimilis* (Schrank, 1781)	-	+	-	+	-
2	*Plateumaris (Juliusiana) braccata* (Scopoli, 1772)	-	-	++	+	-
3	*Donacia (Donacia) bicolor* Zschabch, 1788	-	-	+	+	-
	II. Orsodacninae Thomson 1859					
4	*Orsodacne lineola* (Panzer, 1795)	+	+	-	+	+
5	*Orsodacne cerasi* (Linnaeus, 1758)	-	+	-	-	+
	III. Criocerinae Latreille, 1807					
6	*Oulema (Oulema) melanopus* (Linnaeus, 1758)	+	+++	+++	+	-
7	*Oulema (Haspidolema) galleciana* (Heyden, 1870)	-	+	+	-	+
8	*Oulema (Haspidolema) tristis* (Herbst, 1786)	-	-	+	-	+
9	*Lilioceris merdigera* (Linnaeus, 1758)	++	-	-	+	-
	IV. Clytrinae Kirby, 1837					
10	*Labidostomis longimana* (Linnaeus, 1761)	++	+	+	+	-
11	*Labidostomis humeralis* (Schneider, 17929	-	-	+	+	-

12	*Labidostomis pallidipennis* (Gebler, 1890)	-	-	+	+	-
13	*Lachnaia sexpunctata* (Scopoli, 1763)	-	++	-	-	+
14	*Tituboea macropus* (Illiger, 1800)	-	+	-	+	-
15	*Clytra quadripunctata* (Linnaeus, 1758)	-	++	++	-	+
16	*Clytra laeviscula* Ratzeburg, 1837	-	+	+++	+	+
17	*Clytra appendicina* Lacordaire, 1848	-	-	++	-	+
18	*Smaragdina salicina* (Scopoli, 1763)	-	+	+	-	+
19	*Smaragdina aurita* (Linnaeus 1767)	-	+	+++	-	+
20	*Coptocephala chalybaea* (Germar, 1824)	-	++	+	+	-
21	*Coptocephala unifasciata* (Scopoli, 1763)	-	-	+	+	-
	V. Cryptocephalinae Gyllenhal, 1813					
22	*Pachybrachys tesselatus* (Olivier, 1791)	-	+	+	-	+
23	*Pachybrachys sinuatus* Mulsant et Rey, 1883	-	-	++	-	+
24	*Pachybrachys hippophaes* (Suffrian, 1848)	-	-	+	-	+
25	*Cryptocephalus (Proctophysus) schaefferi* Schrank, 1789	-	+	-	+	-
26	*Cryptocephalus (Burlinius) frontalis* Marsham 1802	+	-	-	-	+
27	*Cryptocephalus (Burlinius) elegantulus* Gravenhorst, 1807	-	+	-	+	-
28	*Cryptocephalus (Burlinius) exiguus* Schneider, 1792	-	+	-	-	+
29	*Cryptocephalus (Burlinius) saliceti* Zebe, 1855	-	+	-	-	+
30	*Cryptocephalus (Burlinius) chrysopus* Gmelin, 1790	-	+	-	-	+
31	*Cryptocephalus (Burlinius) querceti* Suffrian, 1848	-	-	+	-	+
32	*Cryptocephalus (Burlinius) carpathicus* Frivaldszky, 1883	-	-	+	+	-
33	*Cryptocephalus (Burlinius) ocellatus* Drapiez, 1819	-	.	+	.	+
34	*Cryptocephalus (Burlinius) connexus* Olivier, 1808	-	-	+	-	+
35	*Cryptocephalus (Cryptocephalus) bipunctatus* (Linnaeus, 1758)	++	++	++	+	+
36	*Cryptocephalus (Cryptocephalus) aureolus* Suffrian, 1847, ssp. *iliricus*, Franz, 1949	+++	-	-	-	+
37	*Cryptocephalus (Cryptocephalus) sericeus* (Linnaeus, 1758)	+++	-	+++	+	-
38	*Cryptocephalus (Cryptocephalus) hyppochoeridis* (Linnaeus, 1758)	+++	-	-	+	-
39	*Cryptocephalus (Cryptocephalus) violaceus* Laicharting, 1781	+++	++	+	+	+
40	*Cryptocephalus (Cryptocephalus) moraei* (Linnaeus, 1758)	++	+	+++	+	-
41	*Cryptocephalus (Cryptocephalus) quadriguttatus* Richter, 1820	+++	-	-	+	-
42	*Cryptocephalus (Cryptocephalus) flavipes* Fabricius, 1781	++	++	-	-	+
43	*Cryptocephalus (Cryptocephalus) vittatus* Fabricius, 1775	+	-	+	+	-

44	*Cryptocephalus (Cryptocephalus) distinguendus* Schneider, 1792	-	+	+	+	-
45	*Cryptocephalus (Cryptocephalus) octopunctatus* (Scopoli, 1763)	-	+	-	-	+
	VI. Lamprosomatinae Lacordaire, 1848					
46	*Omorphus (Omorphus) concolor* (Sturm, 1807)	-	+	+	+	-
	VII. Eumolpinae Thomson, 1859					
47	*Eumolpus asclepiadeus* (Pallas, 1773)	-	-	+	+	-
48	*Pacnephorus villosus* (Duftschmid, 1825)	-	-	+	+	-
	VIII. Chrysomelinae Latreille, 1802					
49	*Leptinotarsa decemlineata* (Say, 1824)	+	+	+	+	-
50	*Chrysolina (Synerga) coerulans* (Scriba, 1791)	+++	-	+	+	-
51	*Chrysolina (Sphaeromela) varians* (Schaller, 1783)	++	++	-	+	-
52	*Chrysolina (Colaphosoma) sturmi* Westhoff, 1802	+	-	+	+	-
53	*Chrysolina (Menthastriella) herbacea* (Duftschmid, 1825)	-	++	+++	+	-
54	*Chrysolina (Sphaerochrysolina) rufa* Duftschmid, 1825	-	++	-	+	-
55	*Chrysolina (Minckia) chalcites* (Germar, 1824)	-	+	-	-	+
56	*Chrysolina (Minckia) orichalcia* (O.F. Muller, 1776)	-	+	-	-	+
57	*Chrysolina (Hypericia) hyperici* (Forster, 1771)	-	+	-	+	-
58	*Chrysolina (Hypericia) geminata* (Paykull, 1799)	-	+	-	+	-
59	*Chrysolina (Heliostola) carpathica* (Fuss, 1856)	-	+	-	+	-
60	*Chrysolina (Chalcoidea) marginata* (Linnaeus, 1758)	-	+	+	+	-
61	*Chrysolina (Chalcoidea) carnifex* (Fabricius, 1792)	-	-	+	+	-
62	*Chrysolina (Chrysomorpha) cerealis* (Linnaeus, 1767)	-	-	+	+	-
63	*Chrysolina (Euchrysolina) graminis* (Linnaeus, 1758)	-	-	++	+	-
64	*Chrysolina (Erythrochrysa) polita* (Linnaeus, 1758)	-	-	++	+	-
65	*Chrysolina (Chrysolina) staphylea* (Linnaeus, 1758)	-	-	+	+	-
66	*Chrysolina (Ovostoma) olivieri* (Bedel, 1892)	-	-	+	+	-
67	Chrysolina (Fastuolina) fastuosa (Scopoli, 1763)	-	+++	+++	+	-
68	*Colaphus sophiae* (Schaller, 17839	-	-	+++	+	-
69	*Gastrophysa polygoni* (Linnaeus, 1758)	++	++	+	+	-
70	*Gastrophysa viridula* (De Geer, 1775)	-	++	++	+	-
71	*Phaedon (Phaedon) cochleariae* (Fabricius, 1792)	-	++	-	+	-
72	*Plagiodera versicolora* (Laicharting, 1781)	-	-	+	-	+
73	*Chrysomela (Strickerus) vigintipunctata* (Scopoli, 1763)	-	-	+	-	+
74	*Gonioctena (Goniomena) intermedia*, Helliesen, 1913	+	-	-	+	-
75	*Phratora (Phratora) atrovirens* (Cornelius, 1857)	-	+	-	-	+

76	*Phratora (Phratora) vitellinae* (Linnaeus, 1758)	-	-	+	-	+
77	*Timarcha (Timarcha) rugulosa* H. Schaeffer, 1838	-	+	+	+	-
78	*Timarcha (Timarcha) pratensis* (Duftschmid, 1825)	-	-	+	+	-
79	*Timarcha (Timarchostoma) goettingensis* (Linnaeus, 1758)	-	+	+	+	-
	IX. Galerucinae Latreille, 1802					
80	*Galeruca (Galeruca) tanaceti* (Linnaeus, 1758)	+	+	+	+	-
81	*Galeruca (Galeruca) pomonae* (Scopoli, 1763)	-	+	-	+	-
82	*Galeruca (Galeruca) interrupta* (Illiger, 1802) ssp. *circumdata* Duftschmid, 1825	-	-	+	+	-
83	*Phyllobrotica adusta* (Creutzer, 1799)	+	-	-	+	-
84	*Luperus lyperus* Sulzer, 1776	+	-	-	-	+
85	*Luperus longicornis* (Fabricius, 1781)	+	-	-	-	+
86	*Luperus flavipes* Linnaeus 1767	+	-	-	-	+
87	*Calomicrus pinicola* (Duftschmid, 1825)	+++	-	-	-	+
88	*Euluperus xanthopus* (Duftschmid, 1825)	++	-	-	-	+
	X. Alticinae Kutschera, 1859					
89	*Phyllotreta armoraciae* (Koch, 1803)	+	-	-	+	-
90	*Phyllotreta nemorum* (Linnaeus, 1758)	-	-	+	+	-
91	*Aphthona stussineri* (Weise, 1888)	+	++	-	+	-
92	*Aphthona placida* Kutschera, 1864	+	-	-	+	-
93	*Aphthona euphorbiae*, (Schrank, 1781)	+	++	-	+	-
94	*Aphthona atrovirens* (Forster, 1849)	-	+	-	+	-
95	*Aphthona ovata* Foudras, 1861	-	+	-	+	-
96	*Aphthona nigriscutis*, Foudras, 1861	-	++	+++	+	-
97	*Aphthona lacertosa* Rosenhauer, 1847	-	+++	+++	+	-
98	*Aphthona venustula* (Kutschera, 1861)	-	-	++	+	-
99	*Aphthona herbigrada* (Curtis, 1837)	-	-	+++	+	-
100	*Longitarsus apicalis* (Bech, 1817)	+	-	-	+	-
101	*Longitarsus lycopi* (Foudras, 1860)	-	++	+	+	-
102	*Longitarsus nanus* (Foudras, 1860)	-	++	-	+	-
103	*Longitarsus obliteratus* (Rosenhauer, 1847)	-	+	-	+	-
104	*Longitarsus tabidus* (Fabricius, 1775)	-	++	+++	+	-
105	*Longitarsus rubiginosus* (Foudras, 1860)	-	+	+	+	-
106	*Longitarsus ferruginosus* (Foudras, 1860)	-	+	-	+	-
107	*Longitarsus succinaeus* (Foudras, 1860)	-	+	+	+	-
108	*Longitarsus lateripunctatus* (Rosenhauer, 1856)	-	-	+	+	-
109	*Longitarsus foudrasi* (Weise, 1893)	-	-	+++	+	-
110	*Longitarsus ballotae* (Marsham, 1802)	-	-	+	+	-
111	*Altica oleracea* (Linnaeus, 1758)	++	-	+	+	-
112	*Altica quercetorum* Foudras, 1860	+	+	-	-	+
113	*Altica pussila* Duftschmid, 1825	-	+	-	+	-
114	*Altica carduorum* Guerin-Meneville, 1858	-	+	-	+	-
115	*Asiorestia nigritula* (Gyllenhal, 1813)	-	+	-	-	+

116	*Asiorestia norica* (Weise, 1890)	-	+	-	+	-
117	*Asiorestia ferruginea* (Scopoli, 1763)	-	+	++	+	-
118	*Asiorestia transversa* (Marsham, 1802)	-	-	+	+	-
119	*Asiorestia transsylvanica* (Fuss, 1864)	-	-	+	+	-
120	*Crepidodera aurata* (Marsham, 1802)	-	++	+++	-	+
121	*Crepidodera plutus* (Latreille, 1804)	-	-	+	-	+
122	*Podagrica fuscicornis* (Linnaeus, 1867)	-	-	+	+	-
123	*Chaetocnema (Tlanoma) concinna* Marsham, 1802	+	+	++	+	-
124	*Chaetocnema (Tlanoma) tibialis* Illiger, 1802	-	++	+	+	-
125	*Argopus ahrensi* (Germar, 1817)	+	-	-	-	+
126	*Dibolia (Dibolia) cryptocephala* (Koch, 1803)	-	++	-	+	-
127	*Psylliodes attenuata* (Koch, 1803)	-	+	+	+	-
128	*Psylliodes fusiformis* Illiger, 1807	-	+	-	+	-
129	*Psylliodes aerea* (Foudras, 1860)	-	+	-	+	-
130	*Psylliodes circumdata* (Redtenbacher, 1842)	-	+	-	+	-
131	*Psylliodes dulcamarae* (Koch, 1803)	-	-	+	+	-
132	*Psylliodes calcomera* (Illiger, 1807)	-	-	++	+	-
133	*Psylliodes chrysocephala* (Linaeus, 1758)	-	-	+	+	-
	XI. Cassidinae Gyllenhal, 1813					
134	*Hypocassida subferruginea* (Schrank, 1776)	-	+	+	+	-
135	*Cassida (Cassida) nebulosa* Linnaeus, 1758	-	+++	+	+	-
136	*Cassida (Cassida) sanguinolenta* Muller, 1776	-	+	-	+	-
137	*Cassida (Cassida) prasina* Illiger, 1798	-	++	+	+	-
138	*Cassida (Cassida) rubiginosa* O.F. Muller, 1776	-	-	+++	+	-
139	*Cassida (Cassida) berolinensis* Suffrian, 1844	-	-	+	+	-
140	*Cassida (Cassida) denticollis* Suffrian, 1844	-	-	+	+	-
141	*Cassida (Cassida) vibex* Linnaeus, 1767	-	-	+	+	-
142	*Cassida (Cassida) flaveola* Thanberg, 1794	-	-	+	+	-
143	*Cassida (Odontionycha) viridis* Linnaeus, 1758	-	+	+++	+	-
144	*Cassida (Cassidulella) nobilis* Linnaeus, 1758	-	++	++	+	-

Explanations: **Sc.-Bel**.=Scărița-Belioara; **Ch**.= Cheile (gorges); **+** ; **++**; **+++** = existing with low, medium, and high abundance; **-** = lack of; **h** = on herbaceous vegetation; **w** = on wooden vegetation (trees and bushes).

In the three protected zones we identified 144 species of leaf-beetles, belonging to 11 subfamilies, this indicating a rich biodiversity, as compared with the 12 leaf-beetle subfamilies reported in the Central Europe. This means that the researched areas contains almost all the representatives of the terrestrial habitates characteristical to Central Europe, with conditions able to support different types of phytocenosis and, related with these, different species of herbivorous insects, with different ecological demands.

The number of leaf-beetle species in each sampled area and in all three zones varried according to subfamily, in relation with the general ecological demands of each one, as presented in Fig. 1.

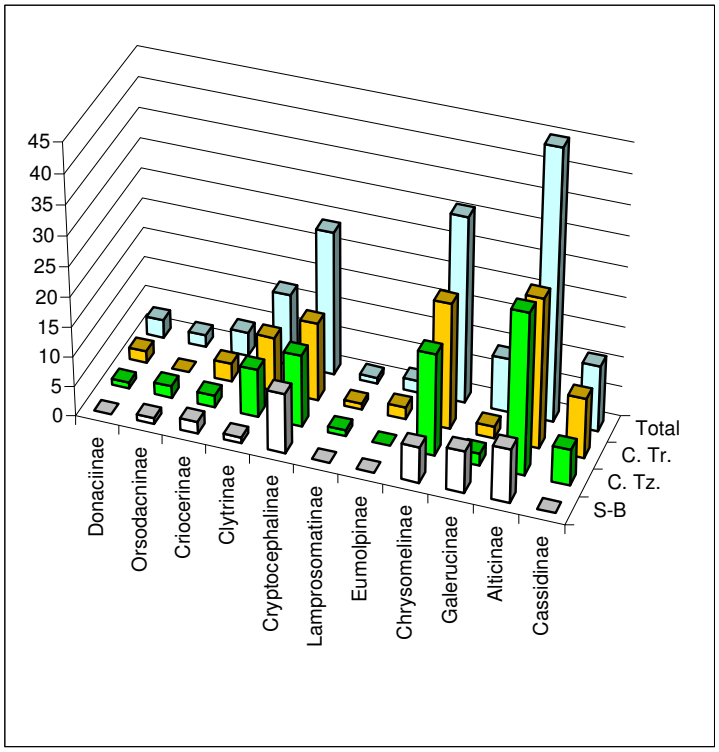

Fig. 1. Diagram of the number of leaf-beetles species registered in Scarita-Belioara (S-B), Cheile Turzii (C-Tz.) and Cheile Turului (C-Tr.), so that on all of the three zones (Total), according to identified subfamilies

The biodiversity was greater in Alticinae, Chrysomelinae and Cryptocephalinae subfamilies, less great in Criocerinae, Clytrinae, Galerucinae and Cassidinae subfamilies and least in the rest of registered sufamilies, both reffering to each researched zone and to the total of the researched territories.

In each protected area leaf-beetle species occupies the adequate habitate, in accord with their biological and ecological demands. Donaciinae, Chrysomelidae and Cassidinae occupied mostly the middle-hygrophilous areas in the proximity of the valleys, both on the herbaceous and wooden vegetation according to the trophical specialisation of each species; Cryptocephalinae, Criocerinae and Clytrinae, and most of Alticinae species occupied the middle-xerophilous areas on the

southern exposed slopes, or on the flat-lands, these assurring the demanded conditions of humidity and insolation for these groups; the species registered in the rest of the identified subfamilies occupied mostly th mesophilous areas or other have more large ecological vallences and were spread more uniformly in the territory.

The fact that any species are present in only one of the three investigated areas, though they are not far one from another, shows the importance of the constituation of such protected zones, for the preservation of Romanian biodiversity.

REFERENCES

Crişan, A. (1993a.) Date asupra familiei Chrysomelidae (Coleoptera) în partea sudică a Deltei Dunării., *An. şt. Inst., Tulcea*, 67-74.

Crişan, A. (1993b.) Cercetări faunistice şi ecologice asupra familiei Chrysomelidae (Coleoptera) în Cheile Turzii în 1992., *Studia Univ. "Babeş-Bolyai", Biol*., **38**, 59-67.

Crişan, A., Teodor, L. (1994) Researches on leaf-beetles (Coleoptera, Chrysomelidae) in "Scărița Belioara" Botanical Reservation., *Bul Inf. Soc. Lepid. Rom.*, **7**, 255-260.

Crişan, A. (1995) Cercetări asupra familiei Chrysomelidae (Coleoptera) în Rezervația Biosferei "Delta Dunării", cu referire specială la *Stylosomus tamaricis* H-Schaeff. şi *Cryptocephalus gamma* H-Schaeff*., Bul Inf. Soc. Lepid. Rom.*, **6**, 145-149.

Crişan, A., Bonea, V. (1995) Studiu faunistic asupra crizomelidelor (Coleoptera, Chrysomelidae) din zona Arcalia, (Jud. Bistrița-Năsăud)., *Bul Inf. Soc. Lepid. Rom.*, **6**, 305-317.

Crişan, A., Teodor, L. (l996a) Researches on leaf-beetles (*Coleoptera: Chrysomelidae*) in "Scarita- Belioara" Botanical Reservation*", Bul Inf. Soc. Lepid. Rom.*, **7**, 255-260.

Crişan, A., Teodor, L. (1996b) Researches on Chrysomelidae (Coleoptera) fauna in "Cheile Turului" in 1995", *Stud. Univ. "Babes-Bolyai", Biol*., **41**, 1-2, 65-72.

Crişan, A., Popa, V., Teodor, L. (1998) Leaf-beetles (Coleoptera: Chrysomelidae) from the area "Cheile Someşului Cald- Ic Ponor", Romania., *Bul Inf. Soc. Lepid. Rom.*, **9**, 127-132.

Crişan, A., Popa, V., Teodor, L. (1999) Studies on leaf-beetle fauna (Coleoptera: Chrysomelidae) in "Someşului Cald Gorges" area, Romania., *Bul Inf. Soc. Lepid. Rom.*, **10** , 131-135.

Crişan, A., Teodor, L.A., Nistor, L. (2000) Data on leaf- beetle fauna (Coleoptera: Chrysomelidae) in the North- West Transylvania (Romania)., *Bul Inf. Soc. Lepid. Rom.*, **11**, 111- 115.

Gruev, P., Merkl O., Vig K. (1993) Geographical distribution of Halticinae (Coleoptera, Chrysomelidae) in Romania., *Ann. Hist. Nat. Mus. Hung.*, **85**, 75-132.

Ieniştea, M.A. (1968) L'entomofaune de l île de Letea (Delta du Danube), ord. Coleoptera (pars)., *Trav. Mus. Hist. Nat. "Gr. Antipa"*., **8**, 81-93.

Ienişta, M.A. (1974) Contribution a la connaisance des coleopteres du Delta du Danube (la "grind" Caraorman)., *Trav. Mus. Hist. Nat. "Gr. Antipa"*., **14**, 239-249.

Ieniştea, M.A., Negru, Ş. (1975) Coleoptera, in *Seria monografică "Porțile de Fier"*, Ed. Acad.Rom., Bucureşti, pp. 193-214.

Ilie, A.L. (2001) Cercetări privind fauna de crizomelide (Coleoptera, Chrysomelidae) din municipiul Craiova şi împrejurimi., *Bul Inf. Soc. Lepid. Rom.*, **12,** 201-208.

Kaszab, Z. (1962-1971) *Magyarország állatvilaga, Bogarak IV/B (Fauna Hungariae, Coleoptera IV/B).,* Akadémiai Kiadó, Budapest

Kippenberg, H., Doberl, M. (1994) Familie Chrysomelidae, in *Die Kafer Mitteleuropas ,Supplementband*, Lohse G.A. and Lucht W.H. eds., Krefeld pp.1-142

Konnert-Ionescu, A. (1963) Halticinae recorded from Romania till 1961., *Trav. Mus. Hist. Nat "Gr. Antipa*"., **4**, 251-268.

Marcu, O. (1927) Neue Coleopteren aus der Bucovina., *Bul. Fac, Şt. Cernăuţi*, **1** (2), 413-423.

Marcu, O. (1928) Beitrage zur Coleopterenfauna der Bucovina., *Bull. Sci. Ec. Polytech., Timişoara*, 4-11.

Marcu, O. (1931). Neue kaferformen aus Rumanien, Zur Coleopetrenfaune der Bucovina, *Bul. Sect. Sci, Acad. Roum*., **9- 10**, 1-10.

Marcu, O. (1933) Coleopterenfaunde aus der Bucovina , *Bul. Sect. Sci., Acad. Roum*, **78**, 1-6.

Marcu, O. (1936) Coleopterenfunde aus der Bucovina., *Bul. Sect. Sci. Acad. Roum.*, **16**, 1-6.

Marcu, O. (1957) Contribuţii la cunoaşterea faunei Coleopterelor Transilvaniei, *Bul. Univ. " V. Babeş" şi "Bolyai", Ser. Şt. nat.,* **I**, 527- 544.

Mohr, K.H. (1966) Chrysomelidae, in *Die Kafer Mitteleuropas* Freude H., Harde K.W., and Lohse G.A. (eds.), Goeke und Evers-Krefeld, Zurich, pp. 95-299.

Negru, Ş. (1957) Contribuţiune la cunoaştrea Faunei colepterologice a Mangaliei şi împrejurimilor ei., *Ann., Univ. "C. I. Parhon", Ser. Şt. nat.,* **165**, 117- 130.

Negru, Ş., Roşca, A. (1967) L' entomofaune des forets du Sud de la Doubroudja, ord.Coleoptera (pars), *Trav. Mus. Hist. Nat. "Gr. Antipa".,* **7**, 119-145.

Nistor, L., Crişan, A., Ruicănescu, A. (2000) Data on leaf- beetle (Coleoptera: Chrysomelidae) and jewel beetle fauna (Coleoptera: Buprestoidea) of the Botanical Reserve " Fânaţele Clujului" (Cluj county, Transylvania region, Romania)., *Bul Inf. Soc. Lepid. Rom.„* **11**, 115-123.

Panin, S. (1951) *Determinatorul coleopterelor dăunătoare şi folositoare din R.P. Romînă*., Ed. Lit. Şt. Did., Bucureşti, pp. 126-150.

Petri, K. (1912) *Siebenburgens Kaferfauna auf Grund ihrer Erforschung bis zum Jahre 1911*., Buchdruckerei Jus. Drotleff, Hermannstadt, pp. 253-286.

Roşca, A. (1973) Contributions a la connaissance du genre *Cryptocephalus* Foucr. (Coleoptera, Chrysomelidae) en Roumanie*., Trav. Mus. Hist. Nat. "Gr. Antipa",* **13**, 143-154.

Roşca, A. (1974) Contributions a la connaissance du genre *Chrysomela* L. (Coleoptera, Chrysomelidae) en Roumanie., *Trav. Mus. Hist. Nat. "Gr. Antipa",* **14**, 250-259.

Roşca, A. (1976) L entomofaune du Nord de la Dobrogea, la zone Măcin – Tulcea-Niculiţel, ord. Coleoptera (pars)., *Trav. Mus. Hist. Nat. "Gr. Antipa".,* **17,** 145-152

Rozner, I. (1996) An update list of the Chrysomelidae of Hungary and the adjoining parts of the Carpathian Basin (Coleoptera), *Folia Entomol. Hung*., **57**, 234-260.

Seidlitz, G., (1891) *Fauna Transsylvanica, die Kafer (Coleoptera) Siebenburgens*., Hartungsche Verlagsdruckerei, Konigsberg, pp. 753-823.

Szel, G., Rozner, I., Kocs, I. (1995) Contribuţii la cunoaşterea coleopterelor din Transilvania (România) pe baza colectărilor din ultimii ani., *Acta Muz. Secuiesc al Ciucului, Muz. Naţ. Secuiesc,* 73-92.

Warchalowski A., (1993) *Fauna Polski- Fauna Poloniae- Chrysomelidae (Coleoptera, Insecta), Tom. 15.*, Pol. Akad. Nauk., Warszawa pp. 2-278

STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LI, 2, 2006 (p. 13-17)

NEW RECORDS ON *COLLEMBOLA* (INSECTA) FROM NATURAL ECOSYSTEMS OF MOLDOVA

GALINA BUSMACHIU[1]

SUMMARY. Eleven species of Collembola from the deciduous forests and banks of rivers were registered as new for fauna of Moldova and four species are registered in new biotopes. The geographical distribution of the species *Thalassaphorura tovtrensis, Micraphorura uralica, Lepidocyrtus szeptyckii* and *Orchesella pontica* was expanded. An annotated list of studied species with their distribution and ecology is given.

KEYWORDS: Collembola, Moldova, new records

Introduction

The problem of biodiversity at the level of ecosystem and species has become acute in the actual condition of the human impact intensification. The biodiversity maintenance on the regional level is necessary for our life insurance and for the future generation (Teleuta *et al.*, 2002). The most part of natural ecosystems are today fragmented and situated near modified agricultural and urban areas. Only the study of the species diversity of natural areas can reflect the taxonomic diversity of ecosystems. This is a priority direction in our days because the majority of species are very vulnerable. The areas with significant taxonomic diversity of faunal communities need to be protected and monitored at national, regional and international level.

This paper is the next step in the study of species diversity of Collembola from the natural ecosystems (Busmachiu, 2004) carried out in Instituto do Ambiente e Vida, Departamento de Zoologia da Universidade de Coimbra, Portugal, thanks to Professor Maria Manuela da Gama Assalino. The list of Collembola from Moldova is increased with another 11 species.

Materials and Methods

*Site description.*The natural ecosystems cover over 15 % of the Moldova Republic and consist of forests, steppes, meadows and floodplains (Teleuta *et al.*, 2002). The studied forests ecosystems are represented by "Codrii" Forest Reserve with dominant trees *Fagus sylvatica, Quercus petraea* and *Quercus robur* mixed

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with *Carpinus betulus*, *Fraxinus excelsior*, *Tilia tomentosa*, *Acer plantanoides* and "Codri Tigheci" forest with monodominant oak (*Quercus petraea*) and oak in combination with hornbeam (*Carpinus betulus*). The first one is European deciduous forests situated in the Central Moldova and the second - plot of native forest with elements of a unique Mediterranean flora adapted to xerothermic conditions in the South Moldova.

A xerophyte ecosystem occurs on calcareous formation along the Răut River where grasses *Sedum acre*, *Potentilla arenaria* and *Thymus moldavicus* are dominated in the petrophyte community on the rocky slope and also sandy beach along the Dniester Rivers with typical plantations of *Populus alba*, *Populus nigra* and *Salix alba*. The paludous ecosystem is in the "Lower Prut" Reserve in the South Moldova. This region has a large temporary inundated territory and several lakes. The banks of river and shore of lakes are rich in organic matter which, in combination with alluvial sediments, offer good condition for many grasses. It is the unique large area in Moldova with reed bed (*Phragmites australis*) and *Typha angustifolia* (Postolache, 1995).

List of localities:

Loc.1. Bahmut village, meadows near forest;
loc. 2. Butuceni village, bank of Răut River;
loc. 3. Cişliţa Prut village, "Lower Prut" Reserve;
loc. 4. Cociulia village, "Codri Tigheci" forest;
loc. 5. Gura Bicului village, bank of Dniester River;
loc. 6. Larguta village, "Codri Tigheci"forest;
loc. 7. Lozova village, „Codrii" Forest Reserve;
loc. 8. Manta village, shores of Manta Lake;
loc. 9. Stejăreni villages, „Codrii" Forest Reserve.

Sampling and extraction. Samples were collected during 2001-2006. Soil samples with 25 cm^2 surface area and 10 cm depth were taken from each habitat. Additionally, the specimens of Collembola were collected from the plants by exhauster. Collembola were extracted with Berlese-Tullgren funnels and water flotation method. The microarthropods were fixed in 80 % ethyl alcohol, sorted and processed to For solution. The specimens were mounted on permanent slides and identified.

Results and Discussion

As a result of investigation 11 species (*) belonging to twelve genera and four families (*Hypogastruridae*, *Onychiuridae*, *Isotomidae* and *Entomobryidae*) were found and mentioned for the first time in Moldova and four species are found in the new biotopes. These species were mainly collected in natural ecosystems unstudied before.

1. **Mesaphorura critica* Ellis, 1976
   Occurrence: Palaearctic.
   Ecology: widely distributed.
   Material: loc.8 (locality), numerous specimens (ssp.) collected on 14.09.05.

2. **Mesaphorura hylophila* Rusek, 1982
   Occurrence: Palaearctic.
   Biology: widely distributed.
   Material: loc.6, numerous ssp. collected on 21.08.05.

3. *Mesaphorura macrochaeta* Rusek, 1976
   Occurrence: cosmopolitan species.
   Ecology: sandy shores, dry meadows and forest soils.
   Material: loc.3, numerous ssp. collected on 13.09.05.

4. **Doutnacia xerophila* Rusek, 1974
   Occurrence: South and Middle Europe.
   Ecology: dry soils.
   Material: loc.2, one specimen, collected on 25.06.05.

5. **Micraphorura uralica* (Khanislamova, 1986)
   Occurrence: Moldova, Russia and Ukraine.
   Ecology: bank of river, caves and forests.
   Material: loc.1, two specimens collected on 01.04.01;
   loc.4, five specimens collected on 15. 01.05.

6. **Thalassaphorura tovtrensis* (Kaprus & Weiner, 1994)
   Occurrence: Moldova, Russia and Ukraine.
   Ecology: xerothermic species.
   Material: loc.2, numerous ssp. collected on 25.06.05.

7. **Brachystomella parvula* (Schaffer, 1896)
   Occurrence: cosmopolitan species.
   Ecology: humid places.
   Material: loc.1, eight specimens collected on 3.07.05.

8. *Xenylla boerneri*Axelson, 1905
   Occurrence: Palaearctic.
   Ecology: dry soil.
   Material: loc.9, 52 specimens, collected on 21.08.05.

9. **Xenylla brevisimilis brevisimilis* Stach, 1949
Occurrence: Europe and South America.
Ecology: litter of forest.
Material: loc.8, seven specimens collected on 15.07.05; loc.9, two specimens collected on 21.08.05; loc.5, 11 specimens, collected on 14.09.05.

10. *Desoria trispinata* (Mac Gillivray, 1896)
Occurrence: cosmopolitan species.
Ecology: soil rich in organic matter.
Material: loc.1, four specimens collected on 13.09.05.

11. **Folsomides angularis* (Axelson, 1905)
Occurrence: Holarctic.
Biology: xerothermic species.
Material: loc.2, one specimen collected on 25.06.05.

12. **Tetracanthella pilosa* Schott, 1891
Occurrence: all Europe.
Biology: forest species.
Material: loc.7, numerous ssp. collected on 11.05.06.

13. **Orchesella pontica* Ionescu, 1915 sensu Stach, 1960
Occurrence: Moldova and Romania.
Ecology: litter in forest.
Material: loc.4, four specimens collected on 5.08.05.

14. **Lepidocyrtus szeptyckii* Rusek, 1985
Occurrence: Moldova and Russia.
Ecology: rotten wood, mosses in taiga, shores of lake and meadow.
Material: loc.3, numerous ssp. collected on 13. 09.05.

15. *Entomobrya violaceolineata* Stach, 1913
Occurrence: Europe.
Ecology: forest species.
Material: loc.3, six specimens collected on 5.08.05.

The species *Thalassaphorura tovtrensis* has been described from the xerothermic grassland of Tovtry Hills, Podillya Region (Ukraine) (Kaprus and Weiner, 1994) and then was found in fluvial sand of the bank of Oka River near Moscow (Russia) (Thibaud et al., 1999). The species *Micraphorura uralica* has been described firstly from the bank of Bystry Tanyp River from Bashkiria (Russia) (Khanislamova, 1986), then was redescribed from the litter of *Carpinetum*

forest near entrance of cave and in the moss on stone near Dniester River from Podillya Region (Ukraine) (Kaprus and Weiner, 1994). The numerous specimens of species *Lepidocyrtus szeptyckii* have been collected and described from biotopes situated near the Baikal Lake (Russia) (Rusek, 1985). The species *Orchesella pontica* was known until now only from Romania (Gruia, 1976).

Conclusions

Eleven species of Collembola from the deciduous natural forests and banks of rivers were registered as new for Moldavian fauna and four are found in new biotopes. Among them thirteen species have a large geographical distribution: cosmopolite (3 species), Palaearctic (3), Holarctic (2), European (4) and one is known from Europe and South America. The species *Doutnacia xerophila, Folsomides angularis* and *Orchesella pontica* are rare, with few individuals found in the studied biotopes. The geographical distribution of four species, namely *Thalassaphorura tovtrensis, Micraphorura uralica, Lepidocyrtus szeptyckii* and *Orchesella pontica* was expanded.

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REFERENCES

Busmachiu, G. (2004) New records on Collembola (Insecta) from Moldova, *Bul. Acad. St. Mold.*, **3**, 44-48

Gruia, M. (1976) Biotopes cavernicoles peuples par des collemboles, *Trav. Inst. Speol.* "Emile Racovitza", **15**, 107 - 122

Kaprus, I., Weiner, W. M. (1994) Two interesting species of *Onychiurinae* (Collembola) from Ukraine and some remarks on *Allaphorura franzi* (Stach, 1946), *Acta zool. cracov.*, **37**, 59 – 64

Khanislamova, G. M. (1986) New species of springtails of the genus *Onychiurus* (*Collembola, Onychiuridae*) from the Ural Mountain Piedmonts, *Zool. J*, **65**, 1470 –1478

Postolache, G. (1995) Vegetația Republicii Moldova, Chișinău, pp 340

Rusek, J. (1985) New palearctic *Lepidocyrtus* and *Pseudosinella* species (Collembola: Entomobryidae), *Vest .cs. Spolec. zool.*, **49**, 132 – 146

Teleuță, A., Capcelea, A., Negru, A. (2002) Biological diversity conservation natural strategy and action plan, Chișinău, Știința, pp 100

Thibaud, J.-M., Babenko, A.B., Potapov, M.B. (1999) Records of arenicolous Collembola in the Moscow Area, *Russian Entomol. J.*, **8**: 71-72

STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LI, 2, 2006 (p. 19-20)

=== SHORT COMMUNICATION ===

NOTEWORTHY FEEDING BEHAVIOUR OF THE HOODED CROW (*CORVUS CORONE CORNIX*)

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On 27th and 28th September 2005, during our stay in Sfântu Gheorghe, Danube Delta Biosphere Reserve, Romania, we observed one individual of the Hooded Crow (*Corvus corone cornix*) interestingly feeding on walnuts (*Juglans regia*). The crow was flying upwards with the walnut in its bill, subsequently dropped it on the concrete pier and obviously repeated this scheme until the walnut was broken. We did not directly observe the successful breaking of the walnut, but we assumed it from some broken nutshells lying on the pier. A similar manipulation with food was observed by Šebela (2002) near Sfântu Gheorghe, but crows were breaking mussels in contrast to walnuts. This method of breaking walnuts was recorded several times in Europe. The majority of papers dealing with this type of feeding behaviour comes from Germany (Bosch, 2001; Creutz, 1953; Deppe, 1992; Förstel, 1993; Giger, 1950) and one is from Poland (Lorek and Oleksik, 1992). This is probably the first record of such behaviour for this area.

It seems this behaviour is widespread throughout Europe during autumn, when walnuts are available. Feeding on energy rich walnut's corns probably exceed energetic costs connected with breaking nutshells. Reichholf (2003) mentioned as few as five walnuts should be sufficient for covering daily energy demands of the crow.

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REFERENCES

Bosch, S. (2001) Krähen knacken Nüsse auf Hubschrauber-Landeplätzen, *Falke*, **48**, 112.

Creutz, G. (1953) Beeren und Früchte als Vogelnahrung, *Beiträge zur Vogelkunde*, **3**, 91-103.

Deppe, H.J. (1992) Nebelkrähe *Corvus corone cornix* öffnet Nüsse durch Abwurf, *Ornithologische Mitteilungen*, **44**, 317.

Förstel, A. (1993) Rabenkrähe *Corvus corone* öffnet Walnuß Juglans regia durch Fallenlassen, *Ornithologischer Anzeiger*, **32**, 165.

Giger, J. (1950) Krähe öffnet Baumnüsse durch Fallenlassen auf eine Strasse, *Ornithologische Beobachter*, **47**, 12.

Lorek, G., Oleksik, I. (1992) Wrony siwe (Corvus corone cornix) rozbijają orzechy zrzucając je na ziemię, *Notatki ornitologiczne*, **33**, 331-332.

Reichholf, J. H. (2003) Umfang und Bedeutung des Nüsse-Abwerfens von Rabenkrähen *Corvus c. corone*, *Ornithologische Mitteilungen*, **55**, 362-366.

Šebela , M. (2002) *Dunajská delta – barvy, vůně a hlasy přírodního ráje*, 1st ed., Moravské zemské muzeum, Brno, pp. 169.

STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LI, 2, 2006 (21-34)

CHLOROPHYLL FLUORESCENCE AND THE ACTIVITY OF XANTOPHYLL CYCLE DURING PHOTOINHIBITION AND IN THE PRESENCE OF PHOTOSYNTHETIC AND RESPIRATORY INHIBITORS IN *Mougeotia sp.*, STRAIN AICB 560

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SUMMARY. The 4000 µmol.m^{-2}.s^{-1} light has decreased the excitation energy capture rate because of the closing of the reaction center, this fact being due to photoinhibition. Nonphotochemical dissipation of the energy is related to xanthophyll cycle activity. DCMU has maintained the plastoquinone reduction state at a high level, while the zeaxanthin has decreased. Sodium azide has inhibited the photochemical efficiency and quantum yield operating on the photosynthetic electrons mobility, leading to the inactivation of the xanthophyll cycle. Propyl gallate and salicylhydroxamic acid inhibit the photochemical efficiency and the quantum yield, and the light photochemical conversion was increased at the level of antenna together with the nonphotochemical dissipation of the excess energy. The xanthophyll cycle components were decreased together with the proteins decrease. In the presence of thenoyltrifluoroacetone and rotenone the closed Q_A acceptors have been enhanced, and the quanta conversion inside of antenna has enhanced activating the xanthophyll cycle, this leading to zeaxanthin enhancement. The dithiothreitol led to decrease of fluorescence, quanta conversion and excess energy dissipation. The xanthophyll cycle was inhibited. The Q^-_A favors the interactions between photosynthesis and chlororespiration while the presence of the inhibitors of mitochondrial respiration influences or stimulates the photosynthetic electrons transport.

KEYWORDS: chlorophyll fluorescence, excitation pressure, HPLC, quenching coefficients, violaxanthin de-epoxidase

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Introduction

Chlororespiration has been defined as the interaction of respirator electrons carriers from mitochondria with the electron transport chain from chloroplast thylacoids (Bennoun, 1982, 1994, 2001, 2002).

The electrons transfer is generated through the reducing and oxidation of plastoquinone by NADH – dehydrogenase specific enzymes (Teicher and Scheller, 1998) and by quinol oxidase (Peltier and Cournac, 2002).

Chlororespiration is important in the photosynthesis regulation by modulating the activity of the cyclic electron chain around of photosystem PS I (Deng *et al.*, 2003; Joët *et al.*, 2002). During the response to the stress factors, the chlororespiration activity and **Ndh** complex are intensified (Bukhov *et al*., 2000). The photooxidative stress increases the nonphotochemical reduction of the intersystemic electron carriers (Havaux, 1996; Casano *et al*., 2000).

In this article the chlorophyll fluorescence parameters in the presence of photosynthetic electrons transfer inhibitors (DCMU) and of mitochondrial respiration inhibitors (n-propyl gallate, rotenone, etc.), during the inducement of photoinhibition by intense light are estimated.

Material and Methods

The green alga *Mougeotia sp.* Agardh (AICB 560) belongs to the Collection of Algae Cultures of I.C.B. Cluj-Napoca (AICB) (Dragoş *et al*., 1997). The strain AICB 560 was grown in Bold nutritive solution (BBM), during continuous air stirring, 400 $\mu mol.m^{-2}.s^{-1}$ continuous illumination, at 20^{o}C. The cultivation period was 23 days.

The 4000 $\mu mol.m^{-2}.s^{-1}$. PAR light intensity (photosynthetic active radiation) was applied for 60 minutes, at room temperature in the presence of diuron (3,27 µM DCMU) and other specific inhibitors: sodium azide (1 mM NaN_3), *n*-propyl gallate (1 mM PG), salicylhydroxamic acid (1 mM SHAM), thenoyltrifluoroacetone (10 µM TTF), 20 µM rotenone and dithiothreitol (20 µM DTT). As light source there were used 500 W halogen bulbs. The suspension was magnetically stirred during the whole period of light exposure in order to homogenize the cells, and a water filter was set between suspension and the light source to avoid the heating.

Carotenoids analysis by HPLC. The algal suspension was concentrated by filtration, and the sediment was saponified with a 30% KOH in ethanol solution (1:1 ratio with the algal suspension), at room temperature, for 24 hours. The carotenoids were extracted with diethyleter, and the extract was washed with water and then dry evaporated. The resulted residue was eluted in 5 ml ethyl acetate and we used the High Performance Liquid Chromatography (HPLC). The used gradient: 0 min: 10% B / 90% A; 20 min: 70% B / 30% A (A is represented by the acetonitrile: water = 9:1 mixture, while B is the ethyl acetate). The chromatograms

display the major carotenoids function of the retention time. The separations investigation was accomplished at 450 nm, 35°C and 144 barr pressure.

The chlorophylls concentrations were estimated according to Arnon (1949), and the proteins according to Lowry *et al.* (1951). Violaxanthin de-epoxidation, respectively the formation of zeaxanthin in connection with the light was analyzed *in vivo* by measuring the absorption changes at 505 nm (ΔA_{505}) (Bilger *et al.*, 1989), while the redox state of the P_{700} reaction center was evaluated by determining the absorption at 700 nm (Melis, 1989).

Chlorophyll fluorescence analysis. The chlorophyll fluorescence was measured with PAM-210 fluorometer according to Schreiber *et al.* (1986). The fluorescence parameters and the quenching analysis were accomplished by saturation pulse method. The quantum yield of the photochemical energy conversion were determined with the Yield = $\Delta F/F_M$, and the F_V/F_M ($F_V/F_M = F_M-F_0/F_M$) display the photochemical quantum yield of the closed PS II reaction centers. The *in vivo* PS II excitation pressure was estimated by $1-q_p$. The parameters of the algal suspensions fluorescence grown in normal conditions were used as control sample.

Results and Discussions

By major carotenoids analysis during the retention time in *Mougeotia* cultures that were considered as control samples, there was detected the dominance of the lutein and various carotenes species. On the other hand, it was observed a low violaxanthin concentration and an obvious enhancement of anteraxanthin amount (Fig. 1).

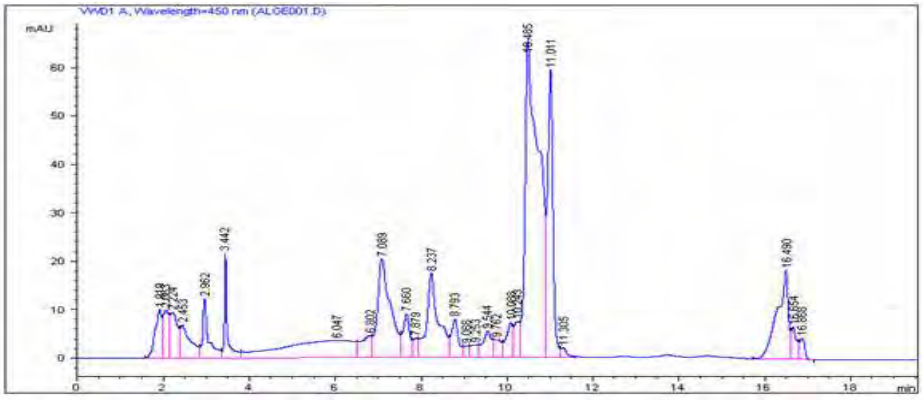

Fig. 1. Major carotenoids chromatogram in *Mougeotia sp.* suspensions grown under normal conditions that were used as control sample

The decrease of the violaxanthin amount is related to the oxidation state of plastoquinone (Pfündel and Bilger, 1994). The zeaxanthin concentration has been maintained at low values and this was due to light medium intensity during the growth that can activate the xanthophyll cycle. The chlorophylls a and b concentration was found at high values because of the medium intensity of growth light (Table 1).

Under 4000 $\mu mol.m^{-2}.s^{-1}$ light the minimal (F_0) and maximal (F_M) fluorescence have enhance according to exposure period (Fig. 2). Comparatively with the control, F_0 has enhanced, while F_M has decreased leading to the variable fluorescence reduction.

The PS II quantic efficiency as well as the photosynthetic performance of the electrons carrier chain have temporary decreased comparatively to control values. Variable fluorescence decrease certify the Q^-_A enhancement, contributing to the decrease of excitation energy capture rate by the antenna because of the reaction centers closure, this being due to the photoinhibition. The interactions between photosynthesis and chlororespiration are favored when the plastoquinone exists in reduced state, while the interactions between photosynthesis and mitorespiration are favored by the oxidation state. The plastid oxidases are more active when plastoquinone becomes very reduced and thus it prevents the overreduction (Cournac et *al.*, 2002).

The efficiency of the photochemical conversion (Q_p) and the nonphotochemical dissipation of the excess energy (q_N) have enhanced with the exposure period, and the excitation pressure of the PS II reaction center was low (Fig. 3). Into the thylacoid membranes the light is absorbed through the light-harvesting antenna complexes that bind the chlorophyll (LHC_S) in association with the reaction centers of the two PS II and PS I photosystems. Under light excess, singlet oxygen radicals are generated, and PS II suffers certain irreversible photooxidative damages. These selective damages affect the PS II reaction center proteins, leading to the potential decrease of the photosynthesis rates and productivity (Powless and Critcheley, 1980; Powles, 1984).

In the presence of diuron (3,27 µM DCMU), the minimal and maximal fluorescence were enhanced, but the variable fluorescence remained at low values. The high level of fluorescence displays the high intensity of the plastoquinone reduction state. The photochemical efficiency and the quantum yield have enhanced the first 15 minutes, and then they have significantly decreased, in comparison with the control (Fig. 2). The photochemical conversion efficiency of the quanta (q_p) and also the excess energy nonphotochemical dissipation (q_N) have enhanced even the excitation pressure remained at low levels (Fig. 3). It is known that DCMU block the transfer of photosynthetic electrons between Q_A (primary quinonic acceptor) and Q_B (secondary quinonic acceptor) and inhibits the oxygen production in PS II.

In the presence of sodium azide (1 mM NaN_3), that is an inhibitor for *c*-cytochrome oxidase, the minimal and maximal fluorescence have enhanced in the first 15 minutes displaying a significant decrease, and then they have enhanced in the end of the exposure. The variable fluorescence has been maintained under control's values. The photochemical efficiency and the quantum yield have significantly decreased (Fig. 2).

The q_p and q_N coefficients values were maintained at high values, while the excitation pressure was maintained at low levels (Fig. 3). If the DCMU effects are removed it can be seen that the sodium azide acts against the electrons activity from the photosynthetic transport.

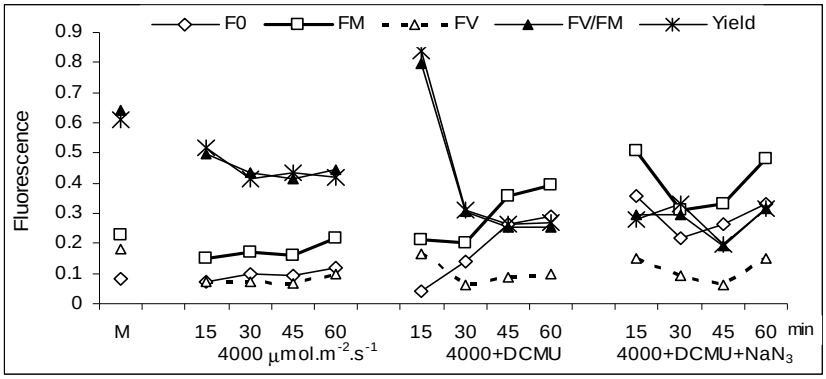

Fig. 2. Evolution of the chlorophyll fluorescence under 4000 $\mu mol.m^{-2}.s^{-1}$ light, in the presence of 3,27µM DCMU, and 1 mM NaN_3. F_0 = minimal fluorescence; F_M = maximal fluorescence; F_V/F_M = photochemical efficiency; Yield = quatum yield; M = control

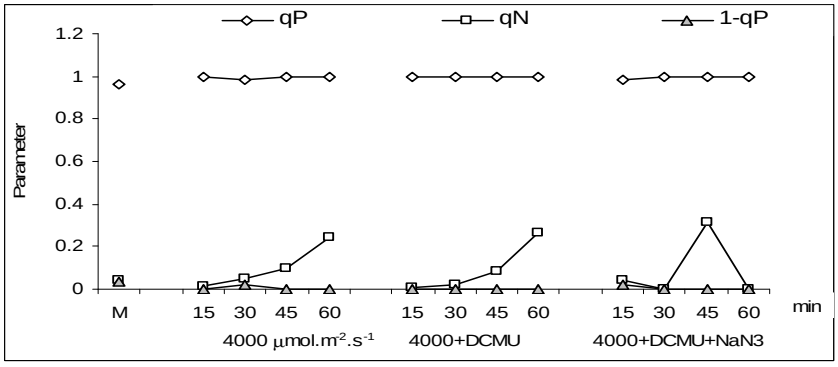

Fig. 3. The evolution of the quenching coefficients and excitation pressure under light excess and in the presence of specific inhibitors. q_p – photochemical quenching; q_N – nonphotochemical quenching; 1- q_p – excitation pressure

Table 1.

The chlorophylls and carotenoids amount and the retention period (minutes) in the *Mougeotia* suspensions under the action of light and various inhibitors

Parameter	Control		4000 µmol. $m^{-2}.s^{-1}$		4000 + DCMU		4000 + DCMU + NaN_3	
	1	*2*	*1*	*2*	*1*	*2*	*1*	*2*
chloropyll a mol.10^{-5}	4.75		3.76		3.36		2.74	
chlorophyll b mol/mol chl. a	0.36		0.356		0,339		0.327	
neoxanthin	3.057	2.962	23.18	3.001	14,70	3.425	6.360	3.449
violaxanthin	2,446	3.442	8.498	3.437	23.34	3.781	9.542	3.583
anteraxanthin	31.41	10.48	51.59	10.52	69.31	10.65	62.98	10.801
lutein	11.63	11.01	18.77	11.10	21.00	11.32	20.15	11.41
zeaxanthin	0.646	11.30	1.632	11.41	0.913	11.63	0.672	11.72
β- criptoxanthin	-	-	0.839	13.88	0.939	14.05	-	14.134
α+β-carotene	7.53	16.49	12.97	16.72	8.71	16.81	15.42	16.846
9Z-β- carotene	1.37	16.65	2.59	16.89	2.90	16.97	3.56	17.011
15 Z-β-carotene	0.68	16.86	0.86	17.10	0.97	17.18	1.19	17.211
a / b	2.76		2.76		2.90		3.01	
Proteins ($g.g^{-1}$ d.w.)	2.03		1.61		1.36		1.35	

1 – amount; ***2*** – retention time

• carotenoids are expressed in mmol/mol chlorophyll a

The zeaxanthin presence was also observed, as a proof for the xanthophyll cycle activity under the tested light intensity. In the DCMU sample the chlorophylls amount has decreased while the carotenoids concentration has increased, especially violaxanthin and anteraxanthin, while the zeaxanthin amount has decreased. In the sample with sodium azide the carotenes, lutein and anteraxanthin amount has enhanced, while the quantity of violaxanthin, neoxanthin and zeaxanthin has decreased. The performing of xanthophyll cycle was inhibited in the presence of these inhibitors. The xanthophylls interconvertibility process from violaxanthin (di-epoxide) → anteraxanthin (mono-epoxide) → zeaxanthin (without epoxide) through the addition or removal of the epoxide was significantly intensified leading to the production of a increased amount of the intermediary product.

The content of cellular proteins has significantly decreased (Table 1). The xanthophylls are connected to the chlorophyll of the proteic complexes that are incorporated in membranes (LHC) that absorb and transfer the excitation energy to the reaction centers in order to be used in electrons transport, these reactions converting the light into chemical energy to be finally used for the CO_2 fixation and production of carbohydrates. The interactions between photosynthesis and mitorespiration require the involvement of the metabolic interaction between chloroplasts and mitochondria (Cournac *et al.*, 2002).

The addition of propyl gallate (1 mM PG) led to the enhancement of minimal and maximal fluorescence and to the decrease of the variable fluorescence, in comparison with the control (Fig. 4). Both the photochemical efficiency and the quantum yield have decreased in the same time with the light capture efficiency (q_p), while the nonphotochemical dissipation of the excess energy and the excitation pressure have enhanced (Fig. 5). The reducing of the variable fluorescence certifies the decrease of the oxidative Q_A contributing to the reducing of excitation energy capture rate, and the F_V/F_M ratio distinguishes a lower quantum yield of the closed PS II reaction centers. By the decrease of F_V/F_M ratio it is demonstrated the inducement of the PS II photosystem photoinactivation and therefore the decrease of the photosynthetic capacity. In these conditions the thylacoid membranes energization state in high, as well as the rate of PS II reaction centers closure in light (Kobližek *et al.*, 2001). The propyl gallate – an inhibitor for chlororespiration oxidase – led to the enhancement of chlorophyll fluorescence because of the inhibition of $\Delta F/F_M$ (electrons flow dependent on PS II) (Cournac *et al.*, 2002).

In the sample with salicylhydroxamic acid (1 mM SHAM), the chlorophyll fluorescence parameters have decreased in the first 30 minutes. The variable fluorescence was maintained at low values as well as the photochemical efficiency and the quantum yield of the photosynthetic chain (Fig. 4). The light photochemical conversion energy has enhanced together with the nonphotochemical dissipation of the excess energy, while the excitation pressure has decreased (Fig. 5).

The thenoyltrifluoroacetone (10 µM TTF) – an inhibitor for the mitochondrial electrons transport chain, led to the enhancement of the minimal fluorescence and to the reduction of the others fluorescence parameters, in comparison to the control. The minimal fluorescence has increased in the light-exposure period, and the maximal fluorescence was maintained at low values emphasizing the reduced quantity of closed Q_A acceptors, thus explaining the prolonged period of excitation (Fig. 4). The efficiency of the quanta conversion was maintained at high levels, while the nonphotochemical dissipation was gradually reduced, the excitation pressure being also reduced (Fig. 5).

By treating the algae with rotenone (20 µM R), the minimal and maximal fluorescence have decreased in the first 30 minutes the same as the variable fluorescence. The photochemical fluorescence was also reduced, while the quantum yield remained constant (Fig. 4). The conversion of light quanta has been

maintained at a high level, and the nonphotochemical dissipation has been slowly enhanced in the first 30 minutes under a low excitation pressure (Fig. 5).

After the dithiothreitol treatment (20 mM DTT) it was observed the decrease of the minimal, maximal and variable fluorescence for the first 30 minutes and then the enhancement of these parameters in comparison to the control sample (Fig. 4). The reduction of the quanta conversion (q_p) and of excess energy dissipation (q_N), as well as the increase of the excitation pressure (Fig. 5) are important.

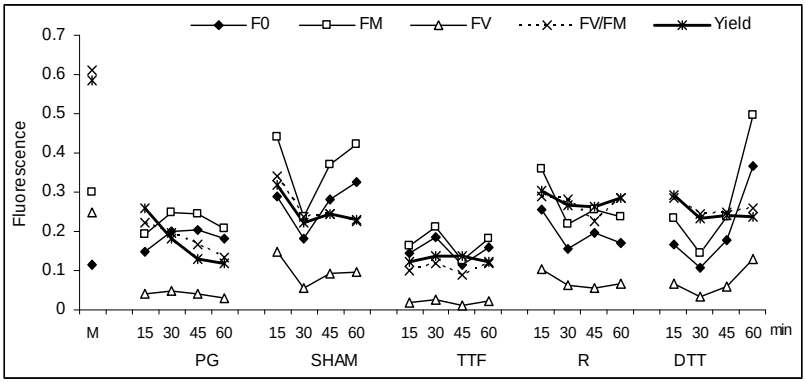

Fig.4. Evolution of the chlorophyll fluorescence under 4000 µmol.m^{-2}.s^{-1} light, in the presence of 1 mM PG, 1 mM SHAM, 10 µM TTF, 20 µM rotenone and 20 mM DTT. F_0 = minimal fluorescence; F_M = maximal fluorescence; F_V/F_M = photochemical efficiency; Yield = quantum yield; M = control. The samples with inhibitors were previously treated with DCMU.

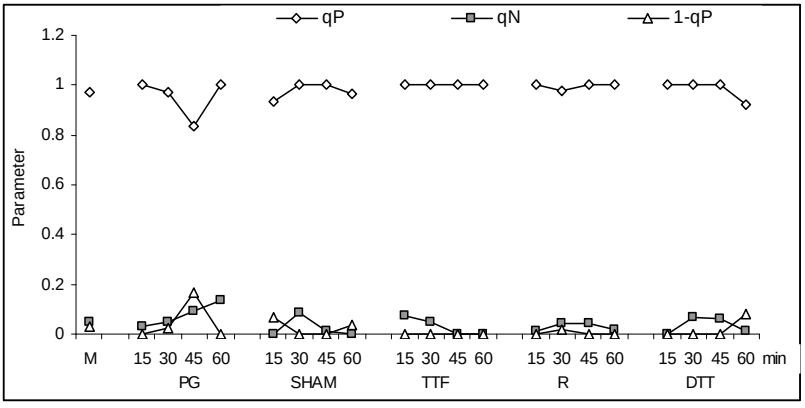

Fig. 5. The evolution of the quenching coefficients and excitation pressure under light excess and in the presence of specific inhibitors. q_p – photochemical quenching; q_N – nonphotochemical quenching; 1- q_p – excitation pressure

The xanthophylls contribute to the pigment-protein (LHC) complexes assembly and stability, and they also have a role in photoprotection. The plants major carotenoids are lutein, β-carotene, violaxanthin and neoxanthin. β-carotene is bound to PS II reaction center, while the xanthophylls are bound to LHCs antenna proteins. The enhancement of lutein amount leads to the decrease in violaxanthin concentration and to the increase of total carotenoids content (Pogson and Rissler, 2000).

Table 2

The chlorophylls and carotenoids amount and the retention period (minutes) in the *Mougeotia* suspensions under the action of light and various inhibitors

Parameter	Control		PG		SHAM		TTF		Rotenone		DTT	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
chlorophyll a mol.10^{-5}	4.75		3.12		2.71		3.62		3.47		3.25	
chlophyll b mol/mol chl. a	0.36		0.34		0.35		0.34		0.35		0.35	
neoxanthin	3.06	2.96	25.14	3.43	22.51	3.43	24.25	2.94	28.46	3.42	9.83	2.93
violaxanthin	2,45	3.44	25.14	3.71	16.09	3.66	8.83	3.09	17.58	3.70	9.83	3.45
anteraxanthin	31.41	10.4	102.34	10.72	93.59	10.65	79.9	10.85	74.80	10.5	69.7	11.0
luteina	11.63	11.0	30.48	11.39	26.04	11.32	31.3	11.39	16.80	11.2	25.4	11.5
zeaxanthin	0.65	11.3	2.95	11.71	1.13	11.63	3.39	11.70	0.89	11.5	0.94	11.8
β- criptoxanthin	-			14.14		14.05		14.06		14.05		-
α+β-carotene	7.53	16.4	19.80	16.85	21.59	16.80	18.8	16.79	15.00	16.7	20.0	16.9
9Z-β-carotene	1.37	16.6	4.17	17.01	3.60	16.96	3.59	16.96	2.81	16.9	4.00	17.1
15Z-β-carotene	0.68	16.8	2.08	17.21	2.40	17.16	1.79	17.15	0.94	17.1	2.00	17.2
a / b	2.76		2.88		2.79		2.86		2.86		2.78	
Proteins ($g.g^{-1}$ d.w.)	2.03		2.19		1.41		1.56		1.34		1.09	

- carotenoids are expressed in mmol/mol chlorophyll a
- *a* = amount; *b* = retention time
- the samples with inhibitors were previously treated with DCMU

After exposing the algae to PG the chlorophylls have been reduced, while the carotenoids concentrations were enhanced. The violaxanthin and anteraxanthin amounts have increased, and the zeaxanthin was maintained at low values, this fact being correlated to the reduction of the excess excitation energy dissipation process in the photosystem antenna (Fig. 5). The high amount of β-carotene explains the excess energy dissipation in the PS II reaction center. The cellular proteins quantity overtook control's values (Table 2).

In the SHAM sample the chlorophyll amount has decreased, the carotenoids were maintained at high values, while the xanthophyll cycle components were reduced. The quantity of cellular proteins was also decreased. In

the presence of TTF the chlorophylls concentration have decreased, and the amount of carotenes and lutein have enhanced. Thus, the violaxanthin and anteraxanthin amount decreased, while the zeaxanthin increased. The cellular proteins amount was reduced comparatively to the control. After the action of the rotenone the quantity of chlorophylls and major carotenoids have decreased as well as the cellular proteins content. In the presence of DTT the chlorophylls and the xanthophyll cycle compounds have decreased while the carotenes and the lutein have enhanced together with the cellular proteins amount diminution (Table 2).

The activity of violaxanthin de-epoxidase monitored by the absorption changes at 505 nm emphasized that under light stress, SHAM and rotenone to support oneself the de-epoxidic activity, while in the case of DCMU and PG this activity was inhibited after an exposure period of 30 minutes. In the presence of sodium azide and DTT violaxanthin de-epoxidation is inhibited, while after the treatment with TTF, xanthophylls de-epoxidation and epoxidation are changed with the exposure period.

The increase of zeaxanthin amount is correlated with the intensification of excitation energy dissipation (q_N) in the most of the experimental variants (Fig. 5). The absorption changes at 505 nm are associated with the xanthophyll cycle activity and they are due to the absorption changes between violaxanthin and zeaxanthin (Niyogi *et al.*, 1997). There is a close connection between the q_E part of the excitation dissipation mechanism and the de-epoxidation activity of the violaxanthin to zeaxanthin (Ruban *et al.*, 1993). The xanthophylls are exclusively located in the pigment-protein light-harvesting complexes (LHC), thus emphasizing the involvement of the PS II photosystem antenna in the excitation dissipation mechanism. In fact q_E derives from the structural changes regarding the binding of pigments to LHC II, being synergic influenced by LHC II polypeptides protonation and violaxanthin replacement by zeaxanthin.

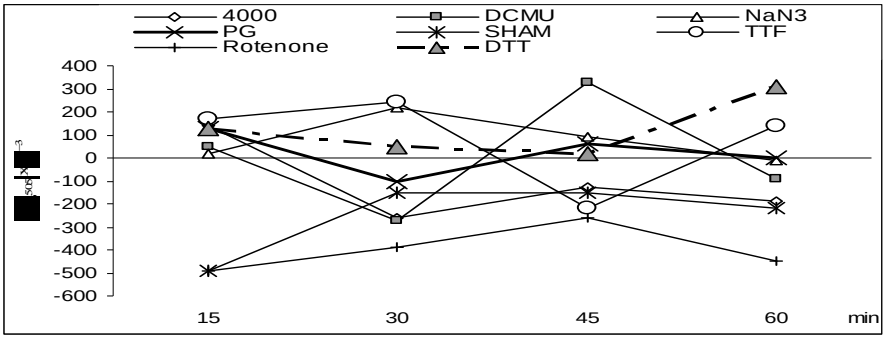

Fig. 6. Absorption changes at 505 nm (de-epoxidase activity) influenced by light and various inhibitors

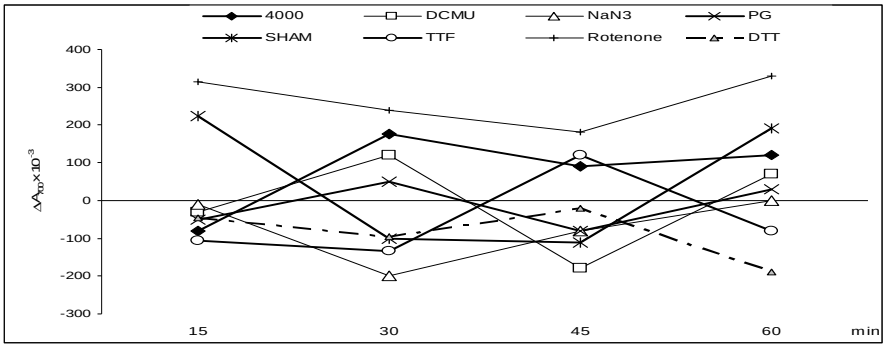

Fig. 7. The absorption changes at 700 nm (P_{700} reaction center) under the influence of light and various inhibitors

The activity of the PS I P_{700} reaction center showed: - the reaction center exists in reduced state with the negative charge dominancy in the majority of the variants, in the first 15 minutes; - under 4000 µmol.m^{-2}.s^{-1} light and rotenone the oxidized state prevails, this being correlated to the decrease of the quantum yield of the electron carrier chain ($\Delta F/F'_M$); - in the presence of DCMU and PG there was observed a bimodal evolution of the PS I reaction center states, with the maximal oxidative peaks after 30 and 60 minute exposure period; - sodium azide, SHAM, TTF and DTT have maintained the reaction centers in reduced state, certifying the electrons mobility during illumination (Fig. 7).

In the presence of DCMU, the photochemical activity is generally based on the PS I photosystem activity whose electrons transport can also derive from chlororespiration operating.

Conclusions

The photosynthesis photochemical activity during the illumination period and in the presence of electrons transport inhibitors from chloroplasts and mitochondria was perturbed due to PS II inhibition and to the maintenance of PS I activity.

Under 4000 µmol.m^{-2}.s^{-1} light the excitation energy harvesting rate by the antenna has decreased because of the reaction centers closure, this being due to the photoinhibition inducement. The photochemical conversion efficiency was high and the nonphotochemical dissipation of the excess energy has increase during the exposure. The chlorophyll concentration has decreased while the xanthophyll cycle activity has been observed.

In the presence of DCMU the high level of plastoquinone reduced state has been maintained, and the photochemical conversion efficiency of the quanta and also the nonphotochemical dissipation of the excess energy have enhanced. The chlorophylls

amount has decreased while the carotenoids content have enhanced, especially violaxanthin and anteraxanthin, whereas the zeaxanthin quantity has decreased.

The sodium azide inhibited the photochemical efficiency and the quantum yield influencing the electrons mobility from the photosynthetic transport leading to the xanthophyll cycle inactivation.

The addition of propyl gallate leads to the decrease of the photochemical efficiency and quantum yield, intensifying the nonphotochemical dissipation of the excess energy. Due to F_V/F_M ratio decrease it is certified the inducement of the PS II photoinactivation process and also it implies the decrease of the photosynthetic capacity, and these conditions caused the reduction of the zeaxanthin concentration.

In the sample treated with salicylhydroxamic acid the photochemical efficiency and the quantum yield have decreased while the light photochemical conversion at the antenna have enhanced together with the nonphotochemical dissipation of the excess energy. The chlorophylls quantity has been reduced, while the components of the xanthophyll cycle have been decreased together with the cellular proteins amount.

In the presence of thenoyltrifluoroacetone the closed Q_A acceptors was reduced, and the quanta conversion in the antenna was maintained at high values, activating the xanthophyll cycle and thus the quantity of zeaxanthin being enhanced. Similar results have been acquired in the presence of rotenone.

The dithiothreitol leads to the decrease of fluorescence, quanta conversion and excess energy dissipation. The chlorophylls and the xanthophyll cycle xanthophylls amounts have also been reduced while the carotenes and lutein quantities were enhanced together with cellular proteins diminution.

The maintenance of Q_A in reduced state favors the interactions between photosynthesis and chlororespiration while the presence of mitochondrial respiration inhibitors affects or stimulates the transport of photosynthetic electrons.

REFERENCES

Arnon, D.I. (1949). Copper enzymes in chloroplasts. Polyphenyloxidase in *Beta vulgaris*, *Plant Physiol.*,**24**, 1-15.

Bennoun, P. (1982). Evidence for a respiratory chain in the chloroplast. *Proc. Natl. Acad. Sci. USA*, **79**, 4352-4356.

Bennoun, P. (1994). Chlororespiration revisited: mitochondrial-plastid interactions in *Chlamydomonas*., *Biochim.Biophys.Acta*, **1186**, 59-66.

Bennoun, P. (2001). Chlororespiration and the process of carotenoid biosynthesis. *Biochim.Biophys.Acta,* **1506**, 133-142.

Bennoun, P. (2002). The present model for chlororespiration., *Photosznth.Res.*,**73**, 273-277.

Bilger, W., Björkman, O., Thayer, S.S. (1989). Light-induced spectral absorbance changes in relation to photosynthesis and the epoxidation state of xanthophyll cycle components in cotton leaves. *Plant Physiol.*, **91**, 542-551.

Bukhov, N.G., Samson, G., Carpentier, R. (2000). Nonphotosynthetic reduction of the intersystem electron transport chain of chloroplasts following heat stress. Steady-state rate. *Photochem.Photobiol.*,**72**, 351-357.

Casano, L.M., Zapata, J.M., Martin, M., Sabater, B.(2000). Chlororespiration and poising of cyclic electron transport- plastoquinone as electron transporter between thylakoid NADH dehydrohenase and peroxidase. *J.Biol.Chem.*, **275**, 942-948.

Cournac, L., Latouche, G., Cerovic, Z., Redding, K., Ravenel, J., Peltier, G.(2002). In vivo interactions between photosynthesis mitorespiration and chlororespiration in *Chlamydomonas reinhardtii*. *Plant Physiol.*,**129**, 1921-1928.

Deng, Y., Ye, J., Mi, H. (2003). Effects of low CO_2 on NAD(P)H dehydrogenase, a mediator of cyclic electron transport around photosystem I in the cyanobacterium *Synechocystis* PCC 6803. *Plant Cell Physiol.*, **44**, 534-540.

Dragoş, N., Péterfi, L.Şt., Momeu, L., Popescu, C. (1997). An introduction to the algae and the culture collection of algae at the Institute of the Biological Research Cluj-Napoca. Cluj Univ.Press.

Havaux, M. (1996). Short-term responses of photosystem I to heat stress. Induction of a PS II-independent electron transport through PS I fed by stromal components. *Photosynth.Res.*, **47**, 85-97.

Joët, T., Cournac, L., Peltier, G., Havaux, M. (2002). Cyclic electron flow around photosystem I in C_3 plants. *In vivo* control by the redox state of chloroplasts and involvement of the NADH-dehydrogenase complex. *Plant Pysiol.*, **128**,760-769.

Koblížek, M., Kaftan, D., Nedbal, L. (2001). On the relationship between the non-photochemical quenching of the chlorophyll fluorescence and the photosystem II light harvesting efficiency. A repetitive flash fluorescence induction study. *Photosynth.Res.*, **68**, 141-152.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J.Biol.Chem.*, **193**, 265-275.

Melis, A. (1989). Spectroscopic methods in photosynthesis: photosystem stoichiometry and chlorophyll antenna size. *Phil.Trans.R.Soc.Lond.B*, **323**, 397-409.

Niyogi, K.K., Björkman, O., Grossman, A.R. (1997). The roles of specific xanthophylls in photoprotection. *Proc.Natl.Acad.Sci.USA*, **94**, 14162-14167.

Peltier, G., Cournac, L.(2002). Chlororespiration. *Annu.Rev.Plant Physiol.*, **53**, 523-550.

Pfündel, E., Bilger, W. (1994). Regulation and possible function of the violaxanthin cycle. *Photosynth.Res.*, **42**, 89-109.

Pogson, B.J., Rissler, H.M. (2000). Genetic manipulation of carotenoid biosynthesis and photoprotection. *Phil.Trans.R.Soc.London*, **355**, 1395-1403.

Powles, S.B., Critchley, C. (1980). Effect of light intensity during the growth photoinhibition of intact attached bean leaflets. *Plant Physiol.*, **65**, 1181-1807.

Powles, S.B. (1984). Photoinhibition of photosynthesis induced by visible light. *Annu.Rev.Plant.Physiol.*, **35**, 15-44.

Ruban, A.V., Young, A.J., Horton, P. (1993). Induction of nonphotochemical energy dissipation and absorbance changes in leaves. Evidence for changes in the state of the light-harvesting system of photosystem II in vivo. *Plant Physiol.*, **102**, 741-750.

Schreiber, U., Schliwa, U., Bilger, W. (1986). Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer, *Photosynth.Res*., **10**, 51-62.

Teicher, H. B. & Scheller, H. V. (1998) The NAD(P)H-dehydrogenase in barley (*Hordeum vulgare* L.) thylakoids is photoactivatable and utilizes NADPH as well as NADH. *Plant Physiol.*, **117**, 525-532

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=== REVIEW ===

ASPECTS OF THE EVOLUTIONARY PROCESS IN SMALL POPULATIONS

MANUELA DORDEA[1] and NICOLAE COMAN [1]

SUMMARY. The Hardy-Weinberg law refers to extremely large populations, where allele and genotype frequencies remain constant from one generation to another, provided that there were no perturbing outside forces, such as selection or migration. Such populations are hypothetical and do not evolve. In smaller populations, allele and genotype frequencies do change over time, because of sampling errors, increase in selection pressure and inbreeding. Consequently, small populations might survive, go to extinction or join other conspecific populations as immigrants. Based on literature as well as on our own research studies, the present paper is a review regarding the effects of population size fluctuations, genetic drift, the founder effect, migration and inbreeding, mechanisms that work in small populations and may cause changes in gene pools, followed by modifications of selection pressures and, implicitly, by evolution.

KEYWORDS: bottleneck effect, founder effect, genetic drift, migration

Introduction

The Hardy-Weinberg theorem states that in a large population, with panmictic reproduction, the frequencies of alleles and genotypes remain constant over the generations, providing there were no perturbing outside forces, like selection or migration. This theorem describes a hypothetical population, of extremely large size, that is not evolving.

But in smaller populations, allele and genotype frequencies do change over time because of several reasons, the most important of which are:

- stochastic fluctuations due to random sampling and, respectively, chance fluctuations in the gene pool, from one generation to the next, known as *genetic drift*;
- increase in selection pressure;

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- increase in inbreeding, with many important effects on the evolutionary process, such as reducing heterozygosity, decreasing genetic variability, which account for even higher selection pressure.

Consequently, small populations have three alternatives:

- survival, providing changes of gene frequencies, that lead to a new gene pool with co-adapted genes. In this case, the population gets more adapted and, consequently, grows in size;
- extinction, due to alterations in gene pool, that make their survival impossible;
- joining other populations, as immigrants.

Effects of population size fluctuations

Environmental conditions vary from a season to another, from the parental population to the offspring one. Temperature, relative humidity, solar radiation, the amount and quality of food, the incidence of several diseases influence the population size, which in turn modifies selection pressures, with all the entailed consequences.

In order to understand the influence of population size on genetic structure, let us review the classical observations of Ford (author of the first *Ecological Genetics* in 1964) on a butterfly population (*Melitaea aurinia*). The butterflies were monitored for 81 years (1981-1962) by members of Ford's family. They lived in an isolated forested habitat, surrounded by agricultural plains, flying no further than 50 m from the forest. The butterfly population, with one generation annually, was two times drastically reduced in size. If till 1884 the butterflies were common in that forest, during 1920-1923 they became rare, rising again in number after 1924. In 1932 the population dropped again, and maintained at this low level till 1935.

During the periods of high density, genetic variation was also high, so that two individuals with similar phenotypes could scarcely be found. Such individuals differed from the normal phenotype regarding the coloration, size and conformation of wings. Some individuals were distorted, with no more ability to fly.

During the periods when the population was drastically reduced, natural selection singled out only a standard phenotype, eliminating the aberrant ones. But there were differences between the standard phenotypes selected during the two periods of decline (1920-1923 and 1932-1935, respectively), regarding the coloration and pattern of wings. Ford emphasized the importance of the population size decline periods on their subsequent evolution, periods when natural selection modifies evidently the gene frequencies and, implicitly, the gene population pools. This action was compared with the pouring of a liquid through a bottleneck, situation known as the bottleneck effect.

Later on, similar observations were recorded in populations of fruit flies - *Drosophila pseudoobscura* (Dobzhansky and Pavlovsky, 1957) and *Drosophila melanogaster* (Coman and Wallace, 1973), voles – *Microtus agrestis* (Chitty and

Phipps, 1961), cheetahs – *Acinonyx jubatus* (O'Brien and Evermann, 1988), mice – *Peromyscus* sp. (Lacy, 1992), lions – *Panthera leo* (Primack, 1993), a tree species from Brazil *Bertholletia excelsa* (Peres *et al*., 2003). A bottleneck effect was supposed to occur also in populations of giant tortoises – *Geochelone nigra vandenburghi* in Galapagos, after the powerful eruption of the prehistoric volcano Alcedo.

A drastically fall in population size enhances the chance of errors, the changes in gene frequencies during successive generations, because of the decrease in new formed zygotes, as compared to theoretical possibilities of combinations. By chance, certain alleles may be overrepresented among the survivors, others may be underrepresented, and some may be eliminated altogether. The smaller the population, the greater the role played by chance fluctuations in allele frequencies from one generation to the next. Such unpredictable fluctuations in gene frequencies are called genetic drift.

The most important impacts of chance fluctuations in gene frequencies are:

- division of the initial population in many subpopulations; for example, it is well known that the human European population is a large population, where marriages between people inside a village, town or country are more frequent. The result was the division of the continental population in many subpopulations (not considering also the migration). When the subpopulations are small, the phenotype variations between them are evident, because of sampling error (Coman, 1977).
- decrease in genetic variation in small populations; in such case, individuals resemble each other more, so that populations become genetically more uniform.
- enhance homozygosity; the risk of many recessive rare genes to become homozygous, that is phenotypically expressed, increases. Usually such genes are deleterious for the carrying individual, lowering the fertility and viability of populations as a whole (Coman and Mixici, 1980).

The bottleneck effect

After a temporary genetic bottleneck, heterozygosity might be re-established, even if the number of alleles is low (Nei *et al*., 1975; Allendorf and Lery, 1986). This was the case of the Indian rhinoceros – *Rhinoceros unicornis* – whose population size dropped to 30 mating partners, but during 1988 returned to hundreds (Dinerstein and McCracken, 1990).

Fig. 1 illustrates, comparatively, the possible effects of a genetic bottleneck in two hypothetical populations with two and ten individuals, respectively (Primack, 1993; Halliburton, 2004). It is obvious that heterozygosity decreases more evidently when population size drops from two individuals (uninterrupted line) than from ten individuals (dotted line). The decrease in heterozygosity is also

affected by the population growth rate (r). When the growth rate per generation is low (r = 0,1), as in elephant populations, heterozygosity decreases more evidently, so that its re-establish is much slower as compared to populations with high growth rate per generation (r = 1), such as rabbits or mice populations.

The influence of size on population heterozygosity was also studied in plants. In small populations of the aquatic species – *Howellia aquatilis* (*Campanulaceae*), genetic variation is very low, probably due also to inbreeding (Lesica *et al*., 1988).

In the subalpine coniferous forests of New Zealand, there are populations of *Holocarpus budwilii* with 10 to 400 000 individuals. In populations with less than 8 000 individuals, the genetic variation is significantly low (Billington, 1991).

One may conclude that the bottleneck and genetic drift that follows usually reduce the overall genetic variability in a population which collapse. The genes of individuals that could survive in the new environmental conditions, under high selection pressures, will be preserved in the gene pool. When the selection pressures diminish, the population starts to rise again, but its gene pool will be to a great extent quite different from that before the collapse.

As we already mentioned, besides the decline of the population size, the bottleneck effect also accounts for reduced heterozygosity. This was the case for many species that survived after the last glacier advance. In order to make up the original genetic variability, populations need hundreds or thousands of generations (Nei and Graur, 1984).

Many experiments confirmed the bottleneck effect in species belonging to very different systematic groups. Thus, McCommas and Bryant (1990) created 3 populations of *Musca domestica* starting from 2, 8 and respectively 32 individuals of a large population. Comparing 4 polymorphic loci, researchers recorded a significantly decrease in alleles, after a bottleneck event. Similar observations were made by Parker *et al*. (1991) regarding lion populations in the Ngorongoro crater (Tanzania). After the outbreak of an infectious disease, in 1962, the population was drastically reduced to 7 females and 8 males. The resulted generation revealed an evident loss in alleles, as compared with the gene pool of the large lion populations in the neighbouring Serengeti Park.

The same drop in genetic variation was also recorded in a cheetah population, after their overhunting (O'Brien *et al*., 1983, 1985).Even though the population has rebounded nowadays, it will take many generations to reach the initial heterozygosity.

The 50/500 rule

If the populations decline too drastically in size, they can no longer recover, revealing a downward trend till extinction. Starting from such observations, researchers have tried to establish the minimal population size at which a species is able to sustain its numbers, survive, and recover its size. Of great

interest are Berger's studies (1990, 1999) on bighorn sheep – *Ovis canadensis*, which live in the south-western part of the United States. Berger stated that small populations, with less than 50 individuals, disappeared over a period of 50 years, while populations larger than 100 individuals survived.

Other studies attempted to establish the minimum viable population size for the north-american grizzly bear– *Ursus horribilis* (Shaffer, 1981, 1983; Shaffer and Samson, 1985; Allendorf, 1997; Allendorf and Servheen, 1986; Kaiser, 1999). The conclusion was that the survival of grizzly populations depends not only on the population size, but also on the area size, population density, amount of food etc.

Based on the experience of domestic animal breeders, Franklin (1980) estimated that 50 might be the minimum size to maintain the genetic variability of a population. For wild populations, the minimum size might be 500 individuals; hence, the 50/500 rule, available for populations to maintain their genetic variability.

In practice, this rule is difficult to apply, because of the different probabilities of adults to reproduce and have offsprings. Therefore, the effective breeding population is less than the total size of that population. Nevertheless, this is not always the case. For example, the large Colorado beetles (*Leptinotarsa decemlineata*) spread in Europe after the 2nd World War, starting from several individuals, introduced accidentally with a transport of potatoes from the United States to England. One can also mention the populations of the tree species *Metasequoia* sp., spread nowadays on all the continents (except Africa), which evolved from the seeds of some trees discovered in an isolated area in China (Dordea and Coman, 2005).

Genetic drift

The random fluctuations of gene frequencies in small populations during successive generations, as a result of sampling errors, respectively of gametes which form zygotes, were called by Wright (1922) genetic drift.

Because of the small number of zygotes, gene frequencies vary unpredictably from one generation to the next. The deviation from the initial frequency will be inversely proportional to the size of the sample. The smaller the sample, the greater the chance of deviation from the predicted result.

In very small populations all loci tend to homo-allelism, so that which of the alleles will be fixed (the term *fixation* in genetics refers to 100 percent frequency) or eliminated from the gene pool is the result of chance. To verify this statement pointed by Wright, we carried out a laboratory experiment (Coman and Gheorghita, 1988). We started with a large population of *Drosophila melanogaster*, mating virgin wild type females (e^+e^+) with mutant males, with black body (ee), a recessive trait. The whole F_1 generation were heterozygotes for the two genes ($p = q = 0,5$). Afterwards, this hybrid generation was divided into three other populations of 1 000 individuals each, respectively into 42 small sub-populations. The large populations, reared in special fly rooms, get stabilized around 3 500

individuals each. The small sub-populations, reared in tubes, resulted from 4 pairs of parental individuals randomly selected.

The large populations, as well as the small ones, were pursued for 29 successive generations. We found out that in large populations the allele frequency remained nearly constant, showing a typical Mendelian 3:1 ratio (3/4 gray body to 1/3 black). The control of gene frequencies was tested every two weeks, adding 8 tubes with fresh medium to the fly room. On this fresh medium, females led eggs for 24 hours. The resulting offsprings were afterwards counted (figs. 2, 3 and 4).

In the subpopulations (figs. 3 and 4), we found out that, starting with generation F_2, more than half do not show the typical Mendelian ratio, the initial allele frequencies, respectively ($p = q = 0,5$).

We noticed that in F_7 only 6 subpopulations showed the Mendelian ratio 3;1, while two were totally homozygous for the allele **e.**

Finally, in F_{29} eleven subpopulations were totally homozygotes for the recessive allele ebony (**ee**), and only three had heterozygotes, but with low frequency.

This means that chance alone causes the fast differentiation of these small populations, each starting with 4 females mated randomly with 4 males, always from the former generation. Consequently, the probability that an allele fixes is the result of a speedy chance process.

In conclusion, when populations are small, genetic drift can cause the frequencies of alleles to wander erratically, so that some of them might accidentally fall to very low frequencies, or even disappear altogether from the population. The process depends on the population size, the initial allele frequencies, as well as the number of successive generations.

Beside genetic drift, natural selection also acts on populations, with different intensities at several loci. Natural selection is unlikely to be consistent in its action, certain genes being sometimes selected, other times not.

Seldom genetic drift and natural selection have similar actions. Initially, population geneticists (Gulick, 1905; Wright 1922, 1931, 1948) considered that phenotypic polymorphism of the color and banding pattern of shells from snails (*Cepaea nemoralis, Cepaea hortensis, Partula sp*., *Achantinella sp*.) was the result only of genetic drift. Later on, geneticists agreed that natural selection also plays an important role in maintaining the coloration polymorphism of snails living in different habitats (Cain and Sheppard, 1952, 1954; Cain *et al*., 1960; Lamotte, 1959).

The different patterns and colors of shells of *Cepaea nemoralis*, which vary largely from hues of yellow to pink and brown, are the result of genetic differences among the individuals due to several alleles, placed in the same locus. The interallelic relations express through the domination of the brown shells against the others, as well as that of the pink shells against the yellow ones. The bands of the shells vary from none to six.

The snails with yellow shells are better camouflaged against predators in grasslands, while those with brown shells in litter and on dark soils. The banded shells are better camouflaged in heterogeneous surroundings with light and

shadows, while the unbanded shells in uniform ones. It is worthy to mention that selection pressure changes form one season to another. Thus, in early spring the dominant color in forests is given by litter and soil. Now, the predators (especially thrushes) feed on snails with yellow shells, the brown ones being better protected.

In some habitats, selection acts on different combinations of shell colors and bandations. For example, in beech forests, most individuals of *Cepaea nemoralis* have brown shells, unbanded. The yellow unbanded snails, survive better during cold and humid periods.

Of great interest in population genetics was the implication of genetic drift in human populations. The studies started from genes considered neutral for selection, like the genes that determine the ABO and MN blood groups. The variability of both blood groups varies largely in different human populations. For example, the Amerindian populations from South America were isogenous for group O (before the arrival of European colonists). If in the Blood and Blackfeet tribes from the northern part of the United States the frequency of the A blood group is 80%, in the Eskimo who live on the Thule Island (Greenland) it is only 9%. Similar differences can be observed also regarding the frequency of B blood group, the frequency varying between 6 and 30%. Initially, the high genetic frequency of human blood groups was assigned to the random genetic drift or to the founder effect (which will be discussed later).

Modern researches reveal that the alleles of human blood groups are also submitted to natural selection. Thus, there were found antigenic differences which cause more frequently premature abortions in mothers with O blood group, as compared with mothers with A or B, and especially with AB blood group.

Several diseases are more frequent in humans with certain blood groups. For instance, gastric and duodenal ulcer is more frequent in humans with O blood group, instead they are more resistant to plague.

Stomach and uterus cancer, pernicious anemia and diabetes mellitus are more frequent in people with the A blood group. Other studies revealed that diseases, as smallpox and syphilis, played a selective role in the dispersal of human blood groups (Vogel and Motulsky, 1986).

From the above mentioned examples, one may conclude that genetic drift and natural selection have sometimes complementary effects, difficult to be distinguished.

In large, better adapted populations, the evolutive role of random genetic drift is small, even neglectful. However, in small and isolated populations, significant genetic drift occurs, so that the genetic pools become more unstable (Coman, 1977, 2003).

The founder effect

The founder effect or the effect of colonization, namely the establishment of a new population starting from few individuals, may result (on account of the sampling errors) in deviations of gene frequencies from those of the parental

population. If the new population is successful, random drift continues to affect the frequency of alleles in the gene pool.

The founder effect accounts probably for the isogeny of the Amerindians from South America, also. The human populations from the Far East, from which the Amerindians are supposed to derive, have all the human blood groups, but the O group is rare. The ancestors who migrated to the American continents, through the Bering Strait, arrived in very small numbers, so that either the founder effect, or the random genetic drift are probably responsible for the isogeny of O blood group in South America, or the prevalence of A group in Blood and Blackfeet populations from North America.

While the inheritance of human blood groups is only a supposition, the change of allele frequencies in dunker populations from Pennsylvania could be followed during their evolution.

This population was founded at the beginning of the 19th century by 27 families, who migrated from Renania (Germany) and colonized Pennsylvania (United States). Isolated from other populations through religious barriers, the dunkers might have kept their blood group frequencies similar to those from Renania, for instance 40 % A group. However, the frequency of A group is around 60 %, meanwhile the B group is nearly extinct. The frequency of MN blood groups was 65 % in dunkers, but only 54 % in Renania (Glass, 1954; Glass *et al*., 1952). The founder effect probably accounts also for other alterations of the allele frequencies in different human populations (Spiess, 1977; Laake *et al*., 1998; Vanschothorst *et al*., 1998).

In conclusion, when few individuals from a large population colonize a new habitat and found another population, this new population will preserve, depending on the number of founders, a certain allele frequency of the initial one. For instance, if more that 10 individuals leave a large population, the new founded population will preserve more that 90 % from the initial variability (fig. 5) (Primack, 1993).

Studies regarding the origin of cultivated maize (*Zea mays mays*) reveal that it comes from an ancestral wild population (*Zea mays parviglumis*), which lived in the north of Mexico 9000 years ago. Starting with 20 founders, it is assumed that the population was bottlenecked for 10 generations (Fedoroff, 2003).

The smaller the number of founders, the smaller the genetic variability of the new founded population, as compared to the source one. Consequently, the new population will have a distinct gene pool, with different allele frequencies from those of the source population.

In a small new founded population, genetic drift can cause the frequencies of alleles to wander erratically. Some rare alleles might accidentally fall to very low frequencies, or even disappear altogether from the population, while others become fixed at random in the population.

Migration

As we have already mentioned, the loss of genetic variation due to genetic drift can also be prevented by migration. Generally, migration will enrich the gene pool of the host population with new alleles, which form new combinations with the local ones. Thus, migration will generally modify the genetic variation in a population which has received immigrants.

The effects of migration on host population differentiation will depend on both the number of immigrants and the difference between the gene pools of those populations. If immigrants have similar gene frequencies with that of the host population, the gene flow will not be efficient, so that it will not significantly modify the genetic variability of the latter. On the contrary, if there are great differences in genetic pools and gene frequencies, then the host population will display a higher genetic variability. If the new genes are suitable for the host population, they will form a pattern of co-adapted genes and the population will became better adapted. If not, the selection pressure might lead the host population to extinction.

In order to support this statement let us remember the undesirable consequences of the deliberate introduction of some individuals of mountain goats from Turkey and Sinai into a population of *Capra ibex* from the Tatra Mountains. Because of overhunting, the population of *Capra ibex* nearly disappeared in the Tatra Mountains. It was assumed that breeding between individuals will re-establish the population of goats from the Tatra Mountains. But it didn't happen, because the goats from Turkey and Sinai were adapted to a much drier and warmer climate, so that the hybrids that were born in Tatra Mountains during winter, namely in February, could not survive (Greig,1979; Templeton, 1997).

Not always was this the case. Thus, the population of the prairie chicken (*Tympanuchus cupido pinnatus*) rebounded after breeding with individuals of other populations from thousands of kilometers away (Westemeier *et al.*., 1998).

In different environmental conditions, natural selection re-shapes the pattern of the co-adapted genes of immigrants and of the host population, during successive generations. Such interactions might lead to geographic variations in an individual trait along latitude, altitude, temperature gradients, air humidity etc. For example, the average body size of many species of vertebrates increases gradually with decreasing temperature, in other words with increasing latitude (Bergman's rule); similarly, the body extremities, like ears, limbs and tails appear to be smaller in individuals living in cold regions, than in warm ones (Allen' rule).

As Mayr (1963) often asserted, migration prevents usually the action of natural selection, implicitly slowing down the speed of evolution. This assertion is probed by the evolution of subpopulations of the common water snake – *Nerodia* (*Natrix*) *sipedon*, living on islands in Lake Erie, United States. Camin and Ehrlich (1958) stated that on mainland the large population of water snakes comprises individuals with striped coloration. On the contrary, on the islands, the frequency

of striped individuals is lowering from young ones to adults. It is obvious that the unstriped individuals are better adapted to islands, where streams are missing and individuals have to cross the sandy beaches to reach the water sources. Moreover, the striped individuals are easier hunted by predators, especially sea birds. Later on, the mark-recapture methods confirmed the conclusions of Camin and Ehrlich (1958), the camouflage of unstriped forms being efficient especially during the first year of life (the frequency of unstriped snakes in the island population remained about 46 %) (King, 1993; King and Lawson, 1995).

If gene flow is common between populations of the same species, it becomes rare between populations of different species. Considering species as "lineage restricted colony" ("*colonie izolată de consângeni"*) {Racovitza, 1912) it is obvious that there is normally a gene flow between populations, which undoubtedly become an important mechanism of evolutionary change in populations.

Speciation is a long dynamic process. In some species "*in statu nascendi*" the reproductive barriers are weak, such as the ecological ones, so that they cannot prevent inbreeding. In such situations, individuals from different species may breed, but hybrids are not viable or have greatly reduced viability. In this latter case, hybrids produce gametes that successfully combine with the gametes of one of the parental species, and the offspring resembles the parental species with which it mated. Suppose that the hybrid resulted from two parental species A and B, mated with species B; then, during the next generations, the hybrids became so similar to species B, that they were practically included in it. The process was called *genetic introgression*, and the species which received new genes *introgressed species*.

Ellstand (1992) describes species of rare plants which living in the nearness of a common related species could be fertilized by these latter ones. The seedlings, sterile or with reduced fertility, cross usually with the common species, bringing new rare alleles in their gene pools.

Inbreeding

Inbreeding is another aspect of small populations. The probability that related individuals mate is higher in smaller populations. If such population keeps its size over successive generations, then inbreeding gets higher.

The consequences of inbreeding on population structure are the increase in homozygosity and decrease in heterozygosity. Assuming that this population turns suddenly from outbreeding to inbreeding, during some generations individuals might become homozygous for all loci. This theoretical assumption is close to reality. It is well known that, if it is rainy and cold during corn blooming, than self-pollination prevails over cross-pollination, since the wind cannot spread the wet pollen.

In small populations inbreeding greatly increases the frequency of rare deleterious genes preserved in the genetic load, decreases the adaptability of the population, increasing also the selection pressure (Barrett and Kohn, 1991).

The genetic load consists of recessive deleterious genes, representing less than 1 % of the population gene pool. When environmental conditions change, some genes of the genetic load may become advantageous, so that their frequency will rise in the population. This was the case of the genes for melanic forms. Industrial melanism is the phenomenon in which black or blackish forms of different species of moths and other organisms (*Biston betularia*, *Mamestra brassicae*, *Pyrausta nubilalis* etc) have come to dominate populations in industrial areas. The genes responsible for the melanic forms appeared accidentally in population through mutations, hundreds or even thousands of generations ago. These genes were preserved with low frequency in the genetic load. In polluted industrial environments, such genes became favorable, the melanic forms being camouflaged against predators. During some generations, the gene frequency for melanic forms increased significantly. The same also happened with mutations providing in some a greater tolerance to lead. When leaded gasoline was used as a fuel for internal combustion engines, these phenotypes became more frequent along the highways (Antonovics, 1976).

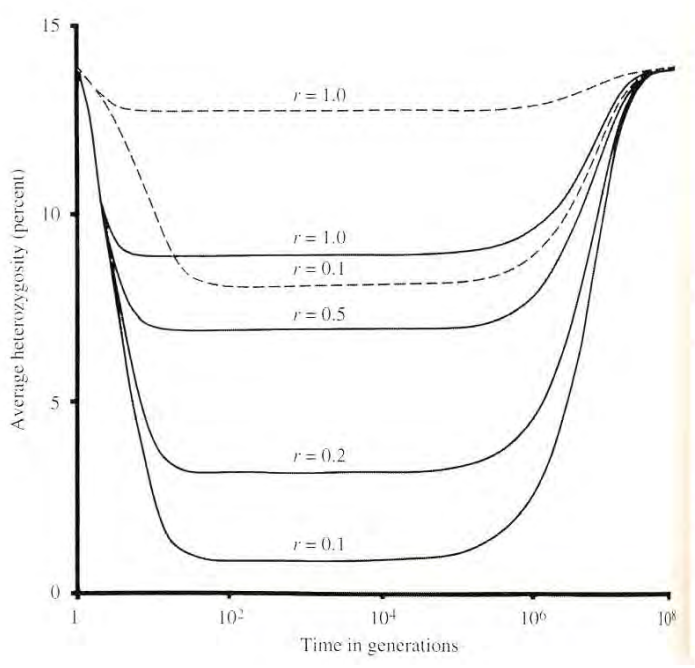


Fig. 1. Possible effects of genetic bottleneck in a population of two and ten individuals with low (r = 0,1), respectively high (r = 1,0) growth rate
(according to Primack, 1993; Halliburton, 2004)

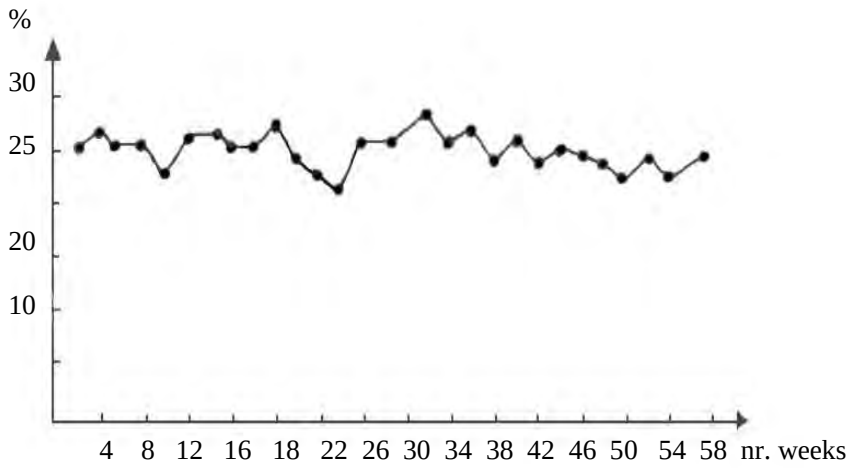


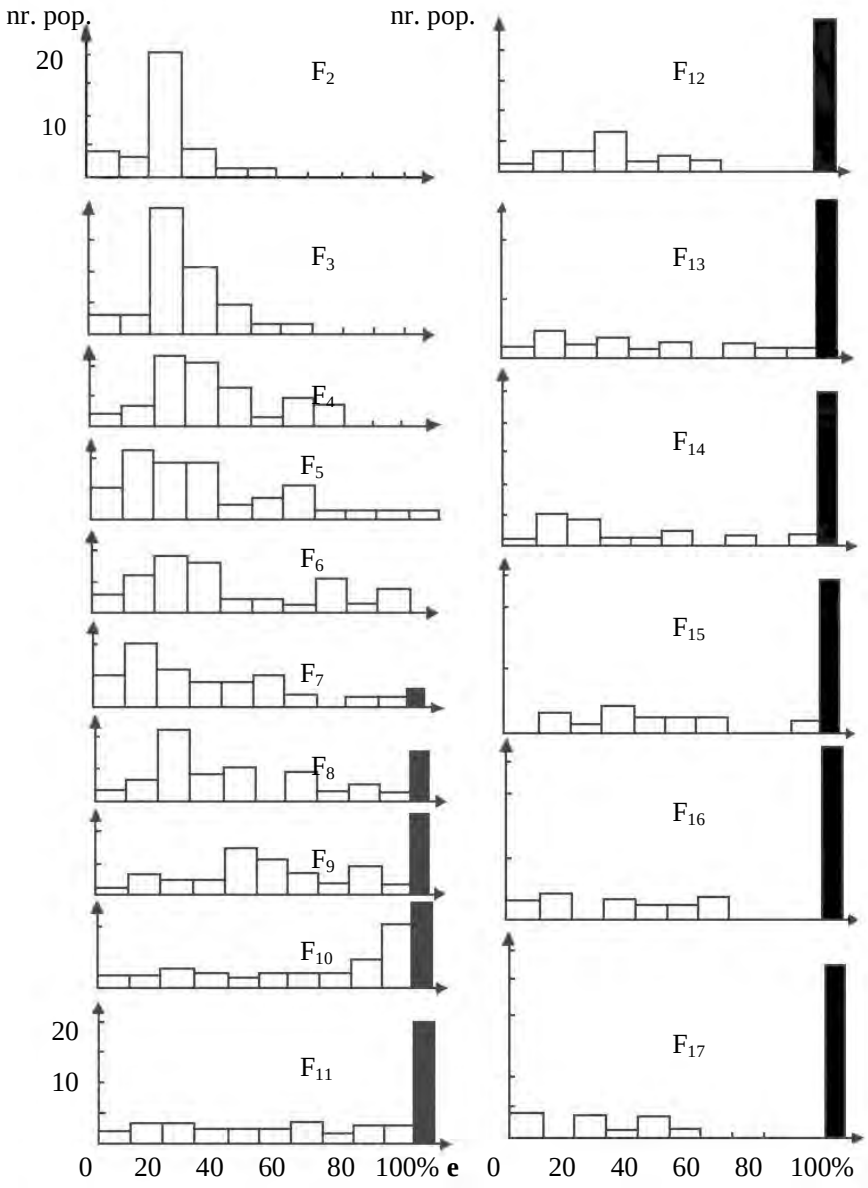
Fig.2. The frequency of allele e in three fly rooms, during 29 successive generations (during the whole experiment the population size remained constant at about 3 500 in each fly room)

Comparing the genetic load with a load, a burden which species are obliged to carry for many generations, Dobzhansky asserted that it is "the price which species pay to evolution (cited by Coman, 2003).

In small populations, with a high inbreeding degree, gene frequencies will vary greatly, together with the enhancement of selection pressures, leading to a weaker expression of characters, including quantitative ones.

The classical example is that of the inbred lines of maize, whose productions are 5-10 times lower as compared with the parental ones (Coman, 2003). In animal species, mating between relatives, namely parents x offspring, brother x sister, is also followed by inbreeding depression, characterized by a lower number of descendents, a higher degree of sterility and abnormalities during development (Coman and Wallace, 1973). Inbreeding depression was recorded in isolated human populations, too (Coman and Mixici, 1980).

In conclusion, mechanisms that work in small populations may cause fast, sometimes drastic changes in gene pools, followed by modifications of selection pressures and, implicitly, by evolution.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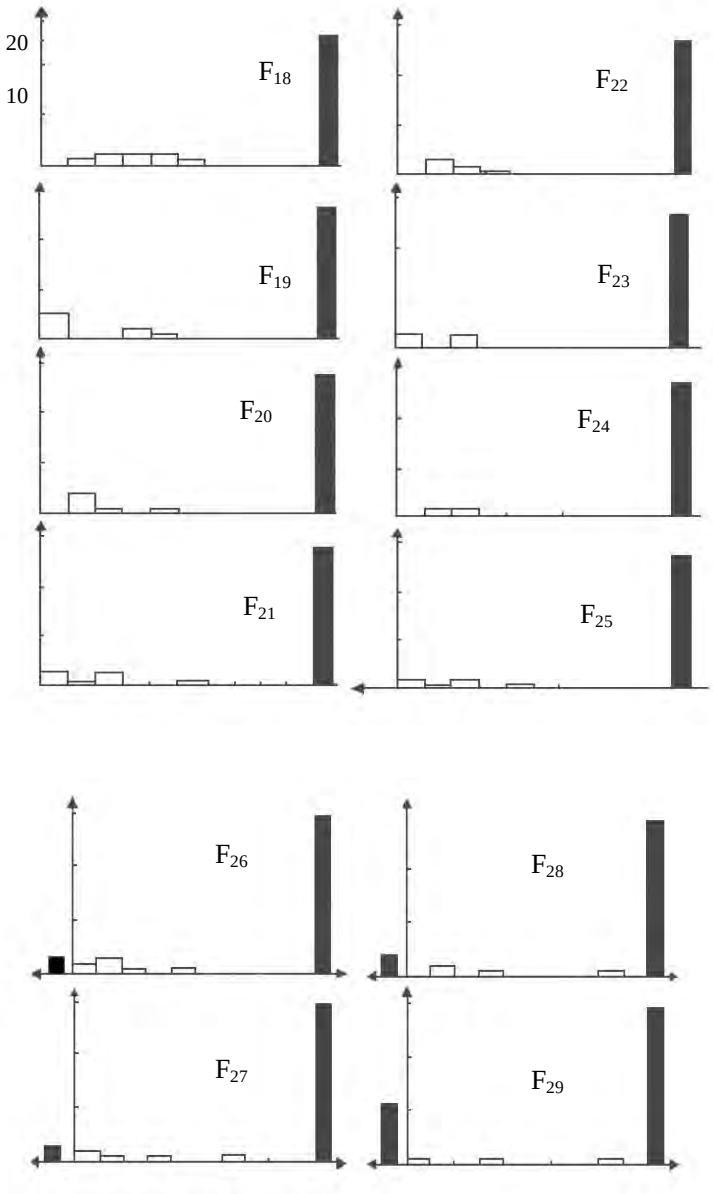

Fig. 4. The frequency of flies with black body (ee) in the 42 subpopulations, during $F_{18} - F_{29}$.

REFERENCES

Allendorf, F.W. (1997) Geneticaly effective sizes of grizzly bear populations. In *Principles of Conservation Biology*, Meffe, C.G., Carroll, C.R. (eds), 2nd ed., Sinauer Assoc. Inc., Sunderland, MA, pp 174-175.

Allendorf, F.W., Leary, R.F. (1986) Heterozygosity and fitness in natural populations of animals. In: *Conservation Biology*: *the Science of Scarcity and Diversity*, Soulé, M.E. (ed.), Sinauer Assoc. Inc., Sunderland, MA, pp 57-76.

Allendorf, F.W., Servheen, C. (1986) Genetics and conservation of grizzly bears, *Trends Ecol. Evol.*, **1**, pp 88-89.

Antonovics, J. (1976) The nature of limits to natural selection, *Ann. Missouri Botan. Garden*, **63**, 224- 247.

Barrett, S.C.H., Kohn, J.R. (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Genetics and Conservation of Rare Plants, Falk, D.A., Holsinger, K.E. (eds),. Oxford Univ. Press, NY, USA, pp 3-30.

Beheregaray, L.B., Ciofi, C., Geist, D., Gibbs, J.P., Caccone, A., Powell, J.R. (2003) Genes record a prehistoric volcano eruption in the Galápagos, *Science*, **302**, 75.

Berger, J. (1990) Persistence of different-sized populations: An empirical assessment of rapid extinctions in bighorn sheep, *Conserv. Biol.*, **4**, 91-98.

Berger, J. (1999) Intervention and persistence in small populations of bighorn sheep, *Conserv. Biol.*, **13**, 432-435.

Billington, H.L. (1991) Effect of population size on genetic variation in a dioecious conifer, *Conserv. Biol.*, **5**, 115-119.

Cain, A.J., Sheppard, P.M. (1952) The effects of natural selection on body color in the land snail *Cepaea nemoralis* (L.), *Heredity* **6**, 617-631.

Cain, A.J., Sheppard, P.M. (1954) Natural selection in *Cepaea*, *Genetics* **39**, 89-116.

Cain, A.J., King, J.M.B., Sheppard, P.M. (1960) New data on the genetics of polymorphism in the snail *Cepaea nemoralis* L., *Genetics*, **45**, 393-411.

Camin, H.J., Ehrlich, P.R. (1958) – Natural selection in water snakes *(Natrix sipedon* L.) on islands in Lake Erie, *Evolution*, **12**, 504-511.

Chitty, D., Phipps, E. (1961) – A declining vole population, *J. Anim. Ecol.* **30**, 490.

Coman, N. (1977) *Genetica populațiilor*, Univ. "Babeş-Bolyai", Cluj-Napoca.

Coman, N. (2003) *Genetică*, vol. I şi II, Presa Univ. Clujeană, Cluj-Napoca.

Coman, N., Gheorghiță, B. (1988) Un modéle expérimental concernant l'effect de la dérive génétique sur quelques populations de *Drosophila melanogaster*, *Evolution et Adaptation*, **III**, 229-238.

Coman, N., Mixici, F. (1980) Efectele consangvinizării într-o populație izolată. *Studia Univ. Babeş-Bolyai*, *Biologia*, **25**, 59-65.

Coman, N., Wallace, B. (1973) Inbreeding depression and environmental stress, *Genetika*, **2**, 157-166.

Dinerstein, E., McCracken, G.E. (1990) Endangered greater one-horned rhinoceros carry high levels of genetic variation, *Conserv. Biol.*, **4**, 417-422.

Dobzhansky, Th., Pavlovsky, O. (1957) An experimental study of interaction between genetic drift and natural selection, *Evolution*, **11**, 311-319.

Dordea, M., Coman, N. (2005) *Ecologie umană*, Casa Cărții de Știință, Cluj-Napoca.

Ellstand, N.C. (1992) Gene flow by pollen: implications for plant conservation genetics, *Oikos*, **63**, 76-86.

Fedoroff, N.V. (2003) Prehistoric GM corn, *Science*, **302**, 1158-1159.

Ford, E.B. (1964) *Ecological Genetics*, Methuen and Co., London, UK.

Glass, B. (1954) Genetic changes in human populations, especially those due to gene flow and genetic drift, *Adv. Genet*., **6**, 95-139.

Glass, B., Sachs, M.S., Jahn, E.F., Hess, C. (1952) Genetic drift in a religious isolate: an analysis of the causes of variation in blood group and other gene frequencies in small population, *Amer. Nat*., **86**, 145-160.

Greig, J.C. (1979) Principles of genetic variation to wildlife management in Southern Africa, *Afr. Tydskr. Naturnav*, **9**, 57-78.

Gulick, J.T. (1905) Evolution and habitudinal, controlled by segregation, *Publ. Carnegie Inst.*, **25**, NY, pp 1-269.

Halliburton, R. (2004) *Introduction to Population Genetics*, Pearson Prentice Hall, Upper Saddle River, NJ, USA.

Kaiser, J. (1999) Study sounds on Yellowstone grizzlies, *Science*, **284**, 5414-5468.

King, J.L. (1993) Color pattern in Lake Erie water snakes: Prediction and measurement of natural selection, *Evolution*, **47**, 1819-1833.

King, R.B., Lawson, R. (1995) Color pattern variation in Lake Erie water snakes: The role of gene flow, *Evolution*, **49**, 885-896.

Laake, K., Telatar, M.N., Geitvik, G.A., Hansen, R.O., Heilberg, A., Andersen, A.M, Gatti, R., Borresendale, L. (1998) Identical mutation in 55% of the ATM alleles in 11 Norvegian AT families: Evidence for founder effect, *Eur. J. Genet*., **6**, 235-244.

Lacy, R.C. (1992) The effects of inbreeding on isolated populations: are minimum viable population size predictable? In: *Conservation Ecology*, Fiedler, P.L., Jain, S.K. (eds), Chapman and Hall, NY, London, pp 277-296.

Lamotte, M. (1959) Polymorphism of natural populations of *Cepaea nemoralis* L. *Cold Spring Harbor Symp. Quant. Biol*., **24**, 65-86.

Lesica, P., Leary, R.F., Allendorf, F.W., Bilderback, D.E. (1988) Lack of genetic diversity within and among populations of an endangered plant, *Howellia aquatilis*, *Conserv. Biol*., **2**, 275-282.

Mayr, E. (1963) *Animal species and evolution*, Harvard Univ. Press, Cambridge, MA.

McCommas, S.A., Bryant, E.H. (1990) Loss the electrophoretic variation in serially bottlenecked populations, *Heredity*, **64**, 315-321.

Nei, M., Graur, D (1984) Extend of protein polymorphism and the neutral mutation theory, *Evol. Biol.* **17**, 73-118.

Nei, M., Maruyama, T., Chakraborty. (1975) The bottleneck effect and genetic variability in populations, *Evolution*, **29**, 1-10.

O'Brien, S.J., Wild, D.E., Goldman, D., Merill, C.R., Bush, M. (1983) The cheetah is depauperate in genetic variation, *Science*, **221**, 1459-1462.

O'Brien, S.J., Roelke, M.E., Marker, L., Newmann, A., Winkler, C.A., Melzer, D., Colley, L., Evermann, J.F., Bush, M., Wild, D.E. (1985) Genetic basis for species vulnerability in the cheetah, *Science*, **227**, 1428- 1434.

O'Brien, S.J., Evermann, J.F. (1988) Interactive influence of infectious disease. and genetic diversity in natural populations, *Trends Ecol. Evol*., **3**, 254-259.

Parker, C., Gilbert, D.A., Pusey, A.E., O'Brien, S.J. (1991) A molecular genetic analysis of kinship and cooperation in African lions, *Nature*, **351**, 562-565.

Peres, C.A., Baider, C., Zuidema, P.A., Wadt, L.H., Kainer, K.A., Gomes-Silva, D.A., Salomão, R.P. (2003) Demographic threats to the sustainability of Brazil nut exploitation, *Science*, **302**, 2112-2114.

Primack, R.B. (1993) *Essentials of Conservation Biology*, 2nd Ed., Sinauer Assoc. Inc., Sunderland, MA,.

Racovitza, E. (1912) Cirolanides (1-re série), Biospeologica XXVII. *Arch. Zool. Exper.Géner*., V^{e} série **10**, 203-329.

Shaffer, M.L. (1981) Minimum population sizes for species conservation, *Bioscience*, **31**(2), 131-134.

Shaffer, M.L. (1983) Determining minimum viable population sizes for the grizzly bear, *Intl. Conf. Bear Res. Manage*, **5**, 133-139.

Shaffer, M.L., Samson, F.B. (1985) Population size and extinction: A note on determining critical population sizes, *Amer. Nat*., **125**, 144-152.

Spiess, E.B. (1977) *Genes in population*, John Wiley & Sons, NY, USA.

Templeton, A.R. (1997) Coadaptation, local adaptation, and outbreeding depression. In: *Principles of Conservation Biology*, Meffe, C.G., Carroll, C.R. (eds), 2nd ed., Sinauer Assoc. Inc., Sunderland, MA, pp 171-172.

Vanschothorst, E.M., Jansen, J.C., Grooters, E., Prins, D.E.M., Wiersinga, A.G.L., Vanommen, G.J.B., Deville, P., Cornelisse, C.J. (1998) Founder effect at PGL1 in hereditary head and neck, *Amer. J. Genet*., **63**, 468-473.

Vogel, F., Motulsky, A.G. (1986) *Human genetics, problems and approaches*. 2nd ed., Springer- Verlag, Berlin.

Westmeier, R.L., Brawn, J.D., Simpson, S.A., Esker, T.L., Jansen, R.W., Walk, J.W., Kersher, E.L., Bouzat, J.L., Paige, C.N. (1998) Tracking the long-term decline and recovery of an isolated population, *Science*, **282**, 1695-1698.

Wright, S. (1922) Coefficients of inbreeding and relationship, *Amer. Nat*., **56**, 330-338.

Wright, S. (1931) Evolution in Mendelian populations, *Genetics*, **16**, 97-159.

Wright, S. (1948) On the roles of directed and random changes in gene frequency in the genetic populations, *Evolution*, **2**, 279-294.

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ON THE OBSERVATIONAL MODEL OF GENOMICS

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SUMMARY. Discussions on biological knowledge, in terms of objects, concepts and rules, suggest a need for mathematical models and novel methodologies to contribute to the conceptual or theoretical framework in studying the dynamics of organisms. Through this paper, the System theory and Factor-Space theory are implied to design and formulate the "Observational Model" of Genomics as a mathematical model based on Fuzzy Relation. In this mode uncertainty is incorporated in formalizing the biological concepts and facts to provide a relational description of dynamics on genomic systems and metabolic systems.

KEYWORDS: observational model; system theory; genomic, factor-space; fuzzy system

Introduction

It seems an ever-increasing move toward inter- and trans-disciplinary attacks upon problems in the life-sciences and new mathematical models in biology, physiology, psychology and even cognitive science (Ashby, 1999) are emerging. In this new order, system theory has a central role to play, which is to first of all understand ways and means of how to encode the natural world into suitable systematic structures. System theory (Klir, 1991) is not a collection of facts but rather a way of thinking and the modeling process itself may be more important than the obtained model. It is concerned with the study of organization and behavior per se. In order to study genomics through a system theoretic approach, a mathematical model is required to organize disparate information into a coherent whole (Bailey, 1999). Then this model is used for a logical analysis of interactions and dynamics also for decision making i.e., prediction, classification and control (Klir and Yuan, 1996).

Genomics is the field of biological research taking us from the DNA sequence of a gene to the structure of the product for which it codes (usually a protein) and continues with the activity of that protein and its function within a cell, a tissue and, ultimately, an organism. The two central questions in genomics are (Paton, 2000):

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- What do genes do?
- How do genes interact?

A typical system theoretic approach to the questions above is to

- **Cluster** genes with known biological function according to similarity in pattern of **expression** (D'Haseleer *et al.*, 1999).
- **Classify** genes with unknown function according to their similarity to the prototypes obtained from the clustering (Brown *et al.*, 2000).
- **Identify** the parameters of a gene-network (dynamic) model using the cluster prototypes obtained previously (Eisen *et al.*, 1998).

However, in the process of compiling the theoretical approaches to the practical methods, we face the following challenges:

- Very large number of variables (thousands of genes).
- Very small number of measurements (say between 8 and 18).
  - Repeated experiments are not usually available.
  - Data are often unreliable, missing, noisy or imprecise.
- Data are collected from a dynamic process under "closed-loop control".
- The processes usually are non-linear and time-variant.
- Information fusion of transcriptome and proteome data is non-trivial.

The first two items lead to the so-called **dimensionality problem** (Zweiger, 1999). To this data, the majority of bioinformatics techniques have been concerned with the assembly, storage, and retrieval of biological information, with data analysis concentrated on sequence comparison and structure prediction. The move to functional genomics demands that both sequence and experimental data are analyzed in ways that permit the generation of novel perspectives on gene and/or protein action and interaction. An approach to this problem is the construction of proper formal mathematical, parametric models that are identified from the data. As the focus in genomics is shifting from molecular characterization to understanding functional activity, system theory is going to play an increasingly important role in providing biologists with better tools to extract information from data, as well as supporting new ways of thinking to characterize molecular systems in a general way, and quite independently of their physical and chemical constitution.

This paper is organized as follows. First, the need for incorporating uncertainty in formalizing biological concepts and facts is introduced as a motivation for this work. Then the sketch of the model is designed by introducing the terminologies and interpreting the main tools. Next the modeling methodology is presented in terms of objects, concepts and rules. Also the mathematical formalization of these concepts and rules is represented next. The last section is devoted to the conclusions of this work.

Motivation

The relevance, applicability and importance of fuzzy set theory and fuzzy logic (Klir and Yuan, 1996) is generally linked to successful applications in the domain of engineering, especially where subjective notions have to be modeled and matched with abstract data structures. The purpose of this paper is to outline the conceptual foundations of a framework, based on the mathematics of fuzzy sets, which can be successfully employed to model some of the most complex phenomena in molecular biology (Edward and Palsson, 1998).

Our formal model describes a genome as a collection of genes. This set is equipped with a mathematical structure for logical inference. To allow reasoning in the presence of uncertainty, we need to formalize biological concepts and facts associated with these. Relationships between concepts and factors are expressed in terms of rules. Though the proposed mathematical language is 'in principle' complete, as accurate as biological knowledge, a working methodology realistically is confined to specific aspects of a natural system. This *uncertainty principle* between the generality and predictive power of a model was summarized by Lotfi Zadeh (Li, 1995): *"As the complexity of a system increases, our ability to make precise and yet significant statements about its behaviors diminishes until a threshold is reached beyond which precision and significance (or relevance) become almost exclusive characteristics."*

Designing the model

Microarray technology provides us with gene expression measurements on the transcriptome level. A typical experiment can provide measurements of the expression level of thousands of genes over a number of experimental conditions or over time. Considering a time-series of *n* samples, we can represent the observation of an individual signal (gene $u \in U$) as a point (Fig. 1) in the *n*-dimensional observation-space $X(f)$. Points that form a cluster have similar expression profiles and are subsequently postulated to have related biological function (Wang, 1990, Li *et al.*, 1995).

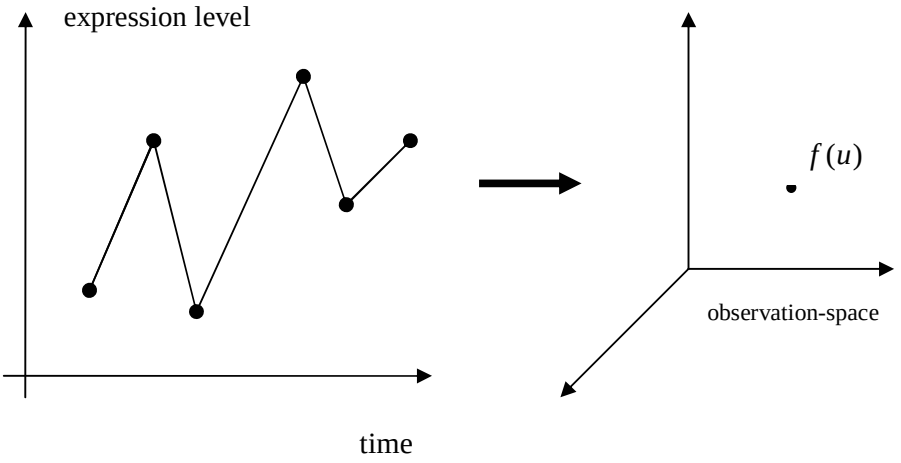


Fig. 1. From time-series to observation or factor-space representation

Here the factor f denotes measurements on the transcriptome level. For a more complete picture of gene expression additional factors, for instance describing measurements on the proteome level, are introduced. A phenomena investigated refers to a specific biological concept *C* which we aim to characterize with the factors defined in (Magee, 1997). The extension of concept *C* in *U* is then the fuzzy mapping $\hat{A}$:

$$\hat{A}: U \to [0,1]$$

$$u \mapsto \hat{A}(u),$$

where $\hat{A}(u)$ is the degree of relevance of *u* with respect to *C* or $\hat{A}$. When $\hat{A}(u) = 1$, *u* definitely accords with *C*, and for $\hat{A}(u) = 0$, *u* does not belong to $\hat{A}$ (a fuzzy attribute of *C*, i.e., the function / expression of gene in a specified context).

Clustering the points in the observation space $X(f)$, using partitional techniques such as the fuzzy-*c*-means algorithm (Wolkenhauer, 1998), we are grouping genes (represented by measurements, i.e., points $f(u)$ in *X*) in order to infer the mapping $\hat{A}$ in *U*. Note that what we observe is a fuzzy set $\hat{B}$ on $X(f)$ (partition of *X*) and it is necessary to establish a relation between the 'model' $\hat{A}$ on *U* and the experimental evidence in $X(f)$. The fuzzy relational framework is

intended to be a *theoretical* construct to complement *experimental* biology. The described *biological principle* is a conditional statement of the form:

$$IF\ \ f(u)\ \ is\ \ \hat{B}\ \ \text{then}\ C\ is\ \ \hat{A}.$$

Let us have a closer look at the formal system described here. In Magee (1997), a factor is defined as a mapping from a set of *abstract objects* $u \in U$ to space *X*. Here *u* denotes a gene, defined as a *conceptual entity* which exists apart from any specific encoding; it is that part of the natural system we wish to encode. In this case, *u* is an **abstract state** of the natural system under consideration. Factor *f* evaluates the genes *u* in an experiment, leading to a numerical representation $x \in X(f)$. We note that any specific act of observation, experiment, is therefore at the same time an act of *abstraction*; theory and experiment are complementary and cannot be separated.

In our scenario, illustrated in Fig. 1, factor $f : U \to X(f)$ is a mapping from the set *U* of abstract states into an element of $X(f)$ which here is a point in the plane $R \times R$ of real numbers. Given any mapping between sets, the mapping *f* induces an **equivalence relation** E_f on its domain, by saying that $E_f(u_1, u_2)$ holds if and only if $f(u_1) = f(u_2)$. Therefore to say that the two genes u_1 and u_2 are related means that both produce the same 'effect' (observation) in our experiment.

If we form the quotient set U / E_f, we find that it is in one to one correspondence with the set of all possible values *f* can assume. This set, called *spectrum*, is denoted $f(U)$. If *x* is a point in $f(U) \subset X(f)$, we associate with *x* the entire equivalence class $f^{-1}(x)$. This means in effect that we can discuss the properties of our model (determined by an appropriate choice of factors *f*), in terms of the equivalence classes on *U*.

We identify an (fuzzy) equivalence class $\hat{A}$ in *U* as a cluster of points in $X(f)$. Genes in *U* are grouped according to their similarity in expression profiles and hence allow us to predict their biological function. If we are to decide upon the similarity of two gene expression profiles by using the inequality $\|f(u_1) - f(u_2)\| \le \varepsilon$ in the observation space, the inequality describes a subset (relation) $R_\varepsilon \subset U \times U$,

$$R_\varepsilon = \{(u_1, u_2) \in U \times U : \|f(u_2) - f(u_2)\| \le \varepsilon\}$$

This relation is not an equivalence relation. We can define a mapping $\hat{E}_\varepsilon$ such that $\hat{E}_\varepsilon(u_1, u_2)$ is greater than $1 - \varepsilon$ if and only if u_1 and u_2 are indistinguishable with respect to the tolerance ε:

$$(u_1, u_2) \in R_\varepsilon \text{ if and only if } \hat{E}_\varepsilon \geq 1 - \varepsilon,$$

where

$\hat{E}_\varepsilon : U \times U \to [0,1]$

$(u_1, u_2) \mapsto 1 - \inf \{\varepsilon \in [0,1] : (u_1, u_2) \in R_\varepsilon\}$

with $\varepsilon \in [0,1]$ and if there is no ε for which the relation holds, we define $\inf \phi = 1$. $\hat{E}_\varepsilon$ is then a **fuzzy equivalence relation**, also referred to as a *similarity relation*. The value $\hat{E}_\varepsilon(u,u) = 1 - \min\{|f(u) - f(u)|, 1\}$ describes the degree to which two objects u_1 and u_2 have similar observable consequences and transitivity of this relation implies that if u_1 and u_2 are similar and u_2 and u_3 are similar in their values in *X*, then u_1 is similar to u_3.

$$u_1 = u_2 \text{ and } u_2 = u_3 \Rightarrow u_1 = u_3$$

Fuzzy clustering algorithms return a matrix that specifies the degree of membership of any *u* in the clusters (equivalence classes). We have seen that the comparison of two real numbers with respect to an error bound ε induces fuzzy equivalence relations (a fuzzy set) and therefore suggests a fuzzy relational framework (H'ohle, 1996). They are however other reasons in support of a fuzzy mathematical approach. In many cases the evidence that we have a gene belong to a cluster will be a matter of degree and W.R.T. functional classes genes may belong to more than one class during an experiment (Bailey, 1998).

By writing $f(u)$, the impression is that f is fixed and u is variable. However, the role of the argument and the mapping are formally interchangeable; we can keep *u* fixed and change the experimental setup. In which case, *u* becomes a mapping, whose arguments are themselves mappings; $\bar{u}(f) = f(u)$. The question "why $f(u)$?" can now be answered by "because *u*" or "because $\bar{u}$" (Magee, 1997). Using fuzzy relations, the obtained formal system allows us to model casual entailment in natural systems (here gene regulatory network).

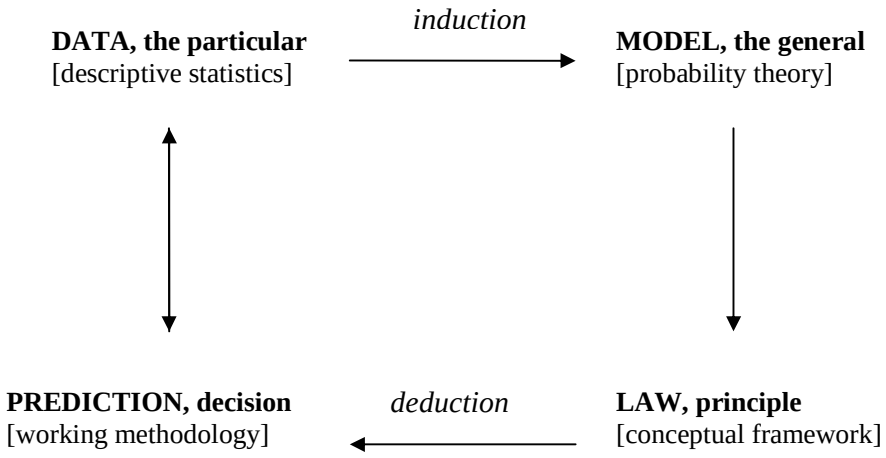


Fig. 2. The modeling process of a scientific investigation illustrating the difference of a conceptual framework and a working methodology

The modeling methodology

The dualism of probability theory and statistics is a useful analogy to illustrate the difference between a conceptual framework and a working methodology (Fig. 2). The motivation for observational modeling is to create a conceptual framework in which problems of genome analysis can be formulated in the way many problems in science and engineering are translated into probability theory, i.e. formulated by means of random variables (Lawvere and Schanuel, 1997). Once this 'translation' has taken place, and is accepted as a reasonable model for the experimental context, we can reason about data and make predictions about events that have not yet been validated experimentally.

Let the *description frame* of a genome be denoted by (U, ς, F), where $C \in \varsigma$ denotes a concept and $f \in F$ describes a characterization in terms of observable objects $u \in U$. We hereafter have two alternative cases (Kyrpides, 1999):

- For the study of a single gene, it is represented as a concept $C \in \varsigma$ while factors $f \in F$ describe different aspects of the expression or function of the gene;
- Studying large numbers of genes, for instance using microarray data, the context is denoted by *C* while the genes are the objects $u \in U$.

For instance in yeast, respiration or fermentation could be the context in which all genes are studied. The result could then be a grouping (clustering) of the genes into these functional classes. We may therefore consider a gene as both an

object or concept. Whichever situation is chosen it does not matter for the formal model. We may call a gene as a concept even if it is represented as an object in our formal model. We should also keep in mind that factors themselves are general in the sense that a factor should cover for a wide range of cases. For example, a factor may represent distances, positions, lengths, a gene's annotation (e.g. its membership in a functional class), expression levels (e.g. light intensity) or peptide masses. The form of the factors however has an effect on the formal model as we shall discuss further below.

The three ingredients (U, ς, F) compose our formal model which is then built from data in the following way. An *object u* is either measured or verbally characterized with respect to a certain *factor f*. For example, *u* may be an ORF and $f(u)$, the *state* (e.g. expression level) is a value in $X(f)$. $X(f)$ is referred to as the state space of factor *f*. The *relevance* of a *symptom* for a particular *phenomenon* is captured by a fuzzy set $\hat{B}$ in $X(f)$. The relevance of object $u \in U$ to the context or concept $C \in \varsigma$ is expressed by the fuzzy set $\hat{A}$ in *U*. In general, we do not know $A: U \to [0,1]$ a *priori*. The purpose of a model is to establish knowledge about $\hat{A}$, which describes a particular phenomenon, by means of observations $f(u)$ establishing symptom $\hat{B}$. The two-way relationship between the formalization of a genome and the modeling of a particular aspect of it is summarized in the Fig. 3.

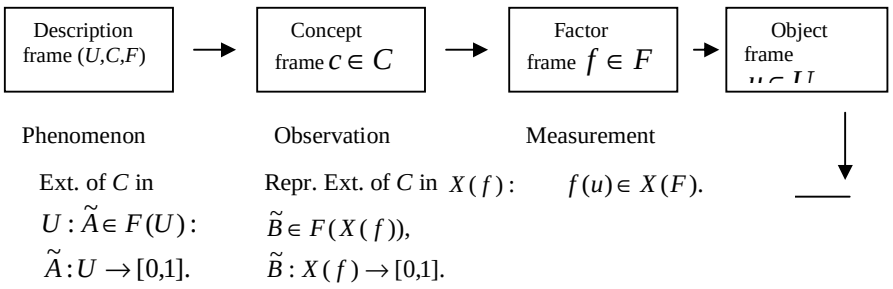


Fig. 3. The formal representation of a genome in terms of genes, factors and objects. The path following the framed boxes describes the key elements of the proposed conceptual framework, whereas the associated 'backward' path describes the working methodology representing gene expression and gene function from data.

In our model, knowledge is represented in terms of *concepts*, *objects*, and *rules.* In their mathematical representation the main tools are factor spaces and fuzzy maps. Factors are used to capture the characteristics of a concept, and fuzzy sets are used to describe the relationship (relevance, association, membership,...) of *objects* to a concept. The description of the essence and attributes of a concept by factors is known as *intension* while the aggregate of objects characterizing a concept is known as *extension*.

The modeling formalization

The formalization of a concept (conceptualization of a gene) is based two aspects: *intension* and *extension* (Wang, 1990). An extension of a concept *C* is an ordinary or fuzzy subset $\hat{A}$ of the objects universe *U*. These atomic data objects may for example be subsequences, ORFs, and so forth[1].

Intension is defined by the collection of factors and their attributes characterizing the concept. The classical definition of a set requires any genome subsequence to be either associated with the gene or not. In other words, given any open reading frame (ORF), for classical set theory we assign truth values 0 or 1 to define a *crisp* set. This application of the *law of the excluded middle* defines crisp sets on which we then build a bivalent logic. In many real world problems it is rather difficult to exactly decide whether an element has the property in question or not. In these cases where either the problem under consideration is a matter of degree or in which these decisions are subject to uncertainty, we use fuzzy sets and possibility distributions. See (Wolkenhauer, 1998) for more details.

As we study processes in a wide range of conditions, we find that there are *relationships* that remain effectively constant. These necessary relationships between objects, events, and conditions at a given time and those at later time are what we call causal entailment or causal laws. We however note that the assumption of causality is usually accompanied by some form of abstraction, i.e., it may imply a simplification by conceptually taking the process considered out of its context, to ignore details and thereby achieving generality. As we almost never are able to include all influential factors in our model, principles must always be complemented by specifying the conditions and context in which we have found that they are applicable. The incompleteness of our model, the context induces uncertainty which we have to consider when drawing conclusions. Our concepts concerning causal relationships will then be true only relative to a certain approximation and to certain conditions. In Table 1 biological concepts, their formalization and considerations of the modeling relation are summarized.

[1] We use here the term *object* instead of *component.* An object can be a member of a set of independent abstract objects. Abstract objects may for example be answers to a question or concepts. Components are understood as interconnected objects or concepts. The factor $f : U \to X(f)$, as a mapping from *U* to $X(f)$, can also be understood as a component.

Table 1.

The formalization of the "modeling relation" for genome analysis.

Problem	Formalization
1. Phenomenon: Gene function, gene expression	concepts
2. Characterization of by means of observable facts	factors
3. Structural components, or concepts	objects
4. The general relationship between 1, 2	representation extension
5. For a known particular expression, relationship between 1, 2	feedback extension
6. Representation of 1 by means of independent factors	*G*-envelope
7. Precision of feedback extension and 6	measure of coincidence

Definition 1 (Fuzzy Sets). Let all objects of a concept under discussion form a *universe U*. A *fuzzy* set $\hat{A}$ on the given universe *U* is defined by a mapping which associates with any object $u \in U$ a real number $\mu_{\hat{A}}(u) \in [0,1]$ in the unit interval, where $\mu_{\hat{A}}$ is called the degree of membership of *u* in $\hat{A}$:

$$\hat{A}: U \to [0,1]$$
$$u \mapsto \mu_{\hat{A}}(u).$$

The set of all fuzzy sets defined on *U* is denoted by $F(U)$. For the sake of simplicity, we make no distinction between fuzzy set $\hat{A}$ and its membership function $\mu_{\hat{A}}$ and write $\hat{A}(u) = \mu_{\hat{A}}$. The definitions of this subsection are identical to those in Li *et al.* (1995) and Wang (1990).

Definition 2 (Factors). A factor *f*, is a common description of its *states* and its *characteristics*. An *object u* is relevant to a factor *f* if there exists a state $f(u)$ of *f* corresponding to *U*. Let *U* be a set of objects and *V* be a set of factors. The pair (U,V) is assumed to satisfy the condition that for any $u \in U$, *V* contains all factors relevant to *u*. Hence (U,V) defines a (crisp) *relation R* between *U* and *V*, where $R(u, f) = 1$ if *u* is relevant to *f*. We define

$$D(f) = \{ u \in U \ : \ R(u, f) = 1 \} \tag{4.1}$$

$$V(u) = \{ f \in V \ : \ R(u, f) = 1 \} \tag{4.2}$$

A factor $f \in V$ is defined as the mapping

$$f : D(f) \to X(f)$$
$$u \mapsto f(u)$$

where $X(f) = \{f(u)\}$, is called the *state space* of *f* and $u \in U$.

Remark. Definition 2 may be generalized to allow for uncertainty in the knowledge about the relevance of an object *u* to a factor *f*. *R* is then defined as a fuzzy relation such that $\hat{R}(u, f) \in [0,1]$.

Remark. We make a distinction between various types of factors. A factor may be *measurable* (the genes position in base-pairs, width, ...) or *ordinal* (e.g. degrees expressed in the unit interval [0,1]). For *nominal* (categorical, qualitative) factors we can evaluate the equality for any two values $f(u) = f(u')$ as being either true or false. For example, a gene may be considered functional or nonfunctional (A nonfunctional copy of a gene is also called a pseudogene).

Another example is the functional class (annotation) of a gene represented by a factor. On the other hand, for *cardinal* (non-nominal, quantitative) factors such as ratios or real-valued measurements, the comparison of two values may not be straightforward.

Without loss of generality, we extend the domain of *f* to the whole set *U* with the understanding that *U* is chosen to coincide with $D(f)$. We consider factors of a gene to be *observable* properties, either direct measurements of properties of sequence data or derived knowledge. A state is a sign or symbol that represents a special instance of a factor. When a state or a characteristic of a factor is used as the condition of producing certain results or effects, we say that the results of effects are attributable to the factor, not the state or the characteristic.

In general, an object is either a concept such as a gene or a structural element such as a segment of the genome, measures or characterizes a sequence or is the measurement of some event in an organism. The latter corresponds then to the definition of an *observable* (Rosen, 1991) in Robert Rosen's *modeling relation* between a *natural system* and a *formal system*, illustrated in Fig. 4.

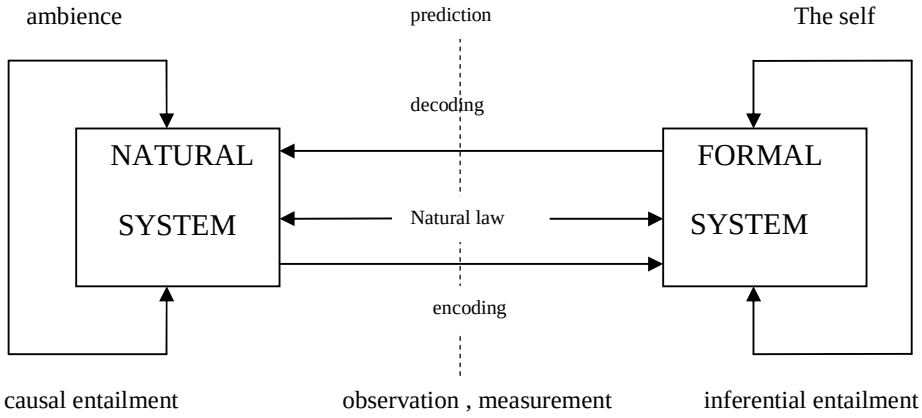


Fig. 4. The modeling relation between a natural system *N* and a formal system *F*. If the modeling relation brings both systems into congruence by suitable modes of encoding and decoding, it describes a natural law. In this case *F* is a model of *N*, *N* is a realization of *F*.

By accepting the existence of the modeling relation, factors become the means by which we encode and observe properties of the natural system under consideration. Using factors and representing them as mappings between the two spaces *U* and $X(f)$, we take the measurement and modeling process itself into account. As we shall see further below, this will allow us to be precise about model uncertainty, something other models avoid by hiding undesirable properties in assumptions about the natural system.

A factor f is equal to a factor g, if they are equal mappings, that is, $D(f) = D(g)$ if and only if $X(f) = X(g)$, and $f(u) = g(u)$ for any $u \in D(f)$.

It is possible for states of a factor g to be a subset of the states of another factor f. A factor g is called a *proper sub-factor* of f, denoted $f > g$, if there exists a (non-empty) set *Y* such that $X(f) = X(g) \times Y$. A factor g is called a *sub-factor* of f, denoted by $f \geq g$, if $f > g$ or $f = g$.

Definition 3 (Factor Spaces). The family of state spaces $\{X(f)\}_{f \in F}$ is called a *factor space* on *U* if *F*, the set of factors, is a Boolean algebra. Therefore, for any $f, g \in F$,

$$X(f \vee g) = X(f - g) \times X(f \wedge g) \times X(g - f)$$

The concept of a state space, given here, is akin to the same concept in control theory, the 'parameter space' in pattern recognition or the 'phase space' in physics (where factors are called observables (Rosen, 1991). The main difference is that a factor space is more general than the usual assumption of an Euclidean or topological space. With the definition of a Boolean algebra, imposing a structure with intersection $\wedge$, disjunction $\vee$ and complement ^{c}, on *F*, we have a basis for logical reasoning with factors (and hopefully a tool for predicting biological properties and function).

Definition 4 (Conjunction, Disjunction of Factors). A factor *h* is called the *conjunction* of factors *f* and *g*, denoted by

$$h = f \wedge g$$

if *h* is the greatest common sub-factor of *f* and *g*. In other words, $h = f \wedge g$, if and only if $X(h)$ is a common subspace of $X(h)$ and $X(g)$. Similarly, a factor *h* is called the *disjunction* of factors *f* and *g*, denoted by

$$h = f \vee g$$

if and only if $X(h)$ contains subspaces of $X(f)$ and $X(g)$, and it is the smallest of such spaces. Both definitions apply to families of factors $g = \bigwedge_{i \in I} f_i$ and $g = \bigvee_{i \in I} f_i$ respectively.

Definition 5 (Independent-, Difference-, and Atomic Factors). Any two factors are called *independent* if their conjunction results in a *zero factor*, denoted *0*, whose only state is the empty state. A factor *h* is called the difference factor between factors *f* and *g*, denoted by

$$h = f - g, \quad \text{if} \quad (f \wedge g) \vee h = f \quad \text{and} \quad h \wedge g = 0.$$

A factor *f* is called an atomic factor if *f* does not have proper sub-factors except the zero factor. The factors in the set of all atomic factor are independent.

A zero factor is equivalent to the empty set in set theory. If a family of factors $\{f_j\}_{j=1,\ldots,r}$ is independent, then

$$X(\bigvee_{j=1,\ldots,r} f_j) = \prod_{j=1}^{r} X(f_j). \tag{4.3}$$

Let *V* be a family of factors and let *F* be a set of factors of *V* such that *F* is sufficient, i.e., satisfying

$$\forall \ u, u' \in U, \ \exists f \in F : \ f(u) \neq f(u'). \tag{4.4}$$

To this point, we therefore assume that we have a sufficient number of factors describing a gene such that for a given object, there exists at least one factor *f* in *F*, such that their state values differ in *f*. That is, in an experimental context we may find that there are objects for which $f(u) = f(u')$, i.e., some objects are indistinguishable for *f*.

The triple (U, ς, F) or equivalently $(U, \varsigma, \{X(f)\}_{f \in F})$ is called a *description frame* of ς and is our formal representation of an experiment or investigation. Let (U, ς, F) be a description frame and $C \in \varsigma$. The *extension* of *C* in *U* is a fuzzy set $\hat{A} \in F(U)$ on *U*, where $\hat{A}$ is a mapping:

$$\hat{A}: U \to [0,1] \qquad (4.5)$$
$$u \mapsto \hat{A}(u)$$

where $\hat{A}(u)$ is the degree of relevance of *u* with respect to *C* or $\hat{A}$. When $A(u) = 1$, *u* definitely accords with *C*, and for $\hat{A}(u) = 0$, *u* does not belong to $\hat{A}$ (a fuzzy attribute of *C*, i.e., the function/expression of a gene or a metabolic pathway). The fuzzy restriction $\hat{A}$ is therefore used to describe the phenomenon under consideration. Obviously, the crisp case, in which knowledge of the association of an object *u* with concept *C* is certain, $\hat{A}(u) = \{0,1\}$, is a degenerate case of the given definition. The fuzzy mapping $\hat{A}$ defined on *U* is the ultimate aim of model as it describes the relationship of subsequences (ORFs') to a gene expression pathway. In general, we do not know $\hat{A}$ a *priori* but must establish knowledge about $\hat{A}$ via observable factors *f* where $f(u) \in X(f)$. For a given description frame (U, ς, F), every state space $X(f)$ is called a representation universe and hence a factor space is just a family of representation universes of ς.

If we consider the objects *u* to be gene segments and factors *f* to be specific processes in a pathway, the fuzzy set $\hat{A}$ on *U* models a physiological effect while the conjunction of segments is modeled using Definition 4 for the conjunction of factors. In analogy to approaches using the co-variation of the nucleotide content of positions in RNA to predict which positions interact with each other, one can use the covariation in the occurrence of proteins to create a model of which proteins depend for their function on each other. Such information could be used to reconstruct metabolic pathways or signaling pathways (Huyen and Bork, 1998).

Remark. The majority of cellular functions are a result of a combination of genes. It becomes therefore important to study the interrelationship of genes. At present our attention is directed at the expression of pathways for individual genes in terms of factors. The reason is that groups of genes, with the expression of one gene linked to that of another, should be more easily studied once the factor space model for individual genes is established. This is due to the fact that individual genes and their expression/function are described by fuzzy sets. Groups of genes can then be dealt with conventional fuzzy mathematics. For example, reasoning about a compound of genes such as an operon (An operon is a group of genes involved in a

single biochemical pathway and expressed in conjunction one another) in bacteria (Kyrpides, 1999), employs triangular norms for the conjunction of fuzzy sets.

Knowledge about $\hat{A}$ is gathered via measurements or observations, $f(u)$, taking values in the *representation universe* $X(f)$. $\hat{A}$ is the *phenomenon* induced by data objects and $f(\hat{A})$ are its observable *symptoms*. Formally, $f(\hat{A})$ is referred to as the extension of C in $X(f)$. The mapping $\hat{A}: U \to [0,1]$ is to capture the essence of a gene's function (or its expression pattern). The extension of C in $X(f)$, $f(\hat{A}) \in F(X(f))$, describing the expression of gene *C*, is defined (using Zadeh's extension principle[1]) as follows:

Definition 6 (Representation Extension of *C* in $X(f)$). For a given description frame (U, ς, F), let $C \in \varsigma$ whose extension is $\hat{A} \in F(U)$. For any $f \in F$, the extension of *f* to deal with fuzzy arguments is defined by

$$f(\hat{A}): X(f) \to [0,1] \qquad (4.6)$$
$$x \mapsto f(\hat{A})(x) = \bigvee_{f(u)=x} \hat{A}(u).$$

Then $f(\hat{A})$ is a fuzzy subset of the representation universe $X(f)$, $f(\hat{A}) \in F(X(f))$, where $f(\hat{A})$ is called the representation extension of *C* in the $X(f)$.

[1] The extension principle is a general principle by which a mathematical object, such as a function, can be extended to work for fuzzy sets.

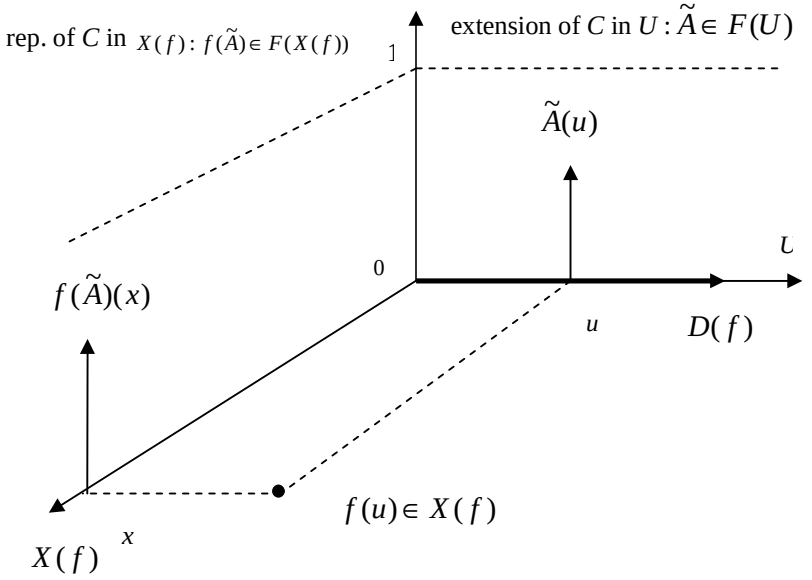


Fig. 5. Example of (U, ς, F) for a specific $f \in F$ and $C \in \varsigma$. The picture illustrates the relationship between the extension of *C* in *U*, $\tilde{A} \in F(U)$ and the representation extension of *C* in $X(f)$, $f(\tilde{A}) \in F(X(f))$.

The relationship between gene function (the phenomenon) and its characterization by means of observable processes (gene expression) is therefore specified by $f^{-1}(f(\hat{A}))$, where $f(\hat{A}) \in F(X(f))$ and $f^{-1}(f(\hat{A})) \in F(U)$. In Definition 6, we have for any $u \in U$,

$$f^{-1}(f(\hat{A}))(u) = f(\hat{A})(f(u)) = \bigvee_{f(u')=f(u)} \hat{A}(u') \geq \hat{A}(u)$$

that is,

$$f^{-1}(f(\hat{A})) \supset \hat{A} \qquad (4.7)$$

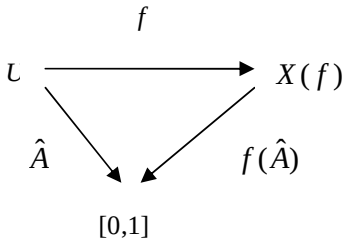
where equality is obtained for *f* being an injection (one-to-one mapping). Relation (4.7) therefore describes the quality of the model depending on the *model structure* – the choice of factors to model $\hat{A}$ on *U*. In Li *et al*. (1995) the following measure is introduced to quantify the *coincidence* of $f(\hat{A})(f(u))$ with $\hat{A}(u)$.

Definition 7 (Measure of Coincidence). Given a description frame (U, ς, F), the mapping

$$\Lambda : F \times F(U) \to [0,1]$$

$$(f, \hat{A}) \mapsto \Lambda(f, \hat{A}) = \sup\{1 - f(\hat{A})(f(u)) + \hat{A}(u) \ : \ u \in U\}$$

is called the *measure of coincidence*. If we are to view a collection of factors as the *intension* of a concept *C*, the measure $1 - \Lambda(f, \hat{A})$ serves as a measures for the precision. The essence of the *modeling relation* in Fig. 4 is therefore captured by the mapping $f : U \to X(f)$. The *natural system* here is the concept of a gene, *C*, and is formalized by a fuzzy restriction $\hat{A} : U \to [0,1]$ on *U*. The basic assumption in modeling is that *C* can also be represented by means of observables in the factor space $\{X(f)\}_{f \in F}$:



We notice that for any factor $f \in F$, the inverse $f^{-1}(f(\hat{A}))$, which we shall discuss further below, is a composition of two mappings *f* and $f(\hat{A})$, that is,

$$f^{-1}(f(\hat{A})) = f(\hat{A}) \circ f \ .$$

and therefore

$$\hat{A} = f(\hat{A}) \circ f \qquad (4.8)$$

If $f^{-1}(x)$ is a single point set for every *x* in $X(f)$, then $f(\hat{A}) = \hat{A} \circ f^{-1}$ and for any state $x \in X(f)$, Definition 6 describes how we can define the fuzzy set $\hat{A}$ by the family of fuzzy sets $\{f(\hat{A})\}_{f \in F}$. With a family of independent factors, (4.3), our model may therefore also be seen as a (compound) rule $(f = \vee f_i)$

$$C: \quad \text{If } f \text{ is } f(\hat{A}), \text{ then } u \text{ is } \hat{A}. \qquad (4.9)$$

We shall pause for a moment in order to reflect how we have proceeded so far. We started of with a natural system described using observable factors which we represented by the mapping $f : U \to X(f)$. Any object $u \in U$ is consequently

assigned a number, say in *R*. As the objects are considered in a context, that is, with respect to a concept *C*, they induce a characteristic distribution (fuzzy restriction) $\hat{A}$ in *U* such that the relevance or association of *u* with *C* is quantified by $\hat{A}(u)$, a value in the unit interval. Since $\hat{A}$ is not known a *priori*, we gather *information* of concept *C* by means of observations or measurements in the image set (range) $X(f)$ of the factor *f*. Formally, we derive our knowledge in *U* via the representation extension of *C* in $X(f)$ leading to fuzzy restriction $f(\hat{A})$. In other words, our discussion of a concept *C* in terms of objects $u \in U$ has shifted to a discussion about the extension of a concept in *U*, $\hat{A}$ and its representation extension $f(\hat{A})$ in $X(f)$ or vice versa. Let us therefore look at the *fuzzy mapping*. $\hat{f}$, now from the set of fuzzy sets in $X(f)$ to the set of fuzzy sets in *U*:

$$\hat{f} : F(X(f)) \to F(U) \tag{4.10}$$

$$f(\hat{A}) \mapsto \hat{f}(f(\hat{A})) = \hat{f} \circ f(\hat{A})$$

where we can obtain $\mu_{\hat{f}(f(\hat{A}))}(u)$ using the extension principle. For a family of independent factors $\{f_j\}$, let $X(f)$ be the Cartesian product of representation spaces $X(f) = X(f_1) \times ... \times X(f_r)$, and $f_1(\hat{A}_1),..., f_r(\hat{A}_r)$ be *r* fuzzy restrictions in $X(f_1) \times ... \times X(f_r)$, respectively. With f^{-1}, a mapping from $X(f)$ to *U*, $u = f^{-1}(x_1,...,x_r)$, the extension principle defines a fuzzy restriction in *U* by

$$\hat{A} = \{\ (u, \hat{A}(u)) : u = f^{-1}(x)\ \}$$

Where

$$\hat{A}(u) = \sup_{(x_1,...,x_r) \in f(u)} \sup \min\{\ f_1(\hat{A}_1)(x_1),..., f_r(\hat{A}_r)(x_r)\ \}. \tag{4.11}$$

For *r = 1*, the extension principle reduces to

$$\hat{A}(u) = \sup_{x \in f(u)} f(\hat{A})(x).$$

As with any mapping an equivalent representation for $\hat{f}$ is the *fuzzy graph* defined by

$$\hat{G} = f(\hat{A}_1) \times \hat{A}_1 \vee f(\hat{A}_2) \times \hat{A}_2 \vee ... \tag{4.12}$$

or more

$$\hat{G} = \bigvee_{k=1} f(\hat{A}_k) \times \hat{A}_k,$$

where the $f(\hat{A}_k)$ and $\hat{A}_k$, $k = 1,2,...$ are fuzzy subsets of $X(f)$ and U, respectively; each Cartesian product $f(\hat{A}_k) \times \hat{A}_k$ is in fact a *fuzzy relation* in $X(f) \times U$; and $\vee$ is the operation of disjunction, which is usually taken to be the union. In terms of membership functions we may then write

$$\hat{G}(x,u) = \bigvee_{j} (f_j(\hat{A}_j)(x) \wedge \hat{A}_j(u))$$

where $x \in X(f_j)$, $u \in U$, $\vee$ and $\wedge$ are any triangular *T*- and *T*-conorm, respectively.

Conclusion

The role of fuzzy restrictions (fuzzy sets) has been the representation of semantic information (or equivalently uncertainty) either observed or induced from measured data. It is important to realize that with factor-space theory, we represent a biological process or an organism in terms of its components, their function and their interaction by relations via the information they provide (Somogyi and Sniegoski, 1996). In this respect our model differs considerably to conventional physical models of organisms. In the factor-space model, a factor represents a component or part of the system fulfilling its function as defined by the mapping that associates objects with some observable consequence. An important extension to this still reductionist perspective is the information captured by fuzzy restriction $\hat{A}(u)$ with respect to some context (concept) of the 'whole' (the genome or description frame). The 'whole' is thus present in the part by 'constraining' not the part itself but the information it carries. In other words the extension from mappings (factors) to associated fuzzy sets allows us to capture external influence on constituent parts of the whole (system).

REFERENCES

Ashby, W.R. (1999). *An Introduction to Cybernetics*,. Chapman and Hall, London, pp.156

Bailey, J.E. (1998). Mathematical modeling and analysis in biochemical engineering: past accomplishments and future opportunities,. *Biotechnol. Prog.*, **14**, 8-20

Bailey, J.E. (1999). Lessons from metabolic engineering for functional genomics and drug discovery, *Nature Biotechnol.*, **17**, 616-618

Brown, M.P.S., Grundy, W.S., Lin, D., Cristianini, N., Sugnet, C.W., Furey, T.S., Ares Jr., M., Haussler, D. (2000). Knowledge-based analysis of microarray gene expression data by using support vector machines. *Proc. Natl. Acad. Sci. USA*, **97,** 262-267

Edward, J.S., Palsson, B.O. (1998). How will bioinformatics influence metabolic engineering. *Biotechnol. Bioengineering*, .**58**, 162-169

Eisen, M.B., Spellman, P.T., Brown, P.O., Botstein, D.. (1998). Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA*, **95**, 14863-14868

D'Haeseleer, P., Wen, X., Fuhrman, S., Somogyi, R. (1999). Linear modeling of mrna expression levels during cns development and injury. *Pacific Symposium on Biocomputing*, **4**, 41–52.

H¨ohle, U. (1996). On the Fundamentals of Fuzzy Set Theory. *J. Mathematical Analysis Applications*, **201**,.786-826.

Huynen, M.A., Bork, P. (1998). Measuring Genome Evolution. P*roc. Natl. Acad. Sci. USA*, **95**. 5849-5856.

Klir, G.J. (1991). *Facets of Systems Science*. Plenum Press, New York, pp.684

Klir, G.J., Yuan, B. eds. (1996). *Fuzzy Sets, Fuzzy Logic, and Fuzzy Systems: Selected Papers by Lot. Zadeh*. World Scientific, River Edge, New Jersey, pp.840

Kyrpides, N.C. (1999). Genomes Online Database (GOLD 1.0). *Bioinformatics,*.**15**, 773-774.

Lawvere, S., Schanuel, S. (1997). *Conceptual Mathematics: A First Introduction to categories*. Cambridge University Press. Pp. 374

Li, H.X., Yen, V.C. (1995). *Fuzzy Sets and Fuzzy Decision Making*. CRC Press, Boca Raton, pp.

Oliver, S.G. (1996). From DNA sequence to biological function. *Nature*, **379,** 597-600.

Paton, N. Khan S.A., Hayes A., Moussouni F., Brass A., Eilbeck K., Goble C.A., Hubbard S.J., Oliver S.G.. (2006). Conceptual Modeling of Genomic Information. *Bioinformatics*, **16**, 548-557

Rosen, R. (1991). *Life Itself*. Columbia University Press,

Rosen, R. (2000). *Essays on Life Itself*. Columbia University Press,.

Somogyi, R., Sniegoski, C.A. (1996). Modeling the complexity of genetic networks. *Complexity*, **1,**.45-63.

Wang, P.Z. (1990). A Factor Spaces Approach to Knowledge Representation. *Fuzzy Sets and Systems*, **36**, 113-124.

Wolkenhauer, O. (1998). *Possibility Theory with Applications to Data Analysi*s. Research Studies Press.

Zweiger, G. (1999). Knowledge discovery in gene-expression microarray data: mining the information output of the genome. *Trends Biotechnol.,*.**17**, 429-436.

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HEPATIC ULTRASTRUCTURE OF CHRONICALLY ETHANOL-FED RATS SUBMITTED TO ACUTE ENDOTOXIN ADMINISTRATION

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SUMMARY. Male Wistar rats were maintained in our animal facility on a normal diet, with free access to water, for about 16 weeks (the non-alcoholic control, C), while. another group of rats (A) was supplemented daily with 1.5 ml of 48% ethanol/100 g body weight. At the end of the period, 24 hrs before the sacrifice, part of the rats in each of the two groups were injected with either 0.5 or 2 mg bacterial endotoxin (LPS)/kg body weight. The four subgroups thus resulting were denoted as 0.5LPS, 2LPS, A+0.5LPS and A+ 2LPS, respectively. After animal sacrifice, most of the liver was used for preparation of mitochondria, on which several functional assays were performed *in vitro*, as previously described (Tarba and Suărăşan, 2003a; 2004a), while a small piece of the original hepatic tissue as well as mitochondrial sediment from the incubation vessel were prepared for electron microscopy. The ultrastructural results obtained on control and alcoholic rats were fully described previously (Tarba and Florea, 2005a; 2006b) while the present paper is devoted to the results obtained with the four endotoxin-treated groups. A certain similarity can be observed between the effects recorded in group A and in the subgroups treated with endotoxin (LPS groups). Thus, the cell nuclei have large nucleoli, with intense activity, the smooth endoplasmic reticulum is highly dilated and vesiculated and lipid droplets are present in cells where peroxysomes are rare. Also, the mitochondria *in situ* have an irregular (polymorphous) shape, especially in group 2LPS. However, unlike in the case of the alcoholic rats, where a matrix rarefaction can be observed, LPS treatment seems to be associated with an increase of matrix electron density, especially in group 2LPS. Administration of the high dose of endotoxin aggravates the situation of ethanol-fed rats (group A+2LPS), while the low dose seems to have little additional effect (group A+0.5LPS), although both doses tend to increase the proportion of apoptotic nuclei.

KEYWORDS: endotoxin, chronic ethanol feeding, hepatic ultrastructure.

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Introduction

As discussed in a lengthier way in our previous articles (Tarba and Suărăşan, 2003a; 2004a) chronic ethanol administration has been generally associated with specific hepatic structural and functional alterations known under the name of alcoholic liver disease (ALD). For decades, ALD has been attributed to necrotic events related, among other things, with the production of proinflammatory cytokines, such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α) (see, for ex., Deaciuc *et al*., 1995; McClain *et al*., 1999; Tsukamoto and Lu, 2001). However, relatively recent data have shown that chronic ethanol consumption or its acute administration is also able to initiate a process of hepatic apoptosis, followed very often by necrosis. Although the mechanism by which alcohol induces the disease is not entirely known, chronic ethanol exposure has been associated with the presence of endotoxin, mitochondrial oxidative stress and partial decrease in ATP synthesis (Halestrap *et al*., 1997; Kurose *et al*., 1997; Thurman *et al.*, 1999; Cunningham and Van Horn, 2003; Wu and Cederbaum, 2003). On the other hand, the same type of factors, to which one could add calcium ions, are implicated in membrane permeabilisation and release of the so-called apoptogenic factors, residing in the mitochondrial intermembrane space, *i.e.*, factors and events that are leading to apoptosis (Kroemer *et al*., 1995; 1997; 1998; Petit *et al*., 1996; Marchetti *et al*., 1996; Scarlet and Murphy, 1997; Susin *et al*., 1997; Hirsh *et al*., 1998; Bernardi *et al*. 1999; 2001; Reed, 2000; Tarba, 2001).

Endotoxin is also known as LPS (lipopolysaccharide). It is an outer membrane component of Gram-negative bacteria whose presence in the circulating blood of higher vertebrates can elicit an immunological response, manifested through inflammation and other negative reactions, which may terminate in septic shock and animal death. It has been discovered that there is a positive correlation between the extent of hepatic injury and the level of endotoxin in the circulating blood of the human alcoholics or ethanol-fed animals (Nanji *et al.*, 1993; Iimuro *et al.*, 1997; Thurman *et al.*, 1997; Mathurin *et al.*, 2000; Rao *et al*., 2004). Moreover, it is now known that the mechanisms of hepatic toxicity of both endotoxin and ethanol have an important convergence point, represented by the Kupffer cells. These are resident hepatic macrophages which constitute a major source of proinflammatory cytokines, the most prominent being TNF-α. The mechanism of activation of these cells and their role in hepatotoxicity has been gradually clarified during the last decade (Thurman, 1998; Su *et al.*, 2000; Uesugi *et al.*, 2002; Wheeler, 2003; McClain *et al.*, 2004). However, controversial and sometimes even conflicting results have been obtained regarding the effects of endotoxin, especially when it was used in association with either acute or chronic ethanol administration, even after careful elimination of possible differences due to the use of different biological material and/or experimental design. In our opinion, these differences may be explained if one takes into consideration two apparently opposing and incompletely understood phenomena: the (hepatic) hypermetabolic state, usually associated with ethanol consumption (Israel and Orrego, 1984; Zhong *et al.*, 1996;

Bradford *et al.*, 1999) but which can also be mimicked by endotoxin administration (Fish and Spitzer, 1984; Arita *et al.*, 1988; Rivera *et al.*, 1998) and the so-called endotoxin tolerance (see, for a review, Yoza *et al.*, 1998, or Fan and Cook, 2004).

The present article is part of a larger study aimed at establishing the order of events leading to the release of the apoptogenic factors from the mitochondrial intermembrane space and uses alcohol and endotoxin, as metabolic stress factors for the induction of apoptosis and/or necrosis in animals kept under more natural conditions (see *Material and Methods*) than usually reported in the literature. In a previous article (Tarba and Florea, 2006b) we dealt mainly with the ultrastructural effects of chronic alcohol consumption, whereas the present study is dedicated to the ultrastructural consequences of acute endotoxin administration, both in control *(i.e.,* non-alcoholic) and alcohol-fed rats. Two preliminary reports of our results have also appeared (Tarba and Florea, 2005b; 2006a).

Materials and Methods

Male white Wistar rats were maintained in our animal facility for 14-18 weeks, starting from an average weight of 120 g/individual, while the evolution of their weight was assessed periodically. The rats were kept on a normal diet (a premix containing all the ingredients of the Larsen diet), with free access to water. One group served as a (non-alcoholic) control (C) while in another group (named here alcoholic control, A) each rat was supplemented daily with 1.5 ml of 48% ethanol/100 g body weight, administered in the morning, on a little piece of bread, before getting access to the food. Twenty four hours before the sacrifice, part of the rats in each of the two groups were injected with either 0.5 or 2 mg bacterial endotoxin (LPS)/kg body weight. The four subgroups thus resulting were denoted as 0.5LPS, 2LPS, A+0.5LPS and A+2LPS, respectively. Following a 24-hr fasting, the rats were sacrificed by decapitation after a slight anaesthesia. Small pieces of liver were taken and prepared for electron microscopy, according to standard techniques, while the rest of the liver was used for the preparation of mitochondria, essentially as previously described (Johnson and Lardy, 1967; Tarba, 1983), in a medium containing 200 mM mannitol, 70 mM sucrose, 5 mM Hepes-KOH (pH 7.37) and 0.5 mM Na-EDTA. The washing and preserving medium lacked the chelating agent (EDTA). The suspending media that are of interest for the present work are designated as MKS (110 mM mannitol, 65 mM KCl, 40 mM sucrose, 1.5 mM $MgCl_2$, 1 mM KP_i, 5 mM Hepes) and KSW (100 mM KCl, 50 mM sucrose, 5 mM KP_i, 10 mM Hepes), also known as the swelling medium. In both cases the pH was 7.35. Mitochondria were suspended in one of the above-mentioned media and NADH-dependent respiration was abolished by the addition of 8 μM rotenone before the introduction of succinate (2.5 mM). In combination with the two media of different ionic content and the addition of different concentrations of calcium, membrane potential and calcium fluxes were each monitored in parallel with the swelling by the use of appropriate probes and a diode array (DA) spectrophotometer, as previously described (Tarba and Suărăşan, 2003a; 2004a).

Aliquots of both supernatant and mitochondrial sediment were taken at specific moments of incubation and saved for later analysis or, in the case of the sediment, also prepared for electron microscopy. In this case, the material was processed as described previously (Tarba and Crăciun, 1990) and the ultrastructural images were obtained using a JEOL JEM 1010 transmission electron microscope.

Results

The control rats. For a better comprehension and as a help for an easier comparison of our results, two images of hepatic ultrastructure are included first, one from a typical non-alcoholic control (Fig. 1) and one from a typical alcoholic control rat (Fig. 2), along with the corresponding images of the *in-vitro* mitochondrial ultrastructure (Fig. 3 and 4, respectively), although many more pictures of this type have been included in our previous papers (Tarba and Florea, 2005a; 2006b). The electron-microscopic images obtained from the liver of the non-alcoholic control rats (see Fig. 1) are representative for the normal state of the liver, the hepatocytes having euchromatic nuclei, with large and active nucleoli. Biliary canaliculi are normal and the microvilli are present in a great number in the space of Disse. The lipids are in general absent while glycogen is present in large quantities, as black granules. The mitochondria *in situ* have an orthodox configuration (conformation), with a normal aspect, both as form and electron density. Isolated (*in-vitro)* mitochondria of (non-alcoholic) controls generally have a condensed configuration, with a normal electron density of the matrix and intact membranes, as shown in Fig. 3. The presence of either orthodox or ultracondensed forms is rare. Even at a rather high concentration of calcium (50 μM), some preparations of the control (C) mitochondria succeed in accumulating this ion and preserving their function and structure, at least for a while (not presented here).

The liver of alcoholic control (ethanol-fed) rats (A) is visibly affected by the chronic ethanol consumption. Thus, in most liver preparations from this group, the nuclei have little heterochromatin and a dilated perinuclear space. The smooth endoplasmic reticulum is very dilated and highly fragmented (vesiculated), whereas the lipids and glycogen are lacking from the cytoplasm. In turn, one can observe a strong peroxysomal proliferation. Mitochondria *in situ* are enlarged and deformed (polymorphous mitochondria), with an electron transparent matrix and lack of cristae, as can be seen from an overall image of a hepatocyte presented in Fig. 2. The majority of the mitochondria tested *in vitro* in the presence of succinate (with no other metabolic challenge) have a condensed or ultracondensed conformation, most of them being either falciform or with the matrix circularly condensed and an electron void central zone. The outer membrane is visible in some cases (due to its detachment) but in many others it seems to be broken. A few organelles, that have an apparently orthodox shape, are more likely on the course of disintegration, since no real internal structure can be seen. Some others, still, are completely collapsed, having lost most of their content. Such details are illustrated in Fig. 4. All these alterations are indicative of the disastrous effects of ethanol.

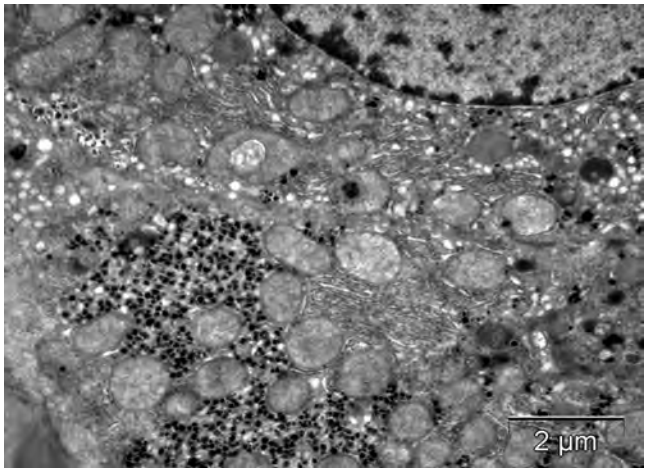

Fig. 1. Ultrastructural aspects of the control liver (group C)

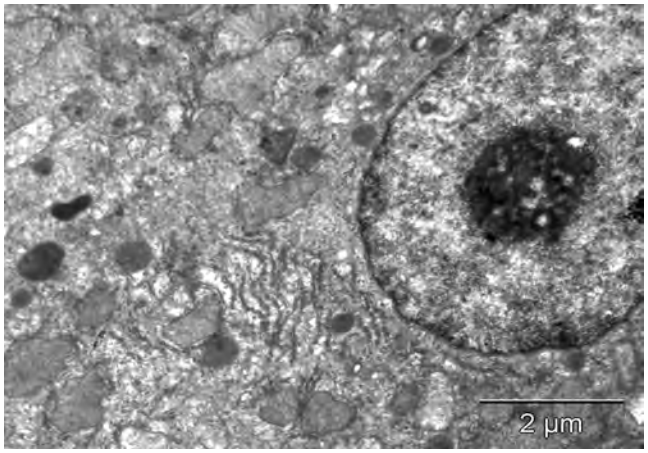

Fig. 2. Overall image of a hepatocyte from a typical ethanol-fed rat

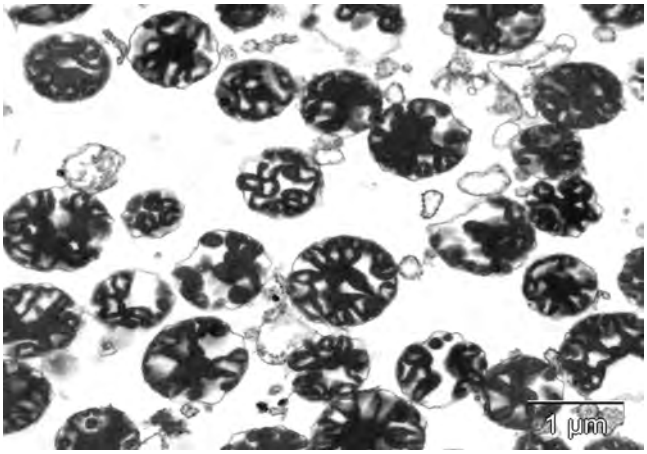

Fig. 3. Ultrastructural aspects of the control (C) mitochondria *in vitro*

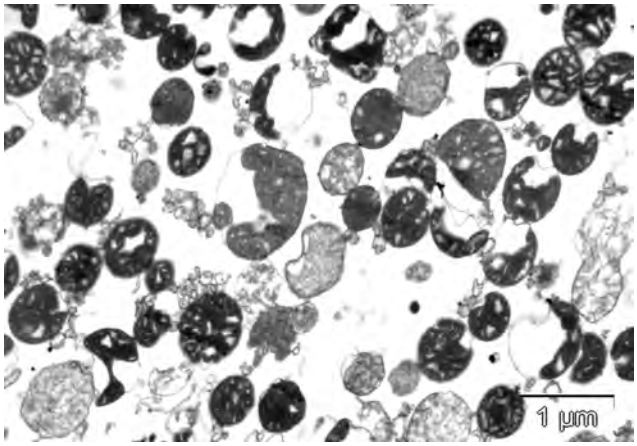

Fig. 4. Aspects of *in-vitro* mitochondria from ethanol-fed rats

Endotoxin-treated (LPS) rats. The hepatic tissue of the rats receiving the low dose of endotoxin (subgroup 0.5LPS) display a rather large variability of responses, from liver zones with an almost normal appearance to clearly affected zones (zonal affection). In such areas, the hepatocytes have nuclei with an irregular contour and a very dilated nuclear space. Peroxysomes are present in a rather large number, mitochondria are slightly polymorphous, lipid droplets can be often seen

and the endoplasmic reticulum is visibly dilated and vesiculated, giving the cell a characteristic "starry" appearance (Fig. 5). The isolated mitochondria are strongly condensed (ultracondensed), but with an apparent normal matrix, which has not lost its structure (Fig. 6). For the high endotoxin dose (subgroup 2LPS), the alterations are more extensive and profound. Very characteristically, the rough endoplasmic reticulum is higly dilated while the smooth one is strongly vesiculated, the cell having a "cloudy" appearance, as can be seen in Fig. 7. The *in-situ* mitochondria present in this figure are usually dilated and polymorphous, but with an apparent dense matrix, filled with some granular material, which can not be observed in the other subgroups. *In-vitro* mitochondria (see Fig. 8) have generally a ultracondensed conformation and the same dense granular material seen *in situ* is also present in many of these organelles.

Endotoxin-treated alcohol-fed rats. This group constitutes the main target of the present study, in which we try to assess the contribution of the endotoxin to the effects produced in the ethanol-fed rats. A general observation for this group is the presence of a rather large variability of the results, especially within the subgroup A+0,5LPS, *i.e.* the ethanol-fed subgroup treated with 0.5 mg LPS/kg.

Subgroup A+0,5LPS. Two hepatic ultrastructural images are included to illustrate both characteristic features and the large variability in this group (Fig. 9 and 10). We consider Fig 9 to be more representative of the effects of ethanol (such as the presence of polymorphous mitochondria with a rarefied matrix, lipid droplets etc.) than of LPS, whereas the contrary is true for Fig. 10, where mitochondria appear almost normal while the smooth endoplasmic reticulum is characteristically vesiculated. The aspect of the *in-vitro* mitochondria is also reminiscent of the effect of ethanol, as can be seen in Fig. 11. If a membrane collapsing concentration of calcium is added (i.e., 50-200 μM), mitochondria become swollen and lose the electron density in a few minutes, as shown in Fig. 12.

Subgroup A+2LPS. Two hepatic ultrastructural images are again included. One (Fig. 13) is presenting a hepatocyte with almost normal mitochondria and a very contorted nucleus, with characteristically condensed chromatin, typical for an apoptotic cell and another (Fig. 14) showing characteristics that suggest necrosis (swollen mitochondria, very dilated and highly vesiculated endoplasmic reticulum etc.). The appearance of *in-vitro* mitochondria in the absence of calcium is shown in Fig. 15, where condensed and ultracondensed mitochondria of normal size can be seen along with megamitochondria and occasionally with completely collapsed organelles. The addition of calcium leads immediately to extensive swelling of mitochondria and loss of electron density (Fig. 16).

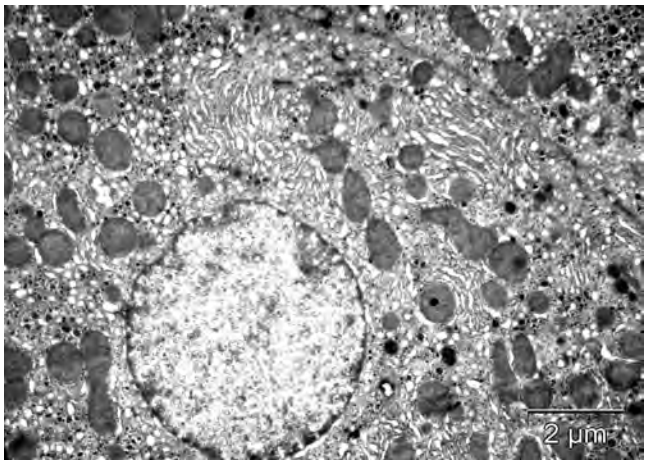

Fig. 5. Hepatic ultrastructural aspects from the 0.5LPS subgroup

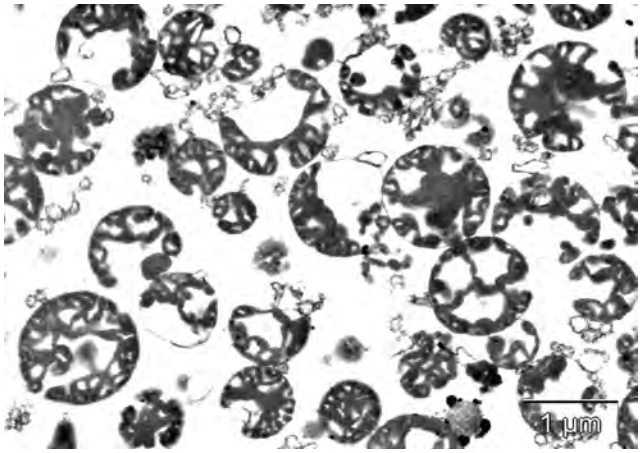

Fig. 6. Aspects of *in-vitro* mitochondria from 0.5LPS rats

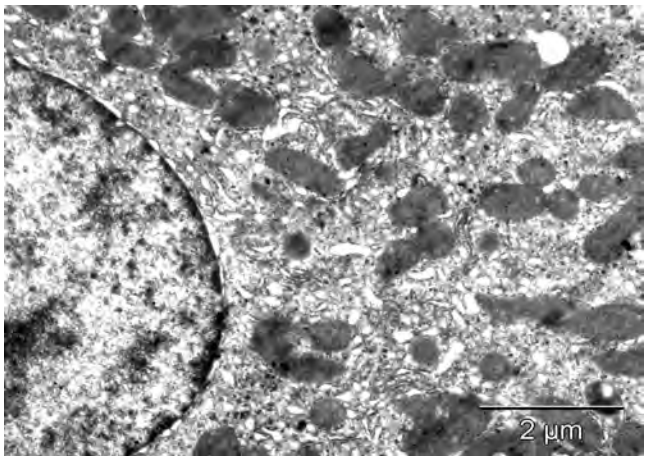

Fig. 7. Hepatocyte ultrastructure characteristic to rats of the 2LPS subgroup

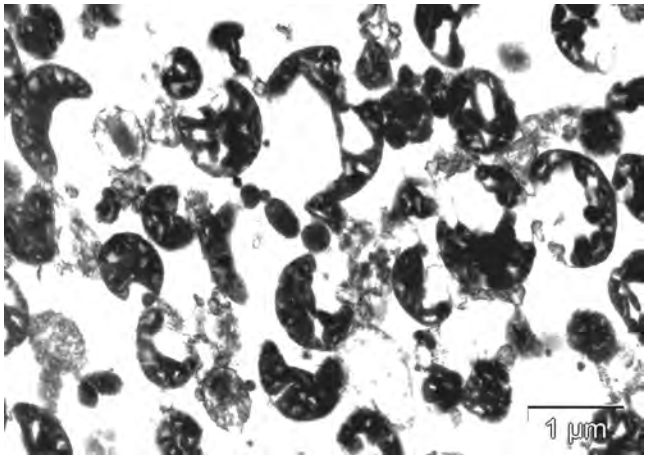

Fig. 8. Ultrastructure of *in-vitro* mitochondria from 2LPS rats

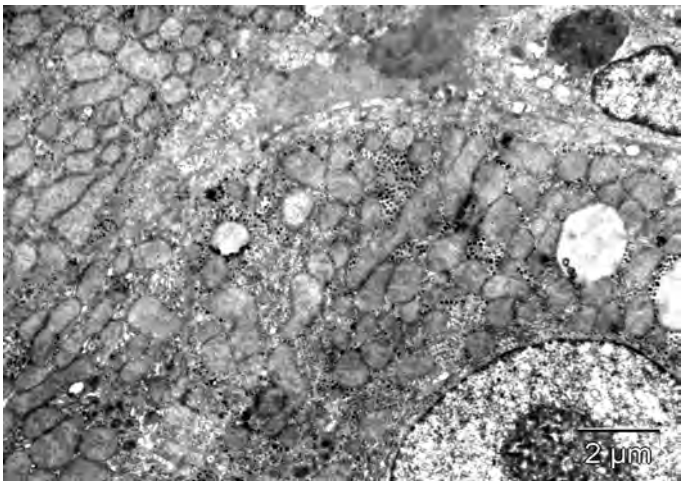

Fig. 9. Hepatic ultrastructure with features of ethanol intoxication in A+0.5LPS rats

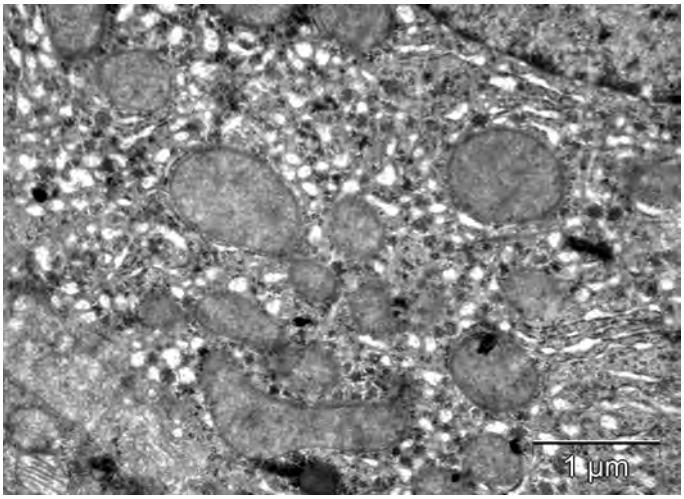

Fig. 10. Hepatic ultrastructure with characteristic endotoxin effects in A+0.5LPS rats

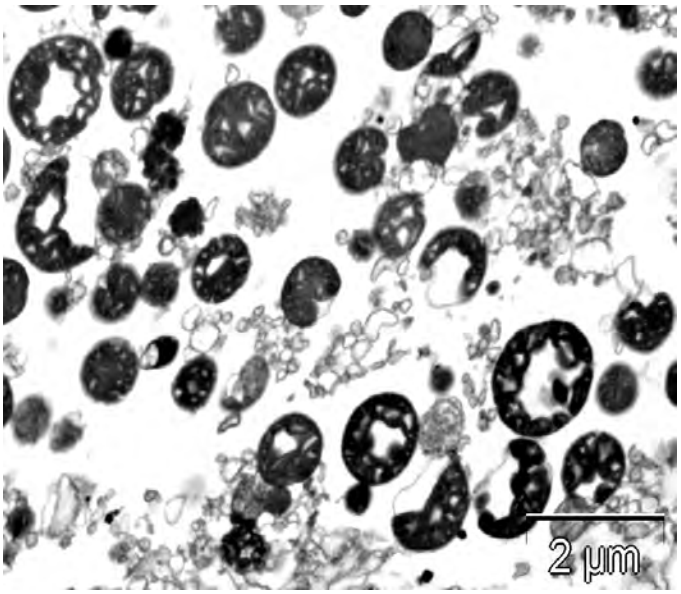

Fig. 11. *In-vitro* mitochondrial ultrastructure of A+0.5LPS rats in the absence of Ca

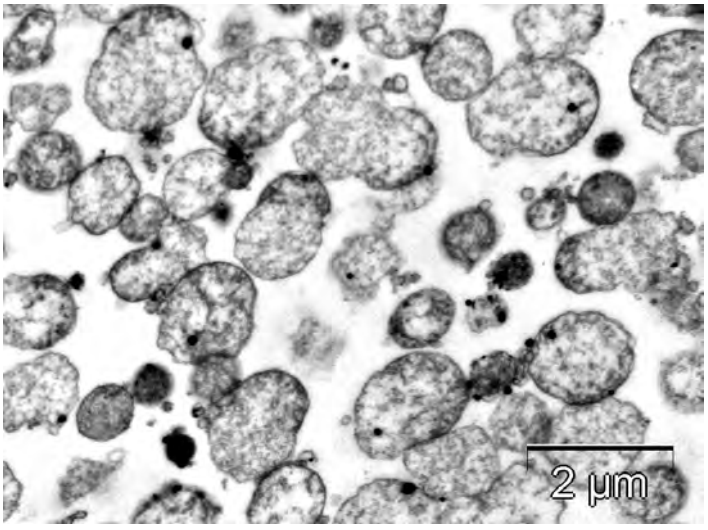

Fig. 12. *In-vitro* mitochondrial ultrastructure of A+0.5LPS rats in the presence of Ca

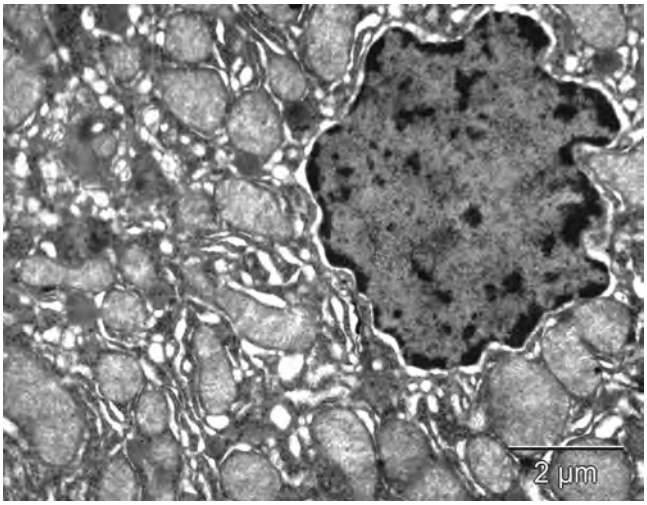

Fig. 13. Hepatocyte with apoptotic features in the liver of the A+2LPS rats

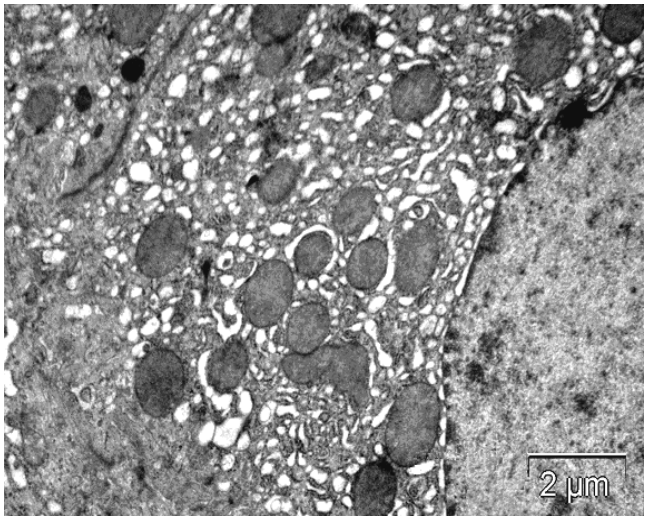

Fig. 14. Hepatocyte with necrotic characteristics in the liver of the A+2LPS rats

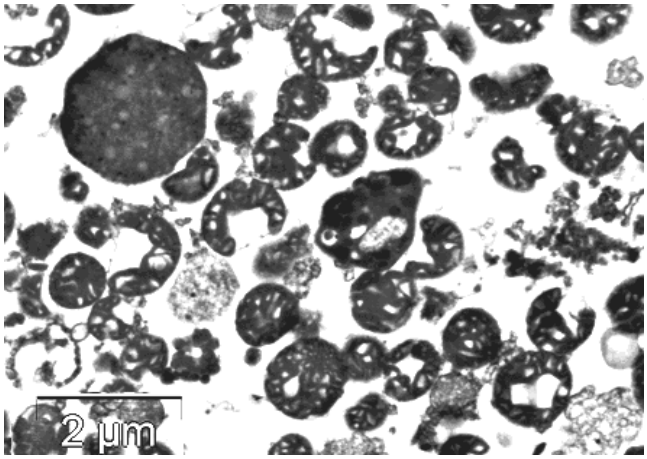

Fig. 15. Ultrastructure of *in vitro* mitochondria from A+2LPS rats in the absence of Ca

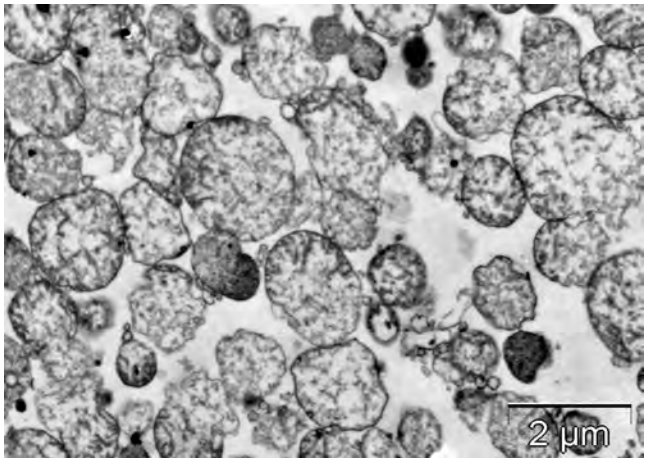

Fig. 16. *In-vitro* mitochondrial ultrastructure of A+2LPS rats in the presence of Ca

Discussion

As mentioned in the introductory section, the results observed in the endotoxin-treated groups may be interpreted in terms of the two complementary phenomena, endotoxin tolerance and hypermetabolism (hypermetabolic state). In the

(non-alcoholic) endotoxin-treated subgroups, the large variability of responses may be assigned to different degrees of endotoxin sensitivity. For some of the rats in this subgroup the small dose of LPS is probably just right for inducing endotoxin tolerance, while for others it could be enough to induce a hypermetabolic state or even inflammation. This can also explain the zonal affection, also present in the A+0.5LPS subgroup, since it is known that in the hypermetabolic state (characterized by increased oxygen consumption) the cells that suffer most are those found in the hepatic centrolobular zones, where the blood has already become hypoxic (see, for example, Cunningham and Van Horn, 2003). As regards the higher dose of endotoxin (2 mg/kg body weight), one would expect a more consistent response, associated mainly with the necrotic factors (hence, the necrotic effects) that are usually elicited by the presence of LPS (see, for example, Su, 2002). However, a search of literature data suggests a very controversial and complex picture for all endotoxin doses employed, ranging from negative effects on hepatic metabolism and structure, such as uncoupling of oxidative phosphorylation, decrease in mitochondrial oxygen consumption and the presence of polymorphous mitochondria with matrix disruption (DePalma *et al.*, 1977; Yoder *et al.*, 1985; Markeley *et al.*, 2002), to apparently positive effects, such as enhancement of mitochondrial functions and mitochondrial respiratory capacity (Dawson *et al.*, 1988; Guidot, 1996; 1998). Moreover, as in the case of ethanol (see our previous papers, Tarba and Florea, 2005a; 2006b), endotoxin seems to be able to induce either apoptosis (Jia *et al.*, 1997; Kosai *et al.*, 1999; Yoshimoto *et al.*, 2005) or necrosis (Liehr *et al.*, 1975; Yoder *et al.*, 1985; Saad *et al.*, 1995; Brown *et al.*, 1996), or both (Jaeschke *et al.*, 1998; Zang *et al.*, 2000; Wu *et al.*, 2004). The situation is due not only to differences in experimental circumstances (including different endotoxin doses), but also to a time-dependent response to LPS, with apoptosis installing first, followed by necrosis, although only one of the above-mentioned studies explicitly addresses this problem (Zang *et al.*, 2000).

The situation is somewhat parallel in the A+LPS subgroups, only even more complicated, because of the chronic ethanol feeding, which has been shown to potentiate the effect of endotoxin and vice-versa (see for example, Nanji *et al.*, 1993; 2002; Thurman *et al.*, 1997; Thurman, 1998; Mathurin *et al.*, 2000; Tsukamoto and Lu, 2001; Deaciuc *et al.*, 2001; Hill *et al.*, 2002; Koteish *et al.*, 2002; Rao *et al.*, 2004). Thus, in the case of the low endotoxin dose (subgroup A+0.5LPS), the main effects seen are those of ethanol, while in the case of the higher dose (subgroup A+2LPS) ethanol effects are indeed amplified by endotoxin, so that mostly strong necrotic features can be observed in our electronmicrographs (very dilated nuclear space, strongly dilated and highly vesiculated endoplasmic reticulum, polymorphous and/or swollen mitochondria etc.).

Conclusions

The results obtained in the present study lend further support to our functional observations on endotoxin-treated rats, submitted or not to a previous

ethanol chronic diet, including a rather large interindividual variability (Suărăşan and Tarba, 2003; Tarba and Suărăşan, 2003b; 2004b).

The action of acute endotoxin administration is dose-dependent, with the higher dose (2 mg LPS/kg b.w.) producing more consistent results, which can be interpreted mostly in terms of necrotic effects, although apoptotic features can sometimes be seen. They partially resemble those obtained in the case of typical alcoholic rats, with one notable exception, the presence of an electron-dense granular material in the matrix of the majority of mitochondria, a fact which has not been explicitly addressed in the literature.

Superimposing an acute endotoxin treatment over the chronic ethanol-feeding of rats leads to the aggravation of the effects of ethanol, especially in the case of the higher dose (subgroup A+2LPS).

Despite the apparent resemblance of the ultrastructural effects of ethanol and endotoxin, which can be explained by the fact that ethanol exerts many (if not most) of its effects through endotoxin, there are certain differences which are more remarkable in the case of mitochondria and which still deserve a closer attention.

REFERENCES

Arita, H., Ogle, C.K., Alexander, J.W., Warden, G.D. (1988) Induction of hypermetabolism in guinea pigs by endotoxin infusion through the portal vein, *Arch. Surg.***123**, 1420-1423.

Bernardi, P., Petronilli, V., Di Lisa, F., Forte, M. (2001) A mitochondrial perspective on cell death, *Trends Biochem. Sci.* **26**, 112-117.

Bernardi, P., Scorrano, L., Colonna, R., Petronilli, V., Di Lisa, F. (1999) Mitochondria and cell death. Mechanistic aspects and methodological issues, *Eur. J. Biochem.* **264**, 687-701.

Bradford, B.U., Enomoto, N., Ikejima, K., Rose, M.L., Bojes, S.K., Forman, D.T., Thurman, R.G. (1999) Peroxisomes are involved in the swift increase in alcohol metabolism, *J.Pharmacol. Exp.Therapeutics* **288**, 254-259.

Brown, A.P., Schultze, A.E., Holdan, W.,L., Buchweitz, J.P., Roth, R.A., Ganey, P.E. (1996) Lipopolysaccharide-induced hepatic injury is enhanced by polychlorinated biphenyls, *Environ.Health Perspectives* **104,** 634-641.

Cunningham, C.C., van Horn, C.G. (2003), Energy availability and alcohol-related liver pathology, *Alcohol Res. Health* **27**, 291-299.

Dawson, K.L., Geller, E.R., Kirkpatrick, J.R. (1988) Enhancement of mitochondrial function in sepsis, *Arch. Surg.* **123**, 241-244.

Deaciuc, I.V., D'Souza, N.B., De Villiers, W.J.S., Burikhanov, R., Sarphie, T.G., Hill, D.B., McClain, C.J. (2001) Inhibition of caspases in vivo protects rat liver against alcohol-induced sensitization to bacterial lipopolysaccharide, *Alcoholism: Clin. Exp. Res.* **25**, 935-943.

Deaciuc, I.V., Fortunato, F., D'Souza, N.B., Hill, D.B., Schmidt, J., Lee, E.Y., McClain, C.J. (1999) Modulation of caspase-3 activity and Fas ligand mRNA expression in rat liver cells in vivo by alcohol and lipopolysaccharide, *Alcoholism: Clin. Exp. Res.* **23**, 349-356

DePalma, R.G., Glickman, M.H., Hartman, P., Robinson, A.V. (1977) Prevention of endotoxin-induced changes in oxidative phosphorylation in hepatic mitochondria, *Surgery* **82** (1), 68-73.

Fan, H., Cook, J.A. (2004) Molecular mechanisms of endotoxin tolerance, *J. Endotoxin Res.* **10** (2), 71-84.

Fish, R.E., Spitzer, J.A. (1984) Continuous infusion of endotoxin from an osmotic pump in the conscious, unrestrained rat: an unique model of chronic endotoxemia, *Circ. Shock* **12** (2), 135-149.

Guidot, D.M. (1996) Endotoxin treatment increases lung mitochondrial scavenging of extramitochondrial superoxide in hyperoxia-exposed rats, *Arch. Biochem. Biophys.* **326**, 266-270.

Guidot, D.M. (1998) Endotoxin pretreatment *in vivo* increases the mitochondrial respiratory capacity in rat hepatocytes, *Arch. Biochem. Biophys.* **354**, 9-17.

Halestrap, A.P., Woodfield, K.Y., Connern, C.P. (1997) Oxidative stress, thiol reagents and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase, *J. Biol. Chem.* **272**, 3346-3354.

Hill, D.B., D'Souza, N.B., Lee, E.Y., Burikhanov, R., Deaciuc, I.V., De Villiers, W.J.S. (2002) A role for interleukin-10 in alcohol-induced liver sensitization to bacterial lipopolysaccaride, *Alcoholism: Clin. Exp. Res.* **26**, 74-82.

Hirsh, T., Susin, S.A., Marzo, I., Marchettti, P., Zamzami, M., Kroemer, G. (1998) Mitochondrial permeability transition in apoptosis and necrosis, *Cell Biol. Toxicol.* **14** (2), 141-145.

Iimuro, Y., Frankenberg, M.V., Arteel, G.E., Bradford, B.U., Wall, C.A., Thurman, R.G. (1997) Female rats exhibit greater susceptibility to early alcohol-induced injury than males, *Am. J. Physiol.* **272** *(Gastrointest. Liver Physiol.* **35**) G1186-G1194.

Israel, Y., Orrego, H. (1984) Hypermetabolic state and hypoxic liver damage, *Recent. Dev. Alcohol.* **2**, 119-133.

Jaeschke, H., Fisher, M.A., Lawson, J.A., Simmons, C.A., Farhood, A., Jones, D.A. (1998), Activation of caspase 3 (CPP32)-like proteases is essential for TNF-α-induced hepatic parenchymal cell apoptosis and neutrophil-mediated necrosis in a murine endotoxin shock model, *J. Immunol.* **160**, 3480-3486.

Jia, L., Dourmashkin, R.R., Newland, A.C., Kelsey, S.M. (1997) Mitochondrial ultracondensation, but not swelling, is involved in TNF-alpha-induced apoptosis in human T-lymphoblastic leukemic cells, *Leukemia Res.* **21**, 973-983.

Johnson, D., Lardy, H. (1967) Isolation of liver or kidney mitochondria, In: *Methods in Enzymology*, Colowick, S.P., Kaplan, N.O. (eds), Acad. Press, New York, vol.**10**, pp.94-96.

Kosai, K., Matsumoto, K., Funakoshi, H., Nakamura, T. (1999) Hepatocyte growth factor prevents endotoxin-induced lethal hepatic failure in mice, *Hepatology* **30**, 151-159.

Koteish,, A., Yang, S.Q., Lin, H.Z., Huang, X., Diehl, A.M., (2002) Chronic ethanol exposure potentiates lipopolysaccharide injury despite inhibiting jun N-terminal kinase and caspase activation, *J. Biol. Chem.* **277**, 13037-13044.

Kroemer, G., Dallaporta, B., Resche-Rigon, M. (1998) The mitochondrial death/life regulator in apoptosis and necrosis, *Annu. Rev. Physiol.* **60**, 619-642

Kroemer, G., Petit, P., Zamzami, N., Vayssiere, J.-L., Mignotte, B. (1995) The biochemistry of programmed cell death, *FASEB J.* **9**, 1277-1287

Kroemer, Zamzami, N., Susin, S.A. (1997) Mitochondrial control of apoptosis, *Immunol. Today* **18**, 44-51

Kurose, I., Higuchi, H., Miura, S., Saito, H., Watanabe, N., Hokari, R., Hirokawa, M., Takashi, M., Zeki, S., Nakamura, T., Ebinuma, H., Kato, S., Ishii, H. (1997) Oxidative stress mediated apoptosis of hepatocytes exposed to acute ethanol intoxication, *Hepatology* **25**, 368-378.

Liehr, H., Grun, M., Thiel, H., Brunswig, D., Rasenack, U. (1975) Endotoxin-induced liver necrosis and intravascular coagulation in rats enhanced by portacaval collateral circulation, *Gut* **16**, 429-436.

Marchetti, P., Castedo, M., Susin, S.A., Zamzami, N., Hirsch, T., Macho, A., Haeffner, A., Hirsh, F., Geuskens, M., Kroemer, G. (1996) Mitochondrial permeability transition is a central coordinating event of apoptosis, *J. Exp. Med.* **184**, 1155-1160

Markley, M.A., Pierro, A., Eaton, S. (2002) Hepatocyte mitochondrial metabolism is inhibited in neonatal rat endotoxaemia: effects of glutamine, *Clinical Science* **102**, 337-334.

Mathurin, P., Deng, Q.-G., Keshavarzian, A., Choudhari, S., Holmes, E.W., Tsukamoto, H. (2000) Exacerbation of alcoholic liver injury by enteral endotoxin in rats, *Hepatology* **32**, 1008-1017.

McClain, C.J., Barve, S., Deaciuc, I, Kugelmas, M., Hill, D.B. (1999) Cytokines in alcoholic liver disease, *Semin. Liver Dis.* **19**, 205-219.

McClain, C.J., Song, Z., Barve, S.S., Kugelmas, M., Hill, D.B., Deaciuc, I. (2004) Recent advances in alcoholic liver disease: IV.Dysregulated cytokine metabolism in alcoholic livrer disease, *Am. J. Physiol. Gastrointest. Liver Physiol.* **287**, G497-G502.

Nanji, A.A., Jokelainen, K., Fotouhinia, M., Rahemtulla, A., Thomas, P., Tipoe, J.L., Su, G.L., Dannenberg, A.J. (2002) Increased severity of alcoholic liver injury in female rats: role of oxidative stress, endotoxin and chemokines, *Am. J. Physiol. Gastrointest. Liver Physiol.* **281**, G1348-G1356.

Nanji, A.A., Khettry, U., Sadrzadeh, S.M., Yamanka, T. (1993) Severity of liver injury in experimental alcoholic liver disease: correlation with plasma endotoxin, prostaglandin E_2, leukotriene B_4 and thromboxane B_2, *Am. J. Pathol.* **142**, 367-373.

Petit, P.X., Susin, S.A., Zamzami, N., Mignotte, B., Kroemer, G. (1996) Mitochondria and the programmed cell death. Back to the future, *FEBS Lett.* **396**, 7-13.

Qu, W., Zhong, Z., Goto, M., Thurman, R.G. (1996) Kupffer cell prostaglandin E_2 stimulates parenchymal cell O_2 consumption: alcohol and cell-cell communication, *Am. J. Physiol. Gastrointest. Liver Physiol.* **270**, G574-G580.

Rao, R.K., Seth, A., Sheth, P. (2004) Recent advances in alcoholic liver disease: I. Role of intestinal permeability in alcoholic liver disease, *Am. J. Physiol. Gastrointest. Liver Physiol.* **286**, G881-G884.

Reed, J.C. (2000) Mechanisms of apoptosis. *Am. J. Pathol.* **157**, 1415-1430.

Rivera, C.A., Bradford, B.U., Seabra, V., Thurman, A.G. (1998) Role of endotoxin in the hypermetabolic state after acute ethanol exposure, *Am. J. Physiol.* **275** *(Gastrointest. Liver Physiol.* **38**) G1252-G1258.

Saad, B., Frei, K., Scholl, F.A., Fontana, A., Maier, P. (1995) Hepatocyte-derived interleukin-6 and tumor necrosis factor alpha mediate the lipopolysaccaride induced acute-phase response and nitric oxide release by cultured rat hepatocytes, *Eur. J. Biochem.* **229**, 349-355.

Scarlett, J.L., Murphy, M.P. (1997) Release of apoptogenic proteins from mitochondrial intermembrane space during the mitochondrial permeability transition, *FEBS Lett.* **418**, 282-286.

Su, G.L. (2002) Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation, *Am. J. Physiol. Gastrointest. Liver Physiol.* **283**, G256-G265.

Su, G., Klein, R., Aminlari, A., Zhang, H., Steinstraesser, L., Alarcon, W., Remick, D., Wang, S. (2000) Kupfffer cell activation by lipopolysaccharide in rats: role for lipopolysaccharide binding protein and toll like receptor 4, *Hepatology* **31**, 932-936.

Suărăşan, F., Tarba, C. (2003) Corelaţii între fluxurile de calciu, tranziţia de permeabilitate şi colapsarea potenţialului de membrană în mitocondriile hepatice de şobolan. II. Efectul tratamentului acut cu endotoxinâ, *Anal. Soc. Naţ. Biol. Cel.* **8**, 413-429.

Susin, A.S., Zamzami, N., Castedo, M., Daugas, E., Wang, H.-G., Geley, S., Fassy, F., Reed, J.C., Kroemer, G. (1997) The central executioner of apoptosis. Multiple links between protease activation and mitochondria in Fas/Apo-1/CD95- and ceramide induced apoptosis, *J. Exp. Med.* **186**, 25-37.

Tarba, C. (1983) Membrane potential in mitochondria estimated by absorption of a cyanine dye, *Rev. Roum. Biochim.* **20**, 61-65

Tarba, C. (2001) Apoptosis and necrosis: differences and similarities; the involvement of mitochondria, *Bull. Mol. Med.* **7-8**, 85-96

Tarba, C., Crăciun, C. (1990) A comparative study of the effects of procaine, lidocaine, tetracaine and dibucaine on the functions and ultrastructure of isolated rat liver mitochondria, *Biochim.Biophys.Acta* **1019**, 19-28

Tarba, C., Florea, A. (2005a) Corelaţii ultrastructurale hepatice privind efectele alcoolizării cronice şi tratamentului acut cu endotoxină bacteriană la şobolan: 1. Efectul tratamentului cronic cu etanol, *Anal. Soc. Naţ. Biol. Cel.* **10**, 188-200.

Tarba, C., Florea, A. (2005b) Corelaţii ultrastructurale hepatice privind efectele alcoolizării cronice şi tratamentului acut cu endotoxină bacteriană la şobolan: 2. Efectul tratamentului acut cu endotoxină, *Anal. Soc. Naţ. Biol. Cel.* **10**, 201-213.

Tarba, C., Florea, A. (2006a) Hepatic ultrastructural aspects of acute endotoxin administration to chronically ethanol-fed rats, in: *The 2nd International Congress of Cellular and Molecular Biology*, Iaşi (Romania), Abstract Book, p.71.

Tarba, C., Florea, A. (2006b) Diverse hepatic ultrastructural morphology induced by chronic ethanol feeding of rats, *Studia Univ. Babeş-Bolyai, Biol.* **51** (1**)**, 57-70.

Tarba, C., Suărăşan, F. (2003a) Corelaţii între fluxurile de calciu, tranziţia de permeabilitate şi colapsarea potenţialului de membrană în mitocondriile hepatice de şobolan. I. Efectul tratamentului cronic cu etanol, *Anal. Soc. Naţ. Biol. Cel.* **8**, 391-412.

Tarba, C., Suărăşan, F. (2003b) Corelaţii între fluxurile de calciu, tranziţia de permeabilitate şi colapsarea potenţialului de membrană în mitocondriile hepatice de şobolan. III. Efectul combinat al alcoolizării cronice şi administrării acute de endotoxină, *Anal. Soc. Naţ. Biol. Cel.* **8**, 430-446.

Tarba, C., Suărăşan, F. (2004a) Functional correlations between the permeability transition, membrane potential collapse and calcium release by liver mitochondria of ethanol-fed rats, *Studia Univ. Babeş-Bolyai, Biol.* **49** (1), 83-103.

Tarba, C., Suărăşan, F. (2004b) Effect of acute endotoxin administration to rats on membrane permeability and related functional parameters of liver mitochondria, *Studia Univ. Babeş-Bolyai, Biol.* **49** (1), 105-128.

Thurman, R.G. (1998) Mechanisms of hepatic toxicity: II.Alcoholic liver injury involves activation of Kupffer cells by endotoxin, *Am. J. Physiol.* **275** *(Gastrointest. Liver Physiol.* **38**) G605-G611.

Thurman, R.G., Bradford, B.U., Iimuro, I., Frankenberg, M.V., Knecht, K.T., Connor, H.D., Adachi, I., Wall, C., Arteel, G.E., Raleigh, J.A., Forman, D.T., Mason, R.P. (1999) Mechanisms of alcohol-induced hepatotoxicity: studies in rats, *Frontiers Biosci.* **4**, e42-46.

Thurman, R.G., Bradford, B.U., Iimuro, I., Knecht, K.T., Connor, H.D., Adachi, I., Wall, C., Arteel, G.E., Raleigh, J.A., Forman, D.T., Mason, R.P. (1997) Role of Kupffer cells, endotoxin and free radicals in hepatotoxicity due to prolonged alcohol consumption: studies in female and male rats, *J. Nutr.* **127**, 903S-906S.

Tsukamoto, H, Lu, S.C. (2001) Current concepts in pathogenesis of alcoholic liver injury, *FASEB J.* **15**, 1335-1349

Uesugi, T., Froh, M., Arteel, G.E., Bradford, B.U., Wheeler, M.D., Gable, E., Isayama, F., Thurman, R.G. (2002) Role of lipopolysaccharide-binding protein in early alcohol-induced liver injury in mice, *J. Immunol.* **168**, 2963-2969.

Wheeler, M.D. (2003) Endotoxin and Kupffer cell activation in alcoholic liver disease, *Alcohol Res. Health* **27**, 300-306

Wu, D., Cederbaum, A.J. (2003) Alcohol, oxidative stress and free radicals, *Alcohol Res. Health* **27**, 277-284.

Wu, Y., Chen, Y., Huang, H., Chen, P. (2004) Recombinant fibronectin polypeptide antagonizes hepatic failure induced by endotoxin in mice, *Acta Pharmacol. Sin.* **25**, 783-788.

Yoder, M.C., Egler, J.M., Yudkoff, M., Cratten, J., Douglas, S.D., Polin, R.A. (1985) Metabolic and mitochondrial changes that mimic Reye syndrome after endotoxin administration to rats, *Infect. Immun.* **47** (1), 329-331.

Yoshimoto, N., Togo, S., Kubota, T., Kamimukai, N., Saito, S., Nagano, Y., endo, I., Sekido, H., Nagashima, Y., Shimada, H. (2005) Role of transforming growth factor-β1 (TGF- β1) in endotoxin-induced hepatic failure after extensive hepatectomy in rats, *J. Endotox. Res.*, **11**, 33-39.

Yoza, B., LaRue, K., McCall, C. (1998) Molecular mechanisms responsible for endotoxin tolerance, *Progr. Clin. Biol. Res.* **397**, 209-215.

Zang, G.Q., Zhou, X.Q., Yu, H., Xie, Q., Zhao, G.M., Wang, B., Guo, Q., Xiang, Y.Q., Liao, D. (2000) Effect of hepatocyte apoptosis induced by TNF-α on acute severe hepatitis in mouse models, *World J. Gastroenterol.* **6**, 688-692.

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CIRCULATORY DITURBANCES INDUCED BY SOME ALKYLATING AGENTS WITH PLATINUM IN WHITE WISTAR RATS

CRISTINA PAŞCA[1] and VICTORIA-DOINA SANDU[1]

SUMMARY. The alterations induced by Cisplatin and Carboplatin (administered in equivalent doses and in monochemotherapy) at the level of the kidney, thymus, heart, liver, pancreas, digestive tract and lungs seem to be due (induced, maintained and then aggravated owing) to the grave circulatory disturbances such as: blood stasis, congestion, oedemas, haemorrhages and disseminate intravascular coagulation phenomena. All these circulatory alterations could be due both to the direct toxic effect of the drugs and thrombocytopenia induced by the two alkylating agents. Finally, they determine at the level of the organs a serious deficient supply with oxygen and nutritive substances, and, simultaneously, an accumulation of some toxic metabolic products which explain the massive destructions of the cells of parenchyma. The intensity, gravity and extension of the circulatory modifications were different, depending on the type of drug, organ and the moment of the sacrification. Thus, Carboplatin seems to possess a higher and longer toxic activity compared to Cisplatin, which appears immediately, aggravates progressively and significantly. In addition, circulatory disturbances induced by Carboplatin were graver, and affected especially the myocardium, thymus, pancreas and digestive tract. Cisplatin had a stronger toxic action on the vessels in the kidneys and lungs. Only moderate alterations of the circulatory system in the liver were induced by these drugs. Both cytostatics generated many vascular (and cellular) alterations with no irreversible character, so that, at the end of the experimental period, without any protective or regenerative treatment, an obvious recovery process could be observed at the level of the most affected organs.

KEYWORDS: Cisplatin, Carboplatin, circulatory disturbances, rats, histopathology, ultrastructure.

Introduction

Platinum compounds are cytotoxic agents used in the treatment of specific cancers. These include nonseminomatous germ cell testicular, advanced ovarian, refractory bladder, lung and squamous cell head and neck carcinomas.

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According to the previous studies, these compounds which are not cell cycle specific, after activation within the cell by displacement of chloride ions, platinum compounds produce inter- and intra-strand cross-links of DNA, which modifies DNA structure. This inhibits DNA replication, transcription and cell division and causes apoptosis of cells. Understanding details on activation of signal transduction by Cisplatin and Carboplatin leading to apoptosis, mostly responsible for the toxic adverse reactions of these platinum compounds, is likely to reveal novel strategies and improve combination therapies and efficacy (Arquilla *et al.,* 1983; Johnson *et al.,* 1989; Farrell, 1990; Hoeschele *et al.,* 1990; Sundquist and Lippard, 1990; Calvert, 1994; Grigg *et al.,* 1996; Millward *et al.,* 1996; Loskotova and Brabec, 1999; Ortiz *et al.,* 2000; Boulikas and Vougiouka; 2002).

Platinum compounds currently used are Cisplatin, Carboplatin and Oxaliplatin, but especially the first two, either used alone or in combination with other chemotherapeutic drugs. Side effects of platinum therapy include general cytotoxic effects, such as nausea and vomiting, myelosuppression and immunosuppression. Specific side effects include nephrotoxicity and peripheral neurotoxicity, the severity of which varies according to which platinum compound is used (Hill and Speer, 1982; Hruban *et al.,* 1991; Calvert, 1994; Grigg *et al.,* 1996; Karpova *et al.,* 2001; Boulikas and Vougiouka; 2002; Caglar *et al.,* 2002).

These agents do not have the same clinical efficacy in all cancer types, and the same intensity of different side effects. Thus, Cisplatin (Platinol or cis-diaminedichloro-platinum(II)) is currently used to treat testicular, ovarian, lung, bladder, head and neck cancers. Although a successful treatment agent, Cisplatin must been used with caution, being extremely nephrotoxic (damages kidneys) and requiring increased water intake and excretion before, during and after therapy. Cisplatin is the major factor affecting dose and kidney function must be carefully monitored. Cisplatin is also associated with severe nausea and vomiting in almost all patients. If nausea and vomiting cannot be controlled, Cisplatin dose may need to be reduced or ceased completely. Cisplatin has a low level of myelotoxicity (bone marrow suppression), which is related to dose (Hruban *et al.,* 1991; Grigg *et al.,* 1996; Karpova *et al.,* 2001).

Patients on Cisplatin therapy must also be monitored for low magnesium and low calcium levels in the blood, hearing problems, red and white cell levels, platelet function, liver function and neurological status. Tinnitus (ringing in the ears) and hearing loss in the high frequency range can occur, but these are usually transient and disappear after treatment has stopped. Peripheral and other nerve damage can occur, especially after prolonged treatment (Hill and Speer, 1982; Loskotova and Brabec, 1999; Zamble and Lippard, 1999; Caglar *et al*., 2002; Hotta *et al.,* 2004).

Carboplatin (Paraplatin) (cis -diamine-1,1-cyclobutanedicarboxylatoplatinum (II)) is a derivative of Cisplatin, and is mostly used in the treatment of advanced ovarian cancer. It can also be used in place of Cisplatin to treat small cell lung cancer. It has not yet been shown whether Carboplatin can be used in the treatment of bladder, cervical, endometrial, head and neck carcinomas.

Carboplatin works in a similar way to Cisplatin. However, its has slightly different side effects with less nephrotoxicity, neurotoxicity, ototoxicity, nausea and vomiting experienced by patients. Carboplatin is, however, more myelotoxic, which is the most common reason for decreasing dose. Other side effects include the liver damage, ototoxicity, electrolyte disturbances experienced with Cisplatin, and hair loss (Farrell, 1991; Belani, 2002; Boulikas and Vougiouka, 2002; Boulikas and Vougiouka, 2003; Hotta *et al.,* 2004). Often, Carboplatin is the drug of choice in view of the improved quality of life that it offers to the patients compared to Cisplatin, but the debate „Carboplatin versus Cisplatin" continues, usually, the decision of the medical oncologist being affected by the pacient cases, more exactly, by the features of the kidney, or liver, or heart, or bone marrow function.

Unfortunately, so far, the dynamics, structural and ultrastructural aspects of the toxic effects of these two platinum compounds at the level of the vascular component and parenchyma of the vital organs, and also the interrelationship between these two are incompletely known. Therefore, our investigations tried to establish comparatively the features, characteristics, dynamics and consequences of the circulatory disturbances induced by Cisplatin and Carboplatin, when they are administered (intravenously) in equivalent therapeutic doses, in monochemotherapy, in white Wistar rats.

Materials and Methods

Our experiments were carried out with the following twelve groups of healthy adult male Wistar rats, weighing 190 $\pm$ 10 g, fed with a standard diet and maintained under conventional (standard) bioclimatic laboratory conditions, starved for 18 hours before the treatment, but having water *ad libitum*:

-groups U_1, U_2, U_3 and U_4 - untreated (control) groups;

-groups: P_1, P_2, P_3 and P_4 treated intravenously with a single dose of Carboplatin (Paraplatin) (LD50), and sacrificed 24 hours, 4, 11 and 18 days after the treatment,

-groups: C_1, C_2, C_3 and C_4 treated intravenously with a single dose of Cisplatin (LD50), and sacrificed 24 hours, 4, 11 and 18 days after the treatment.

Having sacrificed the animals, we took fragments from the kidneys, thymus, spleen, left ventricles, stomach, duodenum, jejunum, ileum, liver, pancreas and lungs. For microscopic examination, the fragments were fixed in 10% neutral formol, processed by the paraffin technique and the sections of 6 μm were stained by the hematoxylin-eosin, and Masson-Goldner trichrome (Mureşan *et al.,* 1974). For ultrastructural investigations, fragments of thymus were prefixed in 2.7% glutaraldehyde solution and postfixed in 2% osmic acid solution. The fragments were dehydrated in acetone and then embedded in Vestopal W. The ultrathin sections were obtained using an LKB III ultramicrotome and were contrasted with uranyl acetate. Examination of the ultrathin sections was performed using a transmission electron microscope (Kay, 1967; Ploaie and Petre, 1979; Brenda, 1981).

On the stained and contrasted sections we studied, by light and electron microscopic examinations, the histological and ultrastructural modifications induced by the drugs at the level of the vascular component of the vital organs we mentioned, in concordance with the moment of the sacrification.

Results

Our light and electron microscopy investigations revealed the appearance of certain serious histopathological and ultrastructural modifications, which affected both the cellular and vascular components of the kidney, thymus, myocardium, liver, pancreas, digestive tract and lungs. The histological alterations seem to be due (induced, maintained and then aggravated owing) to the grave circulatory disturbances such as: blood stasis, congestion, oedemas, haemorrhages and disseminate intravascular coagulation phenomena. Besides, it seems that the features, intensity, gravity and extension of the hemodynamic disturbances were different, depending on the type of drug, organ, and the moment of the sacrification.

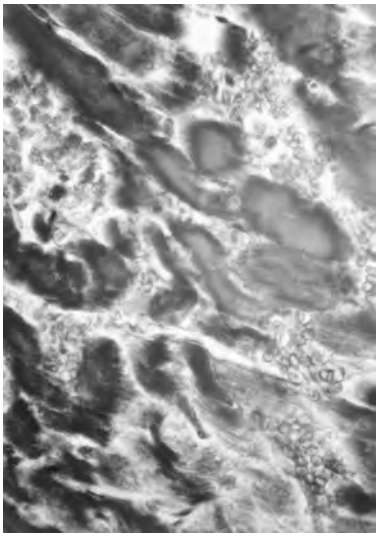

Fig. 1. Haemorrhages, oedemas and myolysis in the rat myocardium (x 1600).

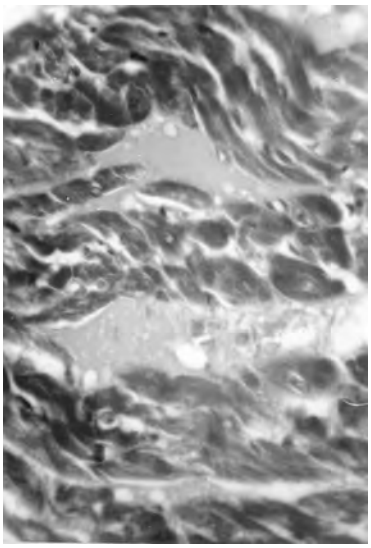

Fig. 2. Masive interfascicular oedemas and microhaemorrhages in myocardium (x 1600).

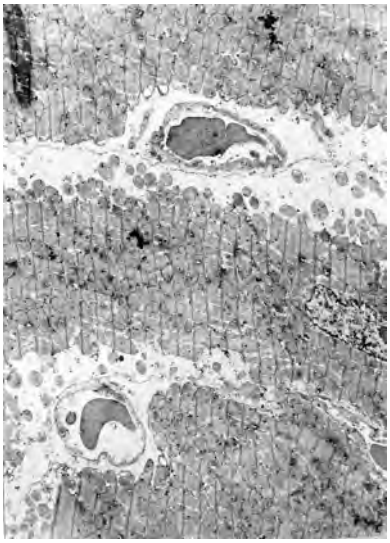

Fig. 3. Congestion, lysis of the sarcolemma and myofibrils, especially in the perifery of the myocytes, and interfascicular oedemas (x 4410).

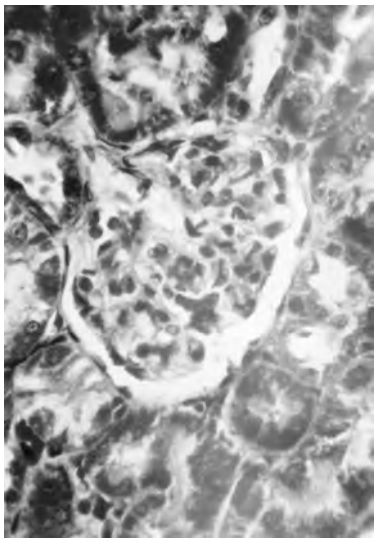

Fig. 4. Glomerular stasis correlated with diffuse intravascular coagulation phenomena (x 1600).

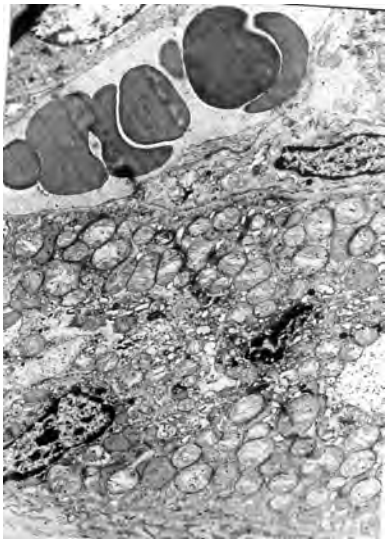

Fig. 5. Congestion in the renal capillaries, serious swelling of the mitochondria and disorganization of their matrix and cristae (x 6720).

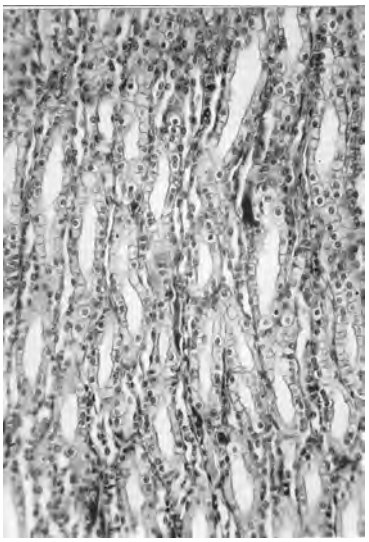

Fig. 6. Phenomena of diffuse intravascular coagulation in capillaries in the medulla of the kidney (x 1200).

Effect of the two alkylating agents at the level of the vascular component of myocardium. We noticed that Carboplatin was significantly more aggressive that Cisplatin. Thus, histopathological modifications already appeared 24 hour after the treatment, consisting of a light congestion and a diffuse oedema correlated with the presence of a cellular infiltrate. All these circulatory alterations got worse progressively during the next 3 days, the stasis, congestion, and oedema being more serious, and, in addition, many interfascicular haemorrhages appeared (Figs. 1-3). The circulatory disturbances correlated with a significant perivascular and interfascicular oedemas persisted, but more discreetly, in group sacrificed 11 days after the treatment, while, at the end of the 18 days of the experiment, the congestion, stasis, oedemas and haemorrhages had just a zonal character and were very discreet.

Also, Cisplatin disturbed the haemodynamic at the level of the myocardium, too, but the modifications (such as: congestion, stasis and slight oedemas) appeared later, were more discreet, had a zonal character and disappeared more quickly.

Effect of the two alkylating agents at the level of the vascular component of kidneys. A completely different situation could be observed at the level of the kidneys, where Cisplatin seemed to disturb more seriously the vascular component both in the cortex and medulla. Thus, 4 days after the treatment, an obvious

glomerular and interstitial stasis correlated with diffuse intravascular coagulation phenomena appeared (Figs. 4 and 5). Besides, such phenomena of diffuse intravascular coagulation could be noticed in the capillaries in the medulla, too (Fig. 6). After 11 days from the treatment, the intravascular coagulation phenomena had disappeared, but it could be observed a mesangial oedema and, in some areas, a mesangial hypercellularity correlated with some necrobiosis processes of the endothelial and mesangial cells. Seven days later, a significant glomerular sclerosis process (including at the level of the arterioles) correlated with the appearance of some interstitial lymphocyte infiltrations could be noticed here and there.

As compared to Cisplatin, Carboplatin disturbs the circulatory system less seriously, and reversibly, inducing just a moderate and zonal stasis, congestion and an extremely discrete and rare phenomena of intravascular coagulation.

Effect of the two alkylating agents at the level of the vascular component of lymphoid organs. Carboplatin seems to possess a higher and longer toxic action compared to Cisplatin at on the central and peripheral lymphoid structures – thymus and spleen, including at the level of their vascular components. Thus, the necrosis and necrobiosis of the thymocytes induced by Carboplatin are correlated with severe vascular disturbances: stasis, congestion, massive haemorrhages and diffuse intravascular coagulation phenomena – all with a reversible character. Besides, a change of the vascular permeability, both in thymus and spleen, determined massive perivascular oedemas (Figs. 7-10). All these alterations could be observed immediately after the treatment, developed progressively and significantly (especially at the level of the thymus) during the first 11 days of the experiment, and, then, decreased moderately.

Effect of the two alkylating agents at the level of the vascular component of digestive tract. The vascular component of the digestive tract (in duodenum and jejunum) seems to be more sensitive to Carboplatin than Cisplatin, and especially at the level of the mucosa, where it could be observe moderate and persistent vascular stasis, congestion, oedemas (Fig. 11) and, zonally, even microhaemorrhages. These aspects appeared in the groups sacrificed 4 and 11 days after the treatment, and decreased significantly at the end of the 18 days of the experiment.

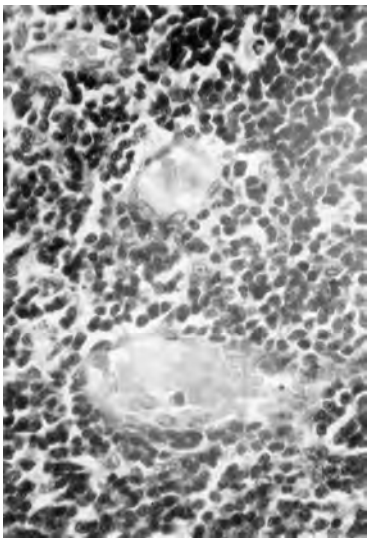

Fig. 7. Obvious congestion and stasis and discreet haemorrhages in the cortex of the thymic lobules (x 1600).

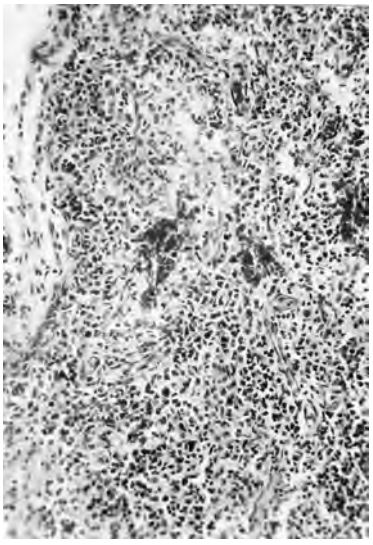

Fig. 8. Masive haemorrhages and diffuse intravascular coagulation phenomena in the cortex of the thymic lobules (x 512).

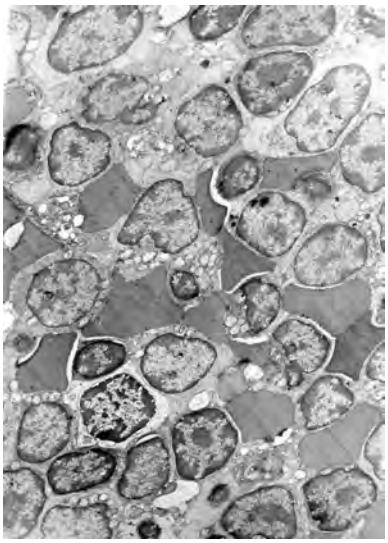

Fig. 9. Many erythrocytes between the thymocytes in the cortex of the thymic lobules (x 5880).

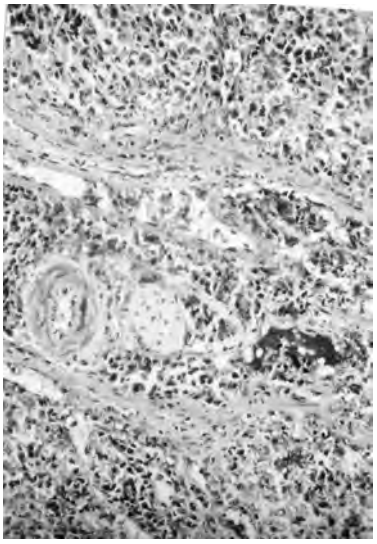

Fig. 10. Congestion, stasis and diffuse intravascular coagulation phenomena in the rat spleen (x 640).

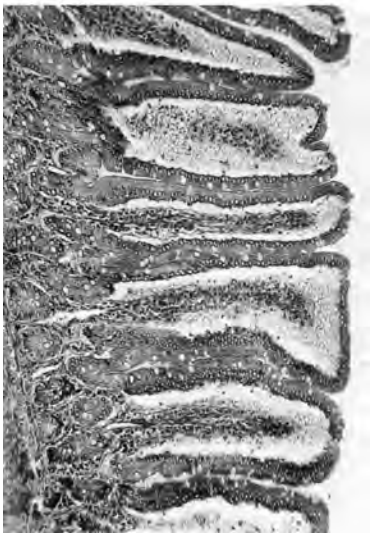

Fig. 11. Obvious oedemas of the villi in duodenum correlated with a significant alteration of the covering epithelium (x 512).

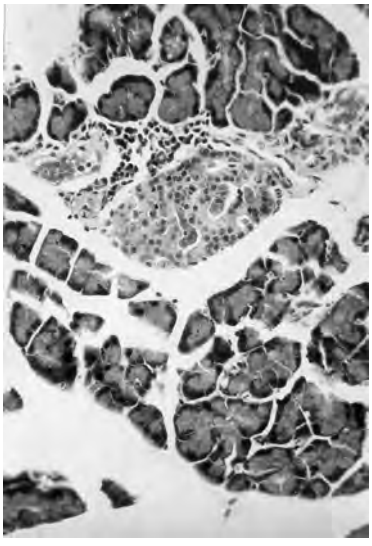

Fig. 12. Congestion, blood stasis, diffuse intravascular coagulation phenomena .and obvious oedemas both at the level of the Langerhans islets and between the pancreatic acini (x 640).

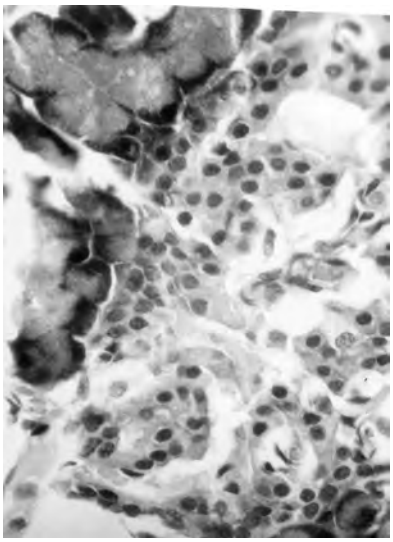

Fig. 13. Blood stasis, diffuse intravascular coagulation phenomena and obvious oedemas both at the level of the Langerhans islets and between the pancreatic acini (x 1600).

Effect of the two alkylating agents at the level of the vascular component of pancreas. Carboplatin seems to affect more serious the pancreas, too, but, the toxic effect are moderate and transitory. The histopathological aterations, both at the level of the exocrine and endocrine secretory units, seem to be due (induced and aggravated owing) to the serious circulatory diturbances – blood stasis, congeston, oedemas and intravascular coagulation phenomena (Figs. 12 and 13) – and to the drastic thrombocytopenia induced by Carboplatin.

Effect of the two alkylating agents at the level of the vascular component of liver. Both in groups treated with Carboplatin, and especially with Cisplatin, hemodynamic alterations in the liver were moderate, transitory and had a zonal character. They consisted of congestion and stasis at the level of the sinusoids and central vein of the lobules, and of the vessels in the Kiernan space. Only Carboplatin, zonally, induced a few microhaemorrhages (Figs. 14 and 15).

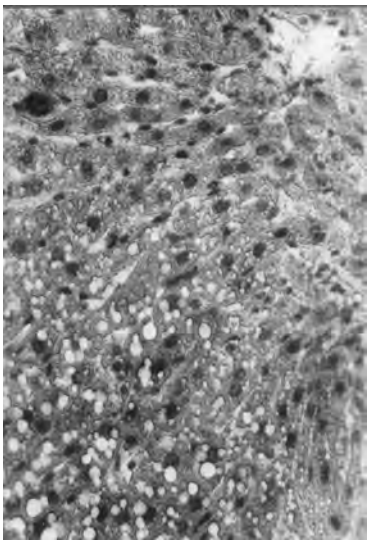

Fig. 14. Stasis and congestion at the level of the sinusoids and central vein of the hepatic lobules and fat degeneration of the hepatocytes (x 1600).

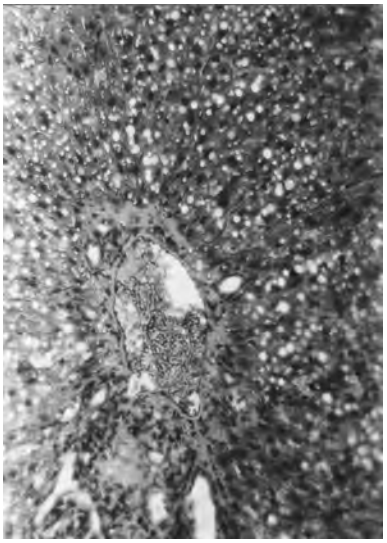

Fig. 15. Obvious congestion, discreet haemorrhages of the vessels in the Kiernan space and fat degeneration of the hepatocytes (x 1600).

Effect of the two alkylating agents at the level of the vascular component of lungs. Only in groups treated with Cisplatin we could notice significant histopathological modifications of the lungs, but they were moderate, affected well circumscribed and not very wide and numerous areas. The destructive phenomena of the lungs induced by Cisplatin seemed to be determined especially by certain circulatory disturbances (capillary stasis, congestion correlated with obvious oedemas and even some microhaemorrhages) which favoured the appearance of pneumocyte alterations and, finally, the losing of the integrity of the alveolar epithelium (haemorrhagic bronchopneumany) (Figs. 16-18).

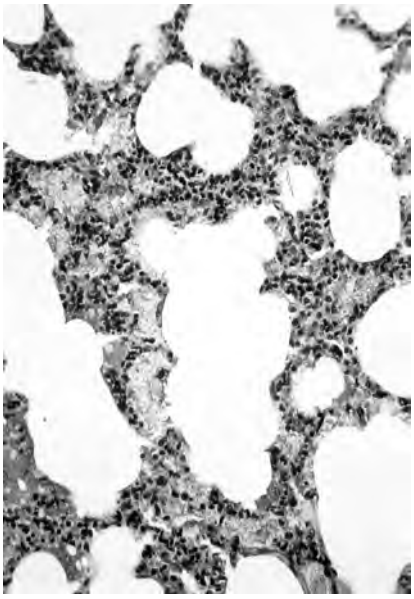

Fig. 16. Histopathological aspect of a serofibrinous and haemorrhagical bronchopneumony (x 512).

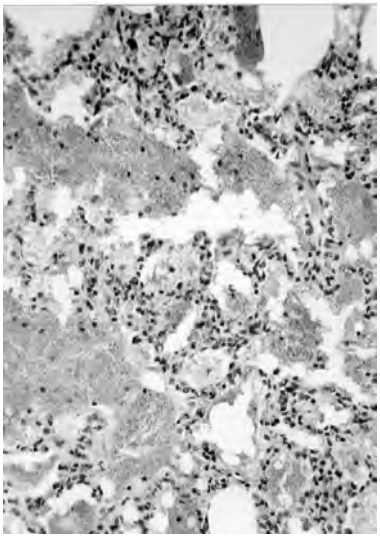

Fig. 17. Obvious vascular congestion in the lung (x 512).

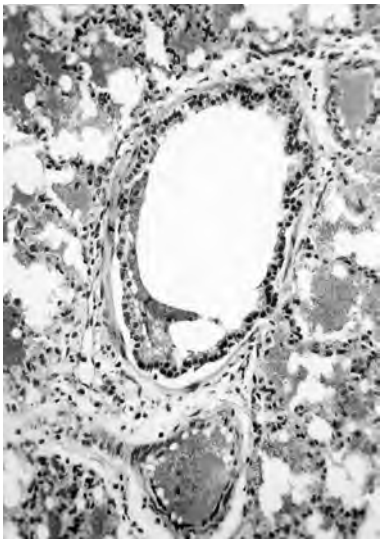

Fig. 18. Generalized congestion, stasis and oedemas in the lung (x 512).

Discussion

All the histopathological and ultrastructural aspects we mentioned above demonstrate that circulatory disturbances induced by Carboplatin were graver, and affected especially the myocardium, thymus, pancreas and digestive tract, while Cisplatin had a stronger toxic action only on the vessels in the kidneys and lungs. Both cytostatics, and especially Cisplatin, induced only moderate alterations of the circulatory system in the liver.

All these circulatory alterations could be due both to the direct toxic effect of the drugs and thrombocytopenia induced by the cytostatics, and finally determine at the level of the organs a serious deficient supply with oxygen and nutritive substances, and, simultaneously, an accumulation of some toxic metabolic products which explain the massive destructions of the cells of parenchyma.

According to the previous studies concerning the toxic effects of alkylating agents with platinum, Cisplatin and especially Carboplatin seem to be responsible for certain hematologic abnormalities that require emergent treatment, as a consequence of an abnormal hematopoiesis and coagulopathy. Underproduction of the blood cell lines is due to the myelotoxicity and results in thrombocytopenia, leucopenia, anemia and disseminated intravascular coagulation (Walker *et al.*, 1989; Hruban *et al.*, 1991; Kotte-Marchant; 1994; Sallah and Kato, 1998; Weijl *et al.*, 2000).

Thrombotic microangiopathies are microvascular occlusive disorders characterized by hemolytic anemia caused by fragmentation of erythrocytes and thrombocytopenia due to increased platelet aggregation and thrombus formation, eventually leading to disturbed microcirculation with reduced organ perfusion. Thrombotic microangiopathic hemolytic anemia has been associated with several chemotherapeutic agents incuding Carboplatin, but not in case of Cisplatin, and often is correlated with widespread microvascular thrombosis, particularly severe in the heart, kidney and brain (Walker *et al.*, 1989; Marani *et al.*, 1996; Moake and Byrnes, 1996; Franchini, 2006). As we already mentioned, such kind of thromboembolic complications caused by hemolytic anemia correlated with thrombocytopenia could be noticed at the level of the most vital organs studied by us (kidney, heart, thymus, spleen, pancreas), only in groups treated with Carboplatin.

Although the pathogenesis of chemotherapy-related thrombotic microangiopathy remains unclear, an elevated von Willebrand factor antigen and pathologic evidence of endothelial hyperplasia in these cases suggest that an abnormality of the endothelium is related to the develop of the clinical syndrome (Walker *et al.*, 1989; Karpova *et al.*, 2001; Franchini, 2006).

Also, our investigations confirm the previous studies according to which depression of the bone marrow activity induced by Carboplatin results in appearance of a diffuse intravascular coagulation phenomena (DIC). DIC represents a massive activation of the coagulation cascade, that results in excessive production of thrombin, systemic intravascular fibrin deposition, and clotting factors and platelet consumption.

The initiating factor is the release of tissue factor from a variety of causes: extensive endothelial injury, the monocytes response to endotoxin exposure or to various cytokines. DIC is a systemic process that causes haemorrhages, microangiopathic hemolytic anemia and thrombosis of varying degrees. Often, disseminated intravascular coagulation caused by the bone marrow hypoplasia, may be the first haematological sign of toxicy, with the most severe coagulopathy occurring about 25 hours after the administration of a large dose of Carboplatin, and may contribute to haemorrhagic and thrombotic events, which have to be immediately treated to improve or save the life of the patient (Siegal *et al.*, 1978; Feinstein, 1982; Carr *et al.*, 1989; walker *et al.*, 1989; Bick, 1992; Weijl *et al.*, 2000). The main clinical manifestation which appeared to be related solely to DIC were (in a decreasing order or frequency): bleeding (64.4%), renal dysfunctions (24.6%), liver dysfunctions (18.6%), respiratory dysfunctions (16.1%), shock (14.4%), thromboembolic phenomena (6.8%) and central nervous system involvement (1.7%) (Siegal *et al.*, 1978).

Nowadays, it is known that alkylating agents with platinum, especially Carboplatin and very discreet Cisplatin, are responsible for the appearance of the DIC phenomena and other circulatory disturbanced, but the investigations concerning the etiology, pathophyiology, clinical and laboratory diagnosis, and management of fulminant and low-grade DIC have to continue to elucidate the incompletely known aspects. Considerable attention have to be devoted to the interrelationships within the hemostasis system. Only by clearly understanding these pathophysiologic interrelationships, the clinician and laboratory scientist can appreciate the divergent and wide spectrum of often confusing clinical and laboratory findings in patients with circulatory disturbances (including DIC). Many therapeutic decisions to be made are controversial and will remain so until more is published about specific therapeutic modalities and survival patterns. Also, therapy must be highly individualized, depending on the nature, degree of DIC, age, etiology of DIC, site and severity of haemorrhages or thrombosis, and hemodynamic parameters. Thus, the alkylating agents could be used more safely in the chemotherapy of different types of malignant diseases.

Conclusions

1. Both antineoplastic alkylating agents – Carboplatin and Cisplatin - in monochemotherapy and administered in a single dose (equivalent to the LD50), induce certain histological and ultrastructural modifications which affected both the cellular and vascular components of the most vital organs of the white Wistar rats.

2. The histological and ultrastructural alterations seem to be due (induced, maintained and then aggravated owing) to the grave circulatory disturbances such as: blood stasis, congestion, oedemas, haemorrhages and disseminate intravascular coagulation phenomena.

3. All these circulatory alterations could be due both to the direct toxic effect of the drugs and thrombocytopenia induced by the cytostatics, and finally determine at

the level of the organs a serious deficient supply with oxygen and nutritive substances, and, simultaneously, an accumulation of some toxic metabolic products which explain the massive destructions of the cells of parenchyma.

4. The intensity, gravity and extension of the circulatory disturbances were different, depending on the type of drug, organ and the moment of the sacrification.

5. Carboplatin seems to possess a higher and longer toxic activity compared to Cisplatin, which appears immediately, aggravates progressively and significantly.

6. Circulatory disturbances induced by Carboplatin were graver, and affected especially the myocardium, thymus, pancreas and digestive tract. Cisplatin had a stronger toxic action on the vessels in the kidneys and lungs. Both Carboplatin and Cisplatin induced only moderate circulatory alterations in liver.

7. Circulatory disturbances induced by the two cytostatics in monochemotherapy had no irreversible character, so that, at the end of the experimental period, without any protective or regenerative treatment, an obvious recovery process could be observed at the level of the most affected organs.

8. The progressive decreasing of the circulatory disturbances allowed and encouraged the recovery processes of the affected tissues.

REFERENCES

Arquilla, M., Thompson, L.M., Pearlman, L.F, Simpkins, H. (1983) Affect of platinum antitumor agents on DNA and RNA investigated by terbium fluorescence, *Cancer Res.*, **43**, 1211-1216.

Belani, C.P. (2002) Docetaxel in combination with platinums (cisplatin or carboplatin) in advanced and metastatic non-small cell lung cancer, *Semin. Oncol*., **29**, 4-9.

Bick, R.L. (1992) Disseminated intravascular coagulation, *Hematol. Oncol. Clin. North Am*., **6**(6), 1259-1285.

Boulikas, T., Vougiouka, M. (2002) Recent clinical trials using cisplatin, carboplatin and their combination chemotherapy drugs, *Int. J. Oncol.*, **21**, 1347-1352.

Boulikas, T., Vougiouka, M. (2003) Cisplatin and platinum drugs at the molecular level, *Oncol. Reports*, **10**, 1663-1682.

Brenda, S.W. (1981) A Beginner's Handbook in Biological Transmission Electron Microscopy, Butler & Tanner, London.

Caglar, K., Kinalp, C., Arpaci, F., Turan, M., Saglam, K., Ozturk, B., Komurku, S., Yavuz, I., Yenicesu, M., Ozet, A., Vural, A. (2002) Cumulative prior dose of cisplatin as a cause of the of nephrotoxicity of high dose chemotherapy followed by autologous stem-cell transplantation, *Nephrol. Dial. Transplant*, **17**, 1931-1935.

Calvert, A.H. (1994) Dose optimization of carboplatin in adults, *Anticancer Res.*, **14**, 2273-2278.

Carr, J.M., McKinney, M., McDonagh, J. (1989) Diagnosis of disseminated intravascular coagulation. Role of D-dimer, *Am. J. Clin. Pathol.*, **91**, 280-287.

Farrell, N. (1991) Structurally novel platinum antitumor compounds. In Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy, (Howell, S.B., ed), 81-91. Plenum Press, New-York.

Farrell, N., Qu, Y., Feng, L., Van Houten, B. (1990) Comparison of chemical reactivity, cytotoxicity, interstrand cross-linking and DNA sequence specificity of bis (platinum) complexes containing monodentate or bidentate coordination spheres with their monomeric analogues, *Biochemistry*, **29**, 9522-9531.

Feinstein, D.I. (1982) Diagnosis and management of disseminated intravascular coagulation: the role of heparin therapy, *Blood,* **60**(2), 284-287.

Franchini, M. (2006) Thrombotic microangiopathies: An update, *Hematol.*, **11**(3), 139-146.

Grigg , A., Szer, J., Skov, K., Barnett, M. (1996) Multi-organ dysfunction associated with high-dose carboplatin therapy prior to autologous transplantation, *Bone Marrow Transplantation***, 17**, 67-74.

Hill, J.M., Speer, R.J. (1982) Organo-platinum complexes as antitumor agents, *Anticancer Res.*, **2**, 173-186.

Hoeschele, J.D., Kraker, A.J., Qu, Y., Van Houten, B., Farrell, N. (1990) Bis (platinum) complexes, chemistry, antitumor activity and DNA-binding. In Molecular Basis of Specificity in Nucleic Acid-Drug Interactions (Pullman, B., Jortner, J., eds.), 301-321. Academic Press, Dordrecht, The Netherlands.

Hotta, K., Matsuo, K., Ueoka, H., Kiura, K., Tabata, M., Tanimoto, M. (2004) Meta-analysis of randomized clinical trias comparing cisplatin to carboplatin in patients with advanced non-small-cell lung cancer, *J. Clin. Oncol.*, **22** (19), 3852-3859.

Hruban, R.H., Sternberg, S.S., Meyers, P., Fleisher, M., Menendez - Botet, C., Boitnott, J.K. (1991) Fatal thrombocytopenia and liver failure associated with carboplatin therapy, *Cancer Invest.* **9**(3), 263-268.

Johnson, N.P., Butour, J.L., Villani, G., Wimmer, F.L., Defais, M., Pierson, V., Brabec, V. (1989) Metal anti-tumor compounds : the mechanism of action of platimun complexes, *Prog. Clin. Biochem. Med.*, **10**, 1-24.

Karpova, G.V., Fomina, T.I., Voronova, O.L., Abramova, E.V., Loskutova, O.P. (2001) Early and delayed effects of Carboplatin on the blood system, *Bull. Exp. Biol. Med.* **132** (5), 1065-1069.

Kay, D. (1967) Techniques for Electron Microscopy, Alden & Mowbray , Oxford.

Kotte – Marchant, K. (1994) Laboratory diagnosis of hemorrhagic and thrombotic disorders, *Hematol. Oncol. Clin. North Am.*, **8**, 809-853.

Loskotova, H., Brabec, V. (1999) DNA interactions of cisplatin tethered to the DNA minor groove binder distamycin, *Eur. J. Biochem.*, **266**, 392-402.

Marani, T.M., Trich, B.M., Armstrong, K.S., Ness, P.M., Smith, J., Minniti, C., Sandler, S. G. (1996) Carboplatin-induced immune haemolytic anemia, *Transfusion,* **(11-12)**, 1016-1018.

Millward, M.J., Webster, L.K., Toner, G.C., Bishop, J.F., Rischin, D., Stokes, K.H., Johnston, V.K., Hicks, R. (1996) Carboplatin dosing based on measurement of renal function – experience at the Peter MacCallum Cancer Institute, *Cancer Res.*, **26**, 372-379.

Moake, J.L., Byrnes, J.J. (1996) Thrombotic microangiopathies associated with drugs and bone marrow transplantation, *Hematol. Oncol. Clin. North Am.*, **10**, 485-497.

Mureşan, E., Gaboreanu, M., Bogdan, A.T., Baba , A.I. (1974) Tehnici de histologie normală şi patologică, Ed. Ceres, Bucureşti.

Ortiz, A., Lorz C., Catalan, M.P., Justo, P., Egido, J. (2000) Role and regulation of apoptotic cell death in the kidney. Y2K Update, *Frontiers in Bioscience*, **5**, 735-749.

Ploaie, P.G., Petre, Z. (1979) Introducere în microscopia electronică cu aplicații la biologia celulară şi moleculară, Ed. Acad. R.S.R , Bucureşti.

Sallah, S., Kato, G. (1998) Evaluation of bleeding disorders. A detailed history and laboratory tests provide clues, *Postgraduate Medicine*, **103**, 209-210.

Siegal, T., Seligsohn, U., Aghai, E., Modan, M. (1978) Clinical and laboratory aspects of disseminated intravascular coagulation (DIC): a study of 118 cases, *Thromb. Haemost.*, **39**(1), 122-134.

Sundquist, W.I., Lippard, S.J. (1990) The coordination chemistry of platinum anticancer drugs and related compounds with DNA, *Coord. Chem. Rev.*, **100**, 293-322.

Walker, R.W., Rosenblum, M.K., Kempin, S.J., Christian, M.C. (1989) Carboplatin associated thrombotic microangiopathic hemolytic anemia, *Cancer*, **64**(5), 1017-1020.

Weijl, N.I., Rutten, M.F.J., Zwinderman, A.H., Keizer, H.J., Nooy, M.A., Rosendaal, F.R, Cleton, F.J., Osanto, S. (2000) Thromboembolic events during chemotherapyfor germ cell cancer: A cohort study and rewiew of the literature, *J. Clin. Oncol.*, **18**(10), 2169-2178.

Zamble, D.B., Lippard, S.J. (1999) The response of cellular proteins to cisplatin-damaged DNA, in Cisplatin Chemistry and Biochemistry of a Leading Anticancer Drug (Lippert, B., ed.), 73-110. VHCA, Wiley-VCH, Zurich.

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MAIZE WATER CONSUMPTION AND WATER USE EFFICIENCY UNDER THE INFLUENCE OF DIFFERENT COMPONENTS OF TECHNOLOGY

CORNEL DOMUȚA[1]

SUMMARY. The results of our research were obtained in Oradea, Western Romania, during 2001-2005. Maize water consumption was determined by using the water balance method on 0-150 cm profile of the soil, in both nonirrigated and irrigated conditions. Determinations made every ten days in the irrigated variant, maintained the soil moisture between easily available water content and field capacity on 0-75 cm profile of the soil. Water use efficiency was calculated by reporting the yield to water consumption. All the components of the technology applied, such as crop rotation (melioration, crop rotation of 3 and 2 years, monocrop), hybrid use, plant density (25, 40, 55, 70 and 85 thousand plants/hectare), soil tillage (plow at 25 cm and 12 cm depth, ciesel, disk), fertilization (manure, manure + $N_{90}P_{45}$), irrigation and weed control, significantly influenced the values of the maize water consumption and of the water use efficiency. These findings underline the importance of optimizing every technological component of the maize crop.

KEYWORDS: maize, technology, water consumption, water use efficiency

Introduction

Plant water consumption is the water quantity used in the soil during a period of time which includes the productive consumption of the plant and the unproductive consumption (water evaporation from soil surface) (Bandici, 2001, Botzan, 1966, Domuța 2003, 2005, Donnen and Westcot, 1988, Pruitt, 1992, Grumeza *et al.*, 1989, Ionescu – Şişeşti, 1986).

Plant water consumption is the basic element of the land reclamation projects (Grumeza *et al.*, 1989). Using a very rigorous data of the plant water consumption in the irrigation design may be an optimum network for water transport with positive economical and ecological consequences (Domuța, 2003). In irrigation scheduling, one of the most used methods in the world is based on the daily soil water balance and the use of crop coefficients. The crop coefficients are established as the ratio between optimum water consumption and the reference evapotranspiration (ET_0). (Doorembos and Kassam,1986)

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First studies regarding maize water consumption in Romania were carried out by Botzan in 1945, in Marculesti and Studina. The investigations were developed after 1969 in the research programme "Irrigation and drainage exploitation" lead by Grumeza at the Research Institute for Irrigation and Drainage Baneasa – Giurgiu. More than 20 long term trials were used in this programme, in the main areas with possibilities of irrigation. One of the problems studied there was the optimum water consumption of the irrigated crops and the water consumption of nonirrigated crops. Direct correlations, based on these data, between maize water consumption (and of the other crops, too) and yield, were quantified using different mathematical expressions (Bălăceanu, 1983, Bora, 1991, Domuța, 1997, Domuța *et al.*, 2002, 2003, 2004 a, 2004 b, Grumeza *et al.*, 1989, Petrescu and Groza, 2003, Renea and Tatariu, 1974, Săndoiu, 1974, Vâjială, 1978) Previous reports also emphasized the differences of the maize water consumption values according to the climate characteristics.

The correlation between temperature and maize water consumption in the Oradea area (Western Romania) was not statistically assured to date. The correlation was established between rainfall from the maize`s vegetation period and water consumption and this proved to be was statistically significant. A statistically significant link between the climate indicators values and maize water consumption was quantified when the climate elements were calculeted by using climate indicators (de Martonne aridity index, Seleaninov coefficient, hydroheliothermic index, Domuța climate index) (Domuța, 1997, Petrescu and Groza, 2003).

Water use efficiency can be calculated as a ratio between yield and water consumption, thus emphasizing the quantity of yield obtained for one unit of water and as a ratio between water consumption and yield, emphasizing the quantity of water for one unit of yield. Maize water use efficiency is determined in the reasearch paper referenced, and in most of the areas and situations, the irrigation determined the improvement of water use efficiency. Naescu and Alionte (2003) and Borza (2005 a) studied the hybrid influence on water use efficiency. Borza (2005 b) and Șandor (2002) determined a better water use efficiency in wheat – maize – soybean crop rotation, compared to maize – wheat crop rotation and monocrop.

The present paper is based on the research carried out during 2001 – 2005 and wants to establish the influence of some of the technology`s components – crop rotation, the type of hybrid, plant density, fertilization, soil tillage, weed control, water supply – on maize water consumption and on water use efficiency.

Materials and Methods

The investigations were carried out in Oradea, Western Romania on preluvosoil with the following horizons: Ap=0-24 cm; El=24-34 cm, Bt_1=34-54 cm; Bt_2=54-78 cm; Bt/c=78-95 cm; C=95-145 cm.

In the horizons Bt_1 and Bt_2, the values of the colloid clay were 39.8% and 39.3%, respectively.

The hydrostability of the macro-aggregates in the ploughed depth was high (47.5%) and the total porosity was medium (46%); hydraulic conductivity was medium on 0 – 20 cm depth (21.0 mm/h) and small (4.4 mm/h) and very small (0.1 mm/h) below. Bulk density was high on all the soil's profile (1.41-1.65 g/cm^3). Field capacity (FC) and wilting point (WP) had medium values in all soil profiles (23.6%-25.1%, respectively 9.2%-14.2%). Easily available water content (Wea) was established by using the following formula:

$$Wea = WP + 2/3(FC - WP);$$

Soil reaction was acidic (pH = 6.8), humus content (1.75%) was low and total nitrogen was small towards medium. The phosphorus (22.0 ppm) and potassium (145.4 ppm) in soil had medium values.

Maize water consumption ($\sum(e + t)$) was determined by soil water balance method, using the following formula:

$$WRi + R + I = WRf + \sum(e + t)$$

where:

Wri = initial soil water reserve
R = rainfall
I = irrigation
WRf = final soil water reserve

Soil water balance was calculated in the 0-150 cm of the soil horizon. The soil moisture was determined by gravimetric method on the soil horizon 0 – 50 cm and by neutron method on the soil horizon 50-150 cm. This scheme for moisture determination assured a good precision of the research data (Domuța 1997).

Optimum water consumption was achieved by maintaining the soil water content between field capacity and easily available water content in the soil horizon 0 – 75 cm. This was performed by determining the soil moisture every ten days and by using irrigation every time it was needed. The following irrigation schemes were used during 2001 – 2005 in the maize irrigation season: 48.0 mm applied once in August 2001, 240 mm applied in 4 rates (2 in June, 1 in July and August) in 2002, 268 mm applied in 5 rates (in June, 2 in July and August) in 2003, 160 mm applied in 3 rates (1 in June, July and August) in 2004, 75 mm applied in 2 rates (August) in 2005.

Water use efficiency was determined as a ratio between the maize yield and water consumption (Doremboos and Kassam, 1986, Pejic *et al.*,2002, Popova and Kercheva, 2002).

The study of the influence of the various technology components on maize water consumption was carried out in specific experiments located in the same research field.

The rainfalls registered during the maize vegetation period were: 450.4 mm in 2001, 289.4 mm in 2002, 194.9 mm in 2003, 384.6 mm in 2004 and 419.3 in 2005. The rainfall registered during the agricultural year (October – September) were: 868.5 mm in 2001, 43.5 mm in 2002, 501.1 in 2003, 737.5 mm in 2004 and 290.2 mm in 2005.

Results and Discussions

Influence of crop rotation. The experiment concerning crop rotation started in 1990, but only the results obtained between 2001 – 2005 are presented in this paper (Table 1).

The influence of the four kinds of crop rotation was studied both in nonirrigated and irrigated conditions: maize – monocrop; maize – wheat; maize – soybean – wheat; alfalfa 1st year – alfalfa 2nd year – maize bean – wheat –sugarbeet – soybean – sunflower – potato.

In the meliorative crop rotation with alfalfa a higher quantity of rainfall was stored in the cold period in comparison with monocrop, with wheat – maize crop rotation and with maize –soybean – wheat crop rotation. The larger amount of rainfall stored in the soil, determined the increase of the total water consumption and the increase in water consumption per plant. Water use efficiency improved in all the crop rotation experiments, in comparison with the monocrop; the highest values of water use efficiency being obtained in the meliorative crop rotation, both in unirrigated and irrigated conditions.

Table 1

Influence of crop rotation on maize water consumption [Σ (e+t)] and on water use efficiency (WUE), Oradea 2001 – 2005

Crop rotation	Variant					
	Nonirrigated			Irrigated		
	Σ (e+t)		WUE	Σ (e+t)		WUE
	mm/ha	l/plant	kg/mm	mm/ha	l/plant	kg/mm
1.Maize– monocrop	448	81	14.4	588	107	14.5
2.Maize –wheat	451	82	15.2	590	107	15.3
3.Maize– soybean – wheat	459	83	15.9	598	109	16.0
4.Meliorative *) on	466	85	18.2	604	110	20.4
LSD 5%	3	0,5	0.2	2	0.4	0.3
LSD 1%	7	1.3	0.6	5	0.9	0.9
LSD 0.1%	16	2.9	1.3	11	2.0	1.4

*) Alfalfa 1st year – Alflafa 2nd year – Maize – Bean – Wheat – Sugarbeet – Soybean – Sunflower – Potato

Influence of hybrid on water consumption. Representative hybrids for every FAO (Food and Agricultural Organization) group were tested: Ciclon, FAO group 100-200; Saturn, FAO group 300-400; ZP 335, FAO group 400-500; Fundulea 376, FAO group 500-600 and Fundulea 365, FAO group >600.

The lowest water consumption was determined in the earliest hybrid, Ciclon, 426 mm/ha (77 l/plant) in nonirrigated conditions and 581 mm/ha (106 l/plant) in irrigated conditions. The increase of the hybrid vegetation period determined the increase of water consumption; the highest value was obtained in the hybrids with the longest vegetation period, 460 mm/ha (84 l/plant) in nonirrigated variant and 627 mm/ha (114 l/plant) in irrigated variant. (Table 2)

Regarding the water use efficiency, the behaviour of the studied hybrids is different. In nonirrigated conditions the biggest value of water use efficiency 16.9 kg/mm was registered in hybrid Fundulea 376 (from 500-600 FAO group), but in irrigated conditions, the biggest water use efficiency (18.2 kg/mm) was registered in the hybrid with the longest vegetation period, Fundulea 365 (from over 600 FAO group).

Table 2

Influence of maize hybrid on water consumption [Σ(e+t)] and on water use efficiency (WUE), Oradea 2001 – 2005

FAO group	Hybrid	Variant					
		Nonirrigated			Irrigated		
		Σ (e+t)		WUE	Σ (e+t)		WUE
		mm/ha	l/plant	kg/mm	mm/ha	l/plant	kg/mm
100-200	Ciclon	426	77	15.8	581	106	14.6
200-300	Turda Super	441	80	16.3	589	107	15.5
300-400	Saturn	444	81	16.1	590	107	16.7
400-500	ZP 335	447	81	16.6	606	110	17.4
500-600	Fundulea 376	457	83	17.2	621	113	18.0
>600	Fundulea 365	460	84	16.9	627	114	18.2
LSD 5%		8	1.5	0.2	7	1.3	0.4
LSD 1%		12	2.18	0.6	11	2.0	0.9
LSD 0.1%		27	4.90	1.3	2.4	4.36	2.4

Plants density. Five types of the plant density were studied. 25000 plants/ha; 40000 plants/ha; 55000 plants/ha; 70000 plants/ha; 85000 plants/ha. The hybrid used was Turda super.

Table 3

Influence of plant density on maize water consumption [Σ (e+t)] and on water use efficiency (WUE), Oradea

Plants /hectare	Variant					
	Nonirrigated			Irrigated		
	Σ (e+t)		WUE	Σ (e+t)		WUE
	mm/ha	l/plant	kg/mm	mm/ha	l/plant	kg/mm
25000	426	170	13.9	570	228	13.6
40000	430	108	15.6	573	143	15.4
55000	437	98	18.8	578	105	17.6
70000	442	63	16.6	587	84	18.8
85000	445	52	14.6	597	70	16.2
LSD 5%	4	17	0.3	3	15	0.4
LSD 1%	7	31	0.7	5	29	0.9
LSD 0.1%	16	59	1.5	13	54	1.7

Calculating the maize water consumption in mm/ha, the values increased along with the increase of plants density, but calculating the maize water consumption in liters/plant, the values decreased with the increase of the plants' density. The variance analysis emphasizes the differences statistically assured in all cases (Table 3).

In nonirrigated conditions, the highest water use efficiency (18.8 kg/mm) was obtained using 55000 plants/hectare. In irrigated conditions, the highest result (18.8 kg/mm) was obtained using 70000 plants/hectare. In both nonirrigated and irrigated variants in the droughty year, the highest values were obtained using a plants' density smaller than the average value of the research period.

Soil tillage. The soil from the research field is heavy and ploughland is the base soil tillage because it improves the air, water and nutrition regime for the plant (Domuța, 2005).

The variants studied were: ploughland 25 cm, ploughland 12 cm, ciesel and disc. The ploughlands and the work with ciesel were made in autumn and the work with roller disk was made in spring, before maize sowing.

By ploughing at 25 cm depth in autumn, a bigger quantity of rainfall, which had fallen in the cold period, was stored in the soil. As a result, the biggest value of the maize water consumption was registered in this variant, 462 mm/ha (84 l/plant). In all the other variants, the values of the maize water consumption are considerably smaller (Table 4).

Maize water use efficiency showed very significant modifications under the soil tillage influence. The value recorded in the variant with ploughland of 25 cm depth (15.3 kg/mm) was 54.5% higher than in the variant with ploughland of 12 cm, 84.3% higher than variant the with ciesel and 142.9% higher than the variant with disk.

Table 4

Influence of soil tillage on maize water consumption [Σ(e+t)] and on water use efficiency (WUE), Oradea 2001 – 2005

Variant	Σ(e+t)		WUE
	mm/ha	l/plant	kg/mm
1.Ploughland 25 cm	462	84	15.3
2.Ploughland 12 cm	436	79	9.9
3.Ciesel	412	75	8.3
4.Disk	402	73	6.3
LSD 5%	3	0.5	1.3
LSD 1%	6	1.1	2.7
LSD 0.1%	15	2.7	5.4

Ferilization influence. Fertilization is an important component of the maize technology from Western Romania (Ciobanu, 2003, Samuel, 2003). The influence of the ferilization on maize water consumption was studied in the next variants: control-without fertilizer; manure 30 t/ha; manure 30 t/ha + $N_{90}P_{45}$.The manure was applied in autumn before ploughland; chemical fertilizers were applied at sowing.

Manure applications determined a higher storage of rainfall in the soil during the cold period. In these conditions, maize water consumption increased significantly, in both nonirrigated and irrigated conditions (Table 5).

Organic fertilization (15.2%; 19.4%) and especially organic+mineral fertilization (45.4%; 51.8%) determined important improvements of the water use efficiency both in nonirrigated and irrigated conditions; the differences in comparison with control variant being statistically significant in all the cases.

Table 5

Influence of fertilization on maize water consumption [Σ (e+t)] and on water use efficiency (WUE), Oradea 2001 – 2005

Fertilization	Variant					
	Nonirrigated			Irrigated		
	Σ (e+t)		WUE	Σ (e+t)		WUE
	mm/ha	l/plant	kg/mm	mm/ha	l/plant	kg/mm
Control	451	82	9.9	584	106	10.8
Manure, 40 t/ha	461	84	11.4	600	109	12.9
Manure 40 t/ha, N90P45	464	84	14.4	604	110	16.4
LSD 5%	3	0.4	0.4	5	1.2	0.7
LSD 1%	7	1.5	0.7	8	2.4	1.8
LSD 0.1%	12	3.6	1.2	15	3.9	3.9

Table 6

Influence of water supply on maize water consumption [Σ (e+t)] and on water use efficiency (WUE), Oradea 2001 – 2005

Variant	Σ (e+t)		Yield kg/ha	WUE kg/mm
	mm/ha	l/plant		
1.Nonirrigated	488	88	6790	13.9
2.Optimum provisionament	681	124	10827	15.9
3.Irrigation suspended in May	654	119	9614	14.7
4. Irrigation suspended in June	621	113	8756	14.1
5. Irrigation suspended in July	639	116	9713	15.2
6. Irrigation suspended in August	640	116	9408	14.7
LSD 5%	7	1.3	310	0.3
LSD 1%	15	2.7	490	0.8
LSD 0.1%	39	7.1	830	1.5

Influence of water supply. The water supply variant studied: nonirrigated; optimum supply – maintaining the soil water reserve between easily available water content on 0-75 cm depth during the irrigation season; irrigation suspended in May; June; July and August.

The lowest maize water consumption was calculated in nonirrigated conditions – 488 mm/ha and 88 l/plant – and the highest maize water consumption was determined in the variant with optimum supply of the water, 681 mm/ha or 124 l/plant. Irrigation suspended during one month of the maize irrigation season determined the decrease of the water consumption in comparison with the variant with optimum supply; the largest difference was registered by suspending irrigation in June, - 60 mm/ha or – 9 l/plant (table 6).

The lowest water use efficiency was obtained in the nonirrigated variant, 13.9 kg/mm; suspending irrigation in June determined a very close value of the water use efficiency, 14.4 kg/mm. The irrigations suspended in other months of maize irrigation season, determined lower values of the water use efficiency than in the variant with optimum water supply. This proved the importance of proper water content throughout the irigation season.

Influence of weeds ontrol. Weeds cause serious damage to maize crops from Western Romania (Ciobanu, 2004). In the variant without control measures, the number of weeds was of 105 plants/m² in nonirrigated conditions and of 119 plants/m² in irrigated conditions. *Amaranthus retroflexus* was the dominant weed species in a density of 74 plants/m² in nonirrigated and 62 plants/m² in irrigated conditions. Other well-spread weed species were *Echinochloa crus galli* (with densities of18 and 14 plants /m²), *Chenopodium album* (11 and 10 plants/ m²),

Polygonum persicaria (11 and 9 plants/ m²), *Solanum nigrum* (5 plants/ m² both in nonirrigated and irrigated conditions); *Convolvulus arvensis* was present only in irrigated conditins. Maize density was of 55000 plants/ha.

In nonirrigated conditions, in the variant with weeds, total water consumption increased compared to the variant without weeds with 4.1 % (461 mm/ha vs. 443 mm/ha). When reporting total water consumption to every plant, a 95% lower value was determined in the variant with weeds than in the variant without weeds (4.17 l/plant and 81 l/plant, respectively) (Table 7).

In irrigated conditions, total water consumption of the variant with weeds increased with 1.3% in comparison with the one without weeds (620 mm/ plants vs. 612 mm/plants). There was a similar situation in nonirrigated conditions when reporting total water consumption to every plant, 4.98 l/plants in weeding variant in comparison with 111 l/plants in the variant without weeds. (relative difference - 96%).

Water use efficiency got worse in the variants with weeds. In nonirrigated conditions water use efficiency of the weeding variant (5.2 kg/mm) was smaller than in the variant without weeds (16.7 kg/mm) with 69%; in the irrigated variant the difference was of 64% (6.3 kg/mm vs 17.4 kg /mm). Both differences are statistically significant.

Table 7

Weeding influence on maize water consumption [Σ (e+t)] and on water use efficiency (WUE), Oradea 2001 – 2005

Variant	Variant					
	Nonirrigated			Irrigated		
	Σ (e+t)		WUE	Σ (e+t)		WUE
	mm/ha	l/plant	kg/mm	mm/ha	l/plant	kg/mm
1. Without weeds	443	81	16.7	612	111	17.4
2. With weeds	461	4.17	5.2	620	4.98	6.3
LSD 5%	3	6	1.2	2	5	1.4
LSD 1%	8	14	2.7	7	12	2.9
LSD 0.1%	19	28	5.4	16	25	5.7

Conclusions.

1. All the technology components studied (crop rotation, hybrid, plant density, soil tillage, fertilization, irrigation and weeds control) influenced the values of the maize water consumption and water use efficiency.

2. Meliorative crop rotation with alfalfa determined a higher quantity of rainfall acumulated in the cold period. As a result, the values of the water consumption were higher than those determined in the crop of rotation of 3, 2 years and in monocrop. Meliorative crop rotation determined the biggest values of the water use efficiency both in nonirrigated conditions (18.2 kg/mm) and in irrigated conditions (20.4 kg/mm).

3. Water consumption values increased from early hybrids to tardive hybrids. The highest values of the water use efficiency were registered in Fundulea 376 (500 – 600 FAO group) in nonirrigated conditions and in Fundulea 365 (> 600 FAO group) in irrigated conditions.

4. Higher values of the plants density determined lower values of the water consumption for every plant. The best values for water use efficiency (18.8 kg /mm) were obtained in the variants recommended as the best technological point of view, 55000 plants/hectar for nonirrigated conditions and 70000 plants/hectar in irrigated conditions.

5. The heavy soil requires plowing at a depth of 25 cm, this variant showing higher values of water consumption than the variants plowed at 0 – 12 cm, with ciesel and disk. Water use efficiency was significantly higher in the variant plowed at 25 cm.

6. Organic and organic + mineral fertilization determined a small increase of the maize water consumption, compared to the control, but, the yield increases from these variants determined significant differences of water use efficiency compared to the control.

7. In all cases, irrigation determined the increase of the maize water consumption, but water use efficiency did not improve when early hybrids were used, and it decreased when plants density was either lower or higher than the optimum recommended. Suspending the irrigation determined the decrease of the plant water consumption and made water use efficiency worse.

8. Weeds (*Amaranhtus retroflexus*, *Echinochloa crus galli*, *Chenopodium album*, *Polygonum persicaria*, *Solanum nigrum*, *Convolvulus arvensis*) determined a very significant decrease (95 % in non-irrigated and 96% in irrigated conditions) of plant`s water consumption (maize or weeds) in all variants. Weeding determined a very significant decrease of the water use efficiency (5.2 kg/mm vs. 16.7 kg/mm in nonirrigated variant and 6.3 kg/mm vs. 17.4 kg/mm in irrigated variant).

REFERENCES

Bandici, G. (2001) *Fiziologia plantelor*, Ed. Dacia Cluj-Napoca, pp 83-100.

Bălăceanu, C. (1983) Influența temperaturii aerului și umidității solului asupra consumului de apă al porumbului pentru boabe, *Cerc. agron. în Moldova*, **1**, 112-124.

Botzan, M. (1966) *Culturi irigate*, Ed. Agro-Silvică, București, pp 70-82.

Bora, C. (1991) Relația consum de apă - producție și prognoza aplicării udărilor pe suprafețele irigate din nordul Olteniei, *An. ICITID*, **VI (XVII)**, 86-97.

Borza, I. (2005) The influence of some maize hybrids belonging to different FAO groups upon water use efficiency, In: *Tehnologii de cultură pentru grâu şi porumb*, Ciobanu, G., Domuța, C., Lazanyi, J. (ed.), Ed. Univ. Oradea, 226-235.

Borza, I. (2005) The influence of crop rotation upon the water use efficiency for the irrigated and nonirrigated maize in conditions from Oradea, In: *Tehnologii de cultură pentru grâu şi porumb*, Ciobanu, G., Domuța, C., Lazanyi, J. (ed.), Ed. Univ. Oradea, 235-243.

Ciobanu, C. (2004) *Biologia şi combaterea speciei Apera spica venti (L) PB*, Ed.Univ. din Oradea, pp 165-169.

Ciobanu, G. (2003) *Agrochimia*, Ed. Univ. din Oradea, pp 315-334.

Domuța, C. (1997) Cercetări şi criterii de apreciere a oportunității irigării porumbului pentru boabe (1987 – 1996) în condițiile zonei moderat subumede din centrul Câmpiei de Vest, *An. ICCPT*, **LXV**, 204-223.

Domuța, C., Sabău, N.C., Tuşa, C., Şandor M. (2002) Research concerning the covering sources of the water consumption in the main crops from Crişurilor Plain during 1976 – 2000, *Tartamkísérletek, tájtermesztés, vidékfejlesztés, nemzetközi konferencia*, **II**, 76-82.

Domuța, C. (2003) *Oportunitatea irigațiilor în Câmpia Crişurilor*, Ed. Univ din Oradea, pp 33-45, 75-129, 165-196.

Domuța, C., Şandor, M., Bandici, G., Sabău, N.C., Borza, I. (2003) Cercetări pentru cuantificarea relațiilor din sistemul sol-apă-plantă la principalele culturi din Câmpia Crişurilor, *Probl.agrofit.teore.aplic.*, **XXV**, 45-58

Domuța, C., Ciobanu, G., Şandor, M., Albu, R. (2004) Irrigation, an important component of sustainable agriculture system in West Romania, *Rom. agric. res.*, **21**, 49-56.

Domuța, C., Sabău, N.C., Bandici, G., Şandor, M., Borza, I. (2004) Corelațiile din sistemul sol-apă-plantă în legătură cu oportunitatea irigațiilor la principalele culturi din Câmpia Crişurilor, *Publ. SNRSS no.34a*, **I**, 254-270.

Domuța, C. (2005) *Irigarea culturilor*, Ed.Univ din Oradea, pp 90-112, 152-186

Donnen, D., Westcot, D.W., (1988) *Irigation practice and water management*, FAO Irrigation and Drainage Paper, Rome, pp 5-8, 14-19, 30-36.

Doorembos, J., Kassam, A.H. (1986) *Yield response to water*, FAO Irrigation and Drainage Paper, Rome, pp 19-26, 37-41.

Doorembos, J., Pruitt, W.O. (1992) *Crop Water requirements* FAO Irrigation and Drainage Paper, Rome, pp 55-59, 71-72.

Grumeza, N., Merculiev, O., Klepş Cr. (1989) *Prognoza şi programarea aplicării udărilor în sistemele de irigații*, Ed. Ceres,Bucureşti, pp 111-163.

Ionescu Şişeşti, Vl. (1986) *Irigarea culturilor*, Ed. Ceres, Bucureşti, pp 123-171.

Năescu, V., Alionte, E. (2003) Cercetări privind eficiența irigării la principalii hibrizi de porumb în condițiile de la Fundulea, *An. ICDA*, **LXX**, 221-231.

Petrescu, E., Groza, N. (2003) Modificări în sistemul sol-apă-plantă sub influența irigației la porumbul pentru boabe în condițiile pedoclimatice ale Câmpiei Caracalului, *An. ICDA*, **LXX**, 247-253

Pejic, B., Bosnjak, D., Bjerkholt, Jarle T. (2002) Water use efficiency coefficients as a base for analyse the effect of irrigation on corn yield, In: *Drought mitigation and prevention of land desertification*, ICID, ERWG, ERWTD, SINCID, Bled, Slovenia, 61-62.

Popova, Z., Kercheva, M. (2002) Optimisation of strategies to mitigate droughts consequences under wheat and maize cultivation in Bulgaria, In: *Drought mitigation and prevention of land desertification*, ICID, ERWG, ERWTD, SINCID, Bled, Slovenia, 61-62.

Renea, St., Tatariu, I. (1974) Consumul de apă la câteva culturi agricole din podişul Central al Dobrogei, *An. ICIF*, **IV (X)**, 198-206.

Sabău, N.C., Domuța, C., Berchez, O. (1999) *Geneza, degradarea şi poluarea solurilor, partea I-a Geneza solului*, Ed. Univ. din Oradea, pp 87-116.

Samuel, A.D. (2003) *Evaluarea microbiologică şi enzimologică a efectelor tehnologiilor agricole asupra biologiei solului*, Ed. Univ. din Oradea, pp 48-50.

Săndoiu, I.D. (1974) Contribuții la metodica de determinare a consumului la culturile agricole în condiții de branciog, *An. ICIF*, **IV (XI)**, 210-221

Şandor, M. (2002) Cercetări privind influența asolamentului asupra unor însuşiri fizice ale solului brun luvic şi asupra producției de porumb în condițiile Câmpiei Crişurilor, *An. Univ din Oradea fascic. Agr. Hort.*, **VIII**, 69-75

Vâjială, M. (1978) Studiul consumului de apă la principalele culturi irigate în zona solului brun roşcat de pădure, *An. ICIF*, **(IV) XI**, 137-149.

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