

***Daphnia* species (Crustacea, Cladocera) and the genetic characteristics of their populations based on allozyme studies in Lake Balaton, Hungary**

J. NÉDLI¹, L. FORRÓ², J. KORPONAI³ & L. G-TÓTH⁴

Abstract. Although the *Daphnia* species of Lake Balaton have long been studied, there was no consensus on the species composition. In this paper we attempted to clarify their specific status using allozyme electrophoresis. Zooplankton samples were collected between 2002 and 2004 from each basin of Lake Balaton. *Daphnia* specimens were counted and analysed using cellulose acetate gelelectrophoresis. The analysis revealed the presence of *Daphnia cucullata*, *D. galeata* and their hybrid in the lake. While *Daphnia galeata* was very rare, *D. cucullata* and the hybrids were abundant during the growing season.

Within the genus *Daphnia* three subgenera may be differentiated, *Daphnia*, *Ctenodaphni*, and *Hyalodaphnia*, of which *Daphnia* and *Hyalodaphnia* are monophyletic sistertaxa, based on phylogenetic studies of the small subunit ribosomal RNA (12S), cytochrome oxidase I and the internal transcribed spacer region (Schwenk *et al.* 2000). Out of the twelve species of subgenus *Hyalodaphnia* three are distributed in both North America and Europe. The remaining species are restricted to one of the continents, as for example *Daphnia cucullata* is widely spread only in European lakes.

D. galeata or *D. hyalina* coexist with *D. cucullata* in several lakes. Interspecific hybrids of them frequently occur, usually together with one or both parental species, and some local populations can even be dominated by hybrids. Morphological characters of the hybrids are stable across lakes (Flößner, 1993).

Wolf and Mort (1986) provided a useful tool for the identification of the species, since their allozyme study showed that morphologically typical specimens of *D. cucullata* and *D. galeata* are fixed for different alleles at the AAT enzyme lo-

cus and interspecific hybrids are heterozygous for these alleles. Although Gießler (1997) showed that the AAT locus *per se* is not sufficient to decide on the taxonomical status of the members of the subgenus *Hyalodaphnia*, in our case it is still applicable to differentiate between taxa. Gießler (1997) agrees that *D. cucullata* is fixed for a diagnostic allele (S) at the AAT locus, and beside examples of this genotype (SS) only a certain genotype of heterozygotes has been found.

Lake Balaton is the largest shallow lake in Europe. It extends 77 km in length, its width ranges from 1.5 to 15 km, and it is 3.5 m deep on the average. It can be divided into five large basins: Keszthely, Szigliget, Zánka, Tihany and Balatonkenese Basins (SW-NE direction). In differences between the basins, trophic level, abundance ratio of zooplankton species and other characteristics can be substantial.

Opinion is divided on the taxonomical status of *Daphnia* occurring in Lake Balaton. Ponyi (1965) recorded two *Daphnia* species: *D. cucullata* and *D. hyalina*, the latter can be found in two forms: *D. hyalina* var. *lacustris* and *D. hyalina* var. *galeata*. Flößner and Kraus (1986) concluded

¹Judit Nédli, Magyar Természettudományi Múzeum Állattára (Department of Zoology, Hungarian Natural History Museum) H-1088 Budapest, Baross u. 13, Hungary. E-mail: judit.nedli@gmail.com

²Dr. László Forró, Magyar Természettudományi Múzeum Állattára (Department of Zoology, Hungarian Natural History Museum) H-1088 Budapest, Baross u. 13, Hungary.

³Dr. János Korponai, Nyugat-dunántúli Környezetvédelmi és Vízügyi Igazgatóság, Kis-Balaton Üzemeltetés (West Transdanubian Water Authority, Department Kis-Balaton), H-8360 Keszthely, Csík Ferenc sétány, Hungary.

⁴Dr. László G-Tóth, MTA Balatoni Limnológiai Kutatóintézet (Balaton Limnological Research Institute of the Hungarian Academy of Sciences), H- 8237 Tihany, Klebelsberg Kuno u. 3, Hungary.

that these are actually the hybrid forms of *D. cucullata* and *D. galeata*. Later, Flößner (1993) named this form *Daphnia (Daphnia) × krausi* hybr. nat. nov., and referred again to the individuals from Balaton as being *D. cucullata* × *D. galeata* hybrids. In his summary, Ponyi (1997) mentioned two species in the lake: *D. cucullata* G. O. Sars, 1862 and *Daphnia galeata* G. O. Sars, 1864.

The main object of the present study was to clarify the controversy over the taxonomical status of *Daphnia* in Lake Balaton. Beside that we aimed to investigate the seasonal dynamics of the different *Daphnia* taxa and to describe the populations' genetic characteristics.

MATERIALS AND METHODS

Allozyme studies

Zooplankton for the genetic analysis were collected with a towed plankton net (60 × 60 cm net-frame, 200 µm mesh size) from each of the five basins of Lake Balaton. We towed the net with a motorboat altering the speed, in order to cover the total water depth. Gravid females were picked up randomly from the sample and stored at -65°C until processing. The analysed populations, sample locations and dates are indicated in Table 1.

To reveal allelic variance at three enzyme loci (aspartate amino transferase - AAT, phosphoglucose-isomerase - PGI and phosphogluco-mutase - PGM) cellulose acetate gelelectrophoresis (Hebert *et al.*, 1989) was applied, followed by the analysis of the dataset with the programmes GenAlEx 6 (Peakall *et al.*, 2005) and TFPGA (Miller 1997). Wright's F- statistics (Wright, 1965) and clustering with Unweighted Pair Group Method, Arithmetic Mean (UPGMA) based on Nei's original genetic distances (Nei, 1972) have been used as a measure of differentiation.

Zooplankton counts

Daphnia cucullata, *Daphnia cucullata* × *galeata* and *Daphnia galeata* individuals were counted based on Flößner (1993) separately, in

three of the five basins: Keszthely, Szigliget and Tihany Basin, to define the abundance of the different taxa.

Sampling was carried out every third week between 15. 04. 2002. and 07. 10. 2002. Samples for the analysis have been collected using a Schindler-Patalas plankton trap of 34 litre, and fixed with 10% formaldehyde.

Samples containing limited numbers of individuals have been counted directly, and the abundance of the different *Daphnia* species was calculated using the following formule: $S_{ind} = Z_e / PAT / 34 \text{ dm}^3$, where Z_e is the number of specimens in the sample, PAT means the number of merging the Schindler-Patalas plankton trap.

Samples, which contained a large number of animals have been diluted with distilled water to 200 ml from which five sub-samples, 5 ml apiece, were used for counting *Daphnia* individuals. If the standard deviation exceeded 10%, additional 5 ml units were included until the SD was less than 10%. In this case Z_e means the average number of individuals in sub-samples multiplied by the quotient of the total volume of the fixed sample (200 ml) and the volume of a sub-sample (5 ml).

RESULTS

Allozyme studies

We analysed 1294 randomly chosen gravid female specimens (Table 1) using cellulose-acetate gelelectrophoresis, 958 of that turned out to be *D. cucullata*, 335 *D. cucullata* × *galeata* and only one specimen *D. galeata* (Keszthely Basin, 26.04. 2004.) according to the species specific alleles of the AAT enzyme locus. Among the male specimens we discovered 9 *D. cucullata* and 7 hybrids.

The analysis revealed four alleles (S, M, F, F⁺) both on the PGI and PGM loci. The F⁺ allele on the PGI locus was found only in hybrid populations and the F⁺ allele on the PGM locus was confined to one *D. cucullata* population. Mean allele frequencies are shown in Table 2.

Table 1. Populations scored for the different alleles at the three loci. Capitals (A, B) next to the number of individuals refer to the different analyses. Zeros indicate that no individual of the given taxon was found on the given sampling date among the randomly chosen animals

Sampling date	Balatonkenese (BK) Basin		Tihany (T) Basin		Zánka (Z) Basin		Szigliget (S) Basin		Keszthely (K) Basin	
	<i>D. cuc.</i>	hybrid	<i>D. cuc.</i>	hybrid	<i>D. cuc.</i>	hybrid	<i>D. cuc.</i>	hybrid	<i>D. cuc.</i>	hybrid
06.05.2002	–	–	7	32	–	–	–	–	22	45
23.06.2002	–	–	–	–	–	–	28	8	–	–
01.07.2002	–	–	18	3	–	–	–	–	–	–
31.07.2002	–	–	21	3	–	–	–	–	–	–
23.08.2002	53	0	48	0	48	0	43	4	42	4
19.06.2003	–	–	36	19	–	–	–	–	–	–
31.07.2003	–	–	29	13	–	–	–	–	40	15
21.08.2003	–	–	54	1	–	–	–	–	–	–
15.09.2003	–	–	44	9	–	–	–	–	–	–
02.10.2003	–	–	47	6	–	–	–	–	–	–
21.10.2003	–	–	35	19	–	–	–	–	–	–
07.11.2003	–	–	45	10	–	–	–	–	–	–
28.11.2003	–	–	35	19	–	–	–	–	–	–
26.04.2004	–	–	–	–	–	–	–	–	0	47
07.10.2004	52	17	65	16	43	16	39	15	60	8
Total number of individuals/ breeding season										
2002.	53 (A)	0	94 (A) 325	38 (B)	48 (A)	0	71 (A)	12	64 (A) 40	49 (B)
2003.	–	–	(A)	94 (B)	–	–	–	–	(A) 60	15 (B)
2004.	52 (A)	17 (B)	65 (A)	16 (B)	43(A)	16 (B)	39 (A)	15 (B)	(A)	55 (B)

Analysis of the genetic structure of the *D. cucullata* population in Lake Balaton was carried out for the samples marked with an „A” in Table 1. Wright’s F_{IS} (Table 3) implied heterozygote excess on both loci, F_{ST} values indicated little genetic differentiation between the samples. Hybrid populations marked with a „B” in Table 1 have been studied to reveal differentiation. Wright’s F -statistics (Table 3) show considerable heterozygote excess at the PGI locus and heterozygote deficiency at the PGM locus. Population differen-

tiation is moderate ($F_{ST}= 0,099$). UPGMA clustering (Fig. 1) reveals that the observed moderate differentiation originates mainly from the separation of two clusters.

Zooplankton counts

Results of the zooplankton counts are summarized on Fig. 2. On the first sampling date in the breeding season almost no *Daphnia* individuals could be found in the water. Number of in-

Table 2. Mean allele frequencies of the different *Daphnia* taxa

		<i>D. cucullata</i>	<i>D. cuc. × gal.</i>
PGI	S	0.395	0.309
	M	0.604	0.205
	F	0.001	0.467
	F ⁺	0.000	0.019
PGM	S	0.697	0.175
	M	0.275	0.583
	F	0.028	0.242
	F ⁺	0.001	0.000

Table 3. Wright's F- statistics for the *Daphnia cucullata* and *Daphnia cucullata galeata* populations

		PGI	PGM	Mean
<i>D. cucullata</i>	F _{IS}	-0,408	-0,041	-0,225
	F _{ST}	0,008	0,070	0,039
<i>D. cu.c. × gal.</i>	F _{IS}	-0,594	0,452	-0,071
	F _{ST}	0,044	0,154	0,099

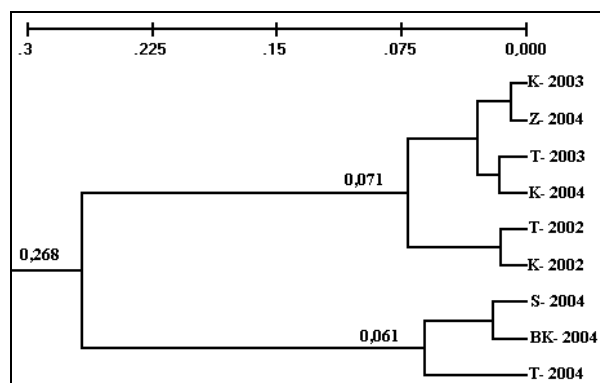


Figure 1. UPGMA clustering of the hybrid populations based on Nei's original genetic distances. K = Keszthely Basin, S = Szigliget Basin, Z = Zánka Basin, T = Tihany Basin, BK = Balatonkenese Basin, numbers combined with the capitals mean the different breeding seasons. Numbers above certain branches show Nei's distance for the given node

dividuals of *D. galeata* was very low throughout the studied period. Early in May the hybrids exceeded the number of *D. cucullata* individuals in two of the three basins (Keszthely and Tihany). *D. cucullata* dominated the assemblage in every basin later. In the middle of the summer the number of the hybrids decreased strongly and rose to a slight extent in September again. However, the abundance of *Daphnia* (every taxa together) never exceeded 28.7 individuum/litre (03. 06. 2002., Szigliget Basin).

DISCUSSION

Based on cellulose acetate gelelectrophoresis carried out in the present study, it has been concluded that the following *Daphnia* taxa are present in Lake Balaton: *Daphnia cucullata* G. O. Sars, 1862, *Daphnia galeata* G. O. Sars, 1864 and *Daphnia cucullata × Daphnia galeata*. The forms mentioned by Ponyi (1965) Flößner and Kraus (1986) and Flößner (1993) are probably really *D. cucullata × galeata* hybrids, as the latter authors claimed.

Spaak (1996) revealed similar mean allele frequencies for *D. galeata* and *D. cucullata × galeata* in Lake Tjeukemeer, but allele frequencies of the hybrid populations were markedly different from those of *D. cucullata*, as it was the case in Lake Balaton. Nei's genetic distance *× galeata* and *D. cucullata* was 0.71 (Spaak, 1996). Similarly, Nei's genetic distance was high (0.65) between the *D. cucullata* and hybrid populations in our case.

D. galeata occurs extremely rarely in Lake Balaton. Only one specimen has been found using electrophoresis and the zooplankton counts revealed *D. galeata* individuals only in samples from April, May and October in low numbers (its highest abundance was 0.3 ind/dm³, Keszthely Basin, 15.04.2002.). Hence the photos of *D. galeata*, presented by Ponyi (1997) depict, in our opinion, *D. cucullata × galeata*. However *Daphnia* taxa are not typical of Lake Balaton since the total abundance throughout the growing season was low.

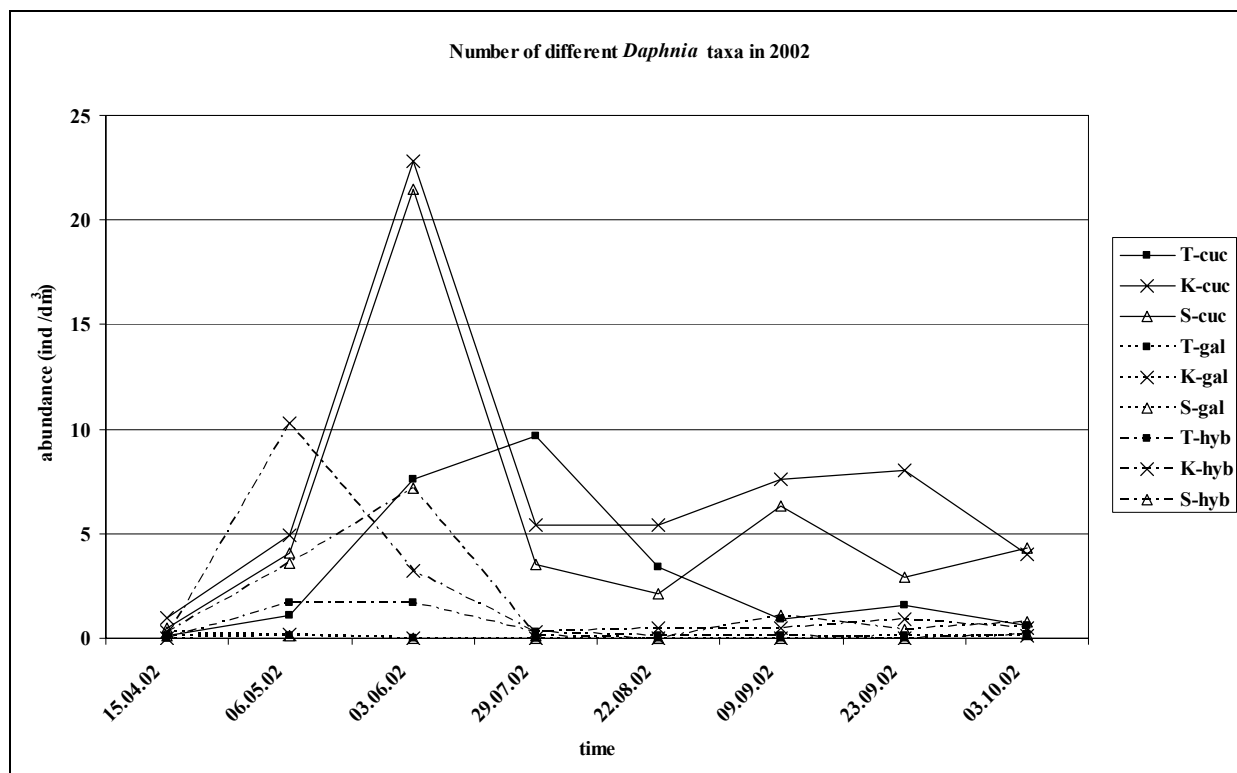


Figure 2. Abundance of different *Daphnia* taxa. Solid line = *D. cucullata*, dashed line = hybrid, dotted line = *D. galeata*, square = Tihany Basin, X = Keszthely Basin, triangle = Szigliget Basin

First year offspring of *Abramis brama* L. consumes solely zooplankton in Lake Balaton, it only occasionally switches to benthic feeding during fall (Specziár & Bíró, 2004). In three permanent lakes in Germany significant positive correlation was found between the intensity of selection and fish density for *D. galeata*, but not for *D. cucullata* × *galeata* hybrids (Müller & Seitz, 1995). Presumably, the abundance of *D. galeata*, the largest and most visible of the taxa is reduced by the predation pressure to a great extent. Since *D. cucullata* × *galeata* occurs together with *D. galeata* in most of the cases more detailed investigations of the ecological factors acting behind the absence of *D. galeata* are necessary.

Acknowledgements – We are grateful to G. Dobos and É. Hajdú for their indispensable help in the field and in the laboratory. Research was financially supported by the Hungarian Scientific Research Fund, T49098 and T32165 contracts.

REFERENCES

- FLÖSSNER, D. & KRAUS, K., (1986): On the taxonomy of the *Daphnia hyalina-galeata* complex (Crustacea: Cladocera). *Hydrobiologia*, 137: 97-115.
- FLÖSSNER, D. (1993): Zur Kenntnis einiger *Daphnia* – Hybriden (Crustacea: Cladocera). *Limnologica*, 23: 71-91.
- GISSLER, S. (1997): Analysis of reticulate relationships within the *Daphnia longispina* species complex. Allozyme phenotype and morphology. *Journal of Evolutionary Biology*, 10: 87-105.
- HEBERT, P. D. N. & BEATON, M. J. (1989): Methodologies for allozyme analysis using cellulose acetate electrophoresis. *Helena Laboratories Beaumont, Texas*, 31 pp.
- MILLER, M. P. (1997): Tools for population genetic analyses (TFPGA) 1.3: A Windows® program for the analysis of allozyme and molecular population genetic data. *Department of Biological Sciences, Northern Arizona University*.

- MÜLLER, J. & SEITZ, A. (1995): Differences in genetic structure and ecological diversity between parental forms and hybrids in a *Daphnia* species complex. *Hydrobiologia*, 307: 25-32.
- NEI, M. (1972): Genetic distance between populations. *American Naturalist*, 106: 283-292.
- PEAKALL, R. & SMOUSE P. E. (2005): GenAlEx 6: genetic analysis in excel. Population genetic software for teaching and research. *Australian National University, Canberra, Australia*.
- PONYI, J. (1965): Crustaceen-Planktonstudien am Balaton II. Beiträge zur Kenntnis der Systematik und Cyclomorphose einiger Arten der Gattung *Daphnia*. *Annales Instituti Biologici (Tihany)*, 32: 159-174.
- PONYI, J. (1997): A Balaton Cladocera és Copepoda rákjai. *Allattani Közlemények*, 82: 69-80
- SCHWENK, K., POSADA, D., HEBERT, P. D. N. (2000): Molecular systematics of European *Hyalodaphnia*: the role of contemporary hybridization in ancient species. *Proceedings of the Royal Society London*, 267: 1833-1842.
- SPAACK, P. (1996): Temporal changes in the genetic structure of the *Daphnia* species complex in Tjeukemeer, with evidence for backcrossing. *Heredity*, 76: 539 -548.
- SPECZIÁR, A. & BIRÓ, P. (2004): Őshonos halfajok ivadékanak táplálkozási stratégiája, trofikus kapcsolatai, növekedése és túlélési esélye a Balatonban. In: Mahunka, S. & Banczerowski, J. (ed.): *A Balaton kutatásának 2003. évi eredményei*. MTA, Budapest, pp. 99-107.
- WOLF, H. G. & MORT, M. A. (1986): Inter-specific hybridization underlies phenotypic variability in *Daphnia* populations. *Oecologia*, 68: 507-511.
- WRIGHT, S. (1965): The interpretation of population structure by F- statistics with special regard to systems of mating. *Evolution*, 19: 395-420.