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Molecular Analysis (RAPD-PCR) of Inter-strain Hybrids of the *Paramecium aurelia* Species Complex (Ciliophora, Protozoa)*

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RAPD-PCR analysis showed that species of the *Paramecium aurelia* complex possessed characteristic band patterns and that the majority were also polymorphic intra-specifically. A comparison of band patterns was performed for some inter-strain hybrids within *P. primaurelia*, *P. tetraurelia*, *P. pentaurelia*, *P. septaurelia*, *P. octaurelia*, *P. decaurelia*, *P. dodecaurelia*, *P. tredecaurelia*, and *P. quadecaurelia* to band patterns characteristic for the parental strains. The investigations, however, did not reveal a close correlation between the degree of inbreeding characteristic for the species and similarity of genotypes. A low similarity of hybrid and parental band patterns was observed in *P. octaurelia, P. dodecaurelia, P. quadecaurelia* and also *P. primaurelia.* A high similarity of band patterns of hybrid and parental strains was found in *P. tetraurelia*, *P. septaurelia*, *P. decaurelia*, and *P. tredecaurelia*.

Key words: *Paramecium aurelia* species complex, structure of species, differentiation of strains, inter-strain hybrids, RAPD-PCR fingerprints.

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The *P. aurelia* complex is composed of 14 species described by SONNEBORN (1975) and a 15th species (*P. sonneborni*) described by AUFDERHEIDE *et al*. (1983). They are characterized by inbreeding (SONNEBORN 1957; LANDIS 1986) causing an increase of intra-specific differentiation. Depending on the degree of inbreeding, strains within species are more or less isolated, showing a higher or lower level of polymorphism within species revealed by RAPD-PCR fingerprints (STOECK *et al*. 1998; PRZYBOŚ *et al.* 2006).

A correlation exists between the degree of inbreeding characteristic for the species with the differentiation of DNA genotypes revealed by RAPD analysis within particular species, e.g. extreme inbreeders – *P. biaurelia*, *P. tetraurelia*, *P. octaurelia*, *P. dodecaurelia* showed substantial variability of band patterns and moderate inbreeders – *P. pentaurelia*, *P. septaurelia*, *P. decaurelia*, *P. tredecaurelia*, *P. quadecaurelia* were highly similar, *P. primaurelia* showed polymorphism of species genotypes but to a lower degree than species from the first group representing extreme inbreeders (PRZYBOŚ *et al*. 2006).

Material and Methods

Material

A list of studied strains and inter-strain hybrids of the *P. aurelia* species complex is given in Table 1.

Methods

1. Strain cultivation and crossing

The methods of SONNEBORN were used (1970) for the cultivation of strains and induction of con-

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Table 1

Strains and inter-strain hybrids of the *Paramecium aurelia* species complex used in the study

Species	Strain designation	Geographical origin
Paramecium primaurelia	90 (standard of the species)	USA, Pennsylvania, Bethayres
	GA	Greece, Athens
	RM	Russia, Moscow
	VH	Vietnam, Hanoi
	IJ	Israel, Qasr-el Yehud, River Jordan
	90 x GA	USA x Greece
	RM x VH	Russia x Vietnam
	GA x VH	Greece x Vietnam
	IJ x VH	Israel x Vietnam
Paramecium tetraurelia	S (standard of the species)	Australia, Sydney
	SM	Spain, Madrid
	J	Japan, Honshu Island
	PK	Poland, Kraków
	J x S	Japan x Australia
	PK x S	Poland x Australia
	SM x S	Spain x Australia
Paramecium pentaurelia	87 (standard of the species)	USA, Pennsylvania
	RAZ	Russia, Astrahan Nature Reserve
	87 x RAZ	USA x Russia
Paramecium septaurelia	38 (standard of the species)	USA, Florida
	RA	Russia, Astrahan Nature Reserve
	38 x RA	USA x Russia
Paramecium octaurelia	138 (standard of the species)	USA, Florida
	IEE	Israel, Ein Efek
	138 x IEE	USA x Isreal
Paramecium decaurelia	223 (standard of the species)	USA, Florida
	JN	Japan, Nara
	223 x JN	USA x Japan
Paramecium dodecaurelia	246 (standard of the species)	USA, southern state
	HHS	Hawaii, Honolulu
	JU	Japan, Ube
	IE	Italy, Elbe Island
	HHS x 246	Hawaii x USA
	JU x 246	Japan x USA
	IE x 246	Italy x USA
	JU x HHS	Japan x Hawaii
Paramecium tredecaurelia	209 (standard of the species)	France, Paris
	321 (standard of the species)	Mexico, Taxco
	IKM	Israel, Kinet Motzkin
	209 x 321	France x Mexico
	209 x IKM	France x Israel
Paramecium quadecaurelia	328 (standard of the species)	Australia, Emily Gap
	AN	Africa, Namibia
	328 x AN	Australi x Namibia

jugation. Paramecia were cultivated on a lettuce medium inoculated with *Enterobcter aerogenes* at a temperature of 24°C. In the inter-strain crosses, the F_1 generation was obtained by conjugation and F_2 by autogamy (using the method of daily isolation lines). The occurrence of the desired stage of autogamy (specimens at the stage of two macronuclear anlagen) was examined on preparations stained with aceto-carmine.

2. Methods used in molecular analysis – random amplified polymorphic DNA-PCR (RAPD-PCR) analysis

Paramecium genomic DNA was isolated from vegetative cells at the end of the exponential phase from parental strains and from inter-strain hybrids (F_2 generation) using QIAamp ^{R} DNA Mini Kit (Qiagen Germany) as described in PRZYBOŚ *et al*. (2003). DNA was stored at –20°C until analysis. All strains used in investigations are listed in Table 1.

PCR amplification for RAPD analysis was carried out using one oligonucleotide 10 mer random primer characterized by the sequence 5'-GCAGAGAAGG - 3'. This primer was chosen from a series of ten 10 mer random primers (Ro 460 Roth, Karsruhe, Germany) because it gave easily distinguishable banding patterns for species and strains in *Paramecium jenningsi* (SKOTARCZAK *et al*. 2004 a, b). The same primer was also used in several studies carried out on *P. aurelia* spp. (STOECK *et al*. 1998, 2000a) and *P. jenningsi* (PRZYBOŚ *et al*. 1999; PRZYBOŚ *et al* 1999; PRZYBOŚ *et al*. 2003). Each 20 μl PCR mixture contained 2 μl of DNA template, 1 x reaction QIAGEN buffer, 2.5 mM $MgCl_2$, 200 μM dNTPs, 1.5 μM primer, 1.5U of Taq DNA polymerase (QIAGEN). PCR reactions were performed according to the program described by STOECK and SCHMIDT (1998).

PCR products (15 μl), along with the pGEM DNA molecular weight marker (Promega) were run on 1.5% TBE agarose gels stained with 0.5 μg ml^{-1} ethidium bromide. Generally, three repetitions of the PCR reaction were performed in order to assess the reproducibility of the data. All RAPD parameters were carefully standardized.

3. Analysis of molecular data

The Bio1D++ program (Vilbert Lourmat, France) was used to calculate intra-species relationships on the basis of the similarity of DNA band patterns obtained by the RAPD method, according to the NEI and LI (1979) similarity coefficient, i.e. $a=2nxy/(nx + ny)$ where nx and ny are the number of bands in lane "x" and "y", respectively, and nxy the number of shared bands between the two lanes. Dendrograms were produced from the similarity values in the matrix using the UPGMA (unweighted pair group match average) algorithm.

Results

RAPD fingerprint analysis

Paramecium primaurelia

Hybrids of *P. primaurelia* strains representing different genotype groups (90 x GA, RM x VH) and the same genotype group (GA x VH, IJ x VH) showed some new bands not present in the parental strains and some bands similar to parental strains. Values of the similarity matrix of hybrid band patterns were rather low (about 30%) when compared to the parental strains. The diagram presents the variability of relationships of hybrid strains to each other and to the parental strains. The similarity of hybrid band patterns 90 x GA and IJ x VH is high (90%), but low in the case of RM x VH and GA x VH hybrids (45%). The similarity of band patterns of the two groups of hybrids is about 35% (Fig. 3A).

Paramecium tetraurelia

P. tetraurelia, each composed of geographically isolated strains. Strains S (Sydney, Australia) and SM (Spain) form one genotype group, strains J (Japan) and PK (Poland) form the second cluster.

The hybrids of strains (JxS, PKxS, SMxS) have similar band patterns. When their band patterns were compared with parental strains some differences could be seen, the most characteristic is lack of bands at 2150 and 2400 bp characteristic for S and SM strains. The similarity of band patterns of hybrids and parental strains differs, depending on hybrid, from 70 to 90% (Figs 1D, 2D, 3D).

Paramecium pentaurelia

P. pentaurelia strains used in the present study (strain 87 from USA, Pennsylvania and strain RAZ from Russia, Astrahan Nature Reserve) revealed the same genotypes, as well as their hybrid (Figs 1B, 2B).

Paramecium septaurelia

Both strains of the species, one from the USA (strain 38, Florida) and the second from Russia (RA, from Astrahan Nature Reserve) are characterized by similar band patterns showing 94% similarity and differ by only one extra band at about 1300 bp in the pattern of the strain from Russia (Figs 1B, 2B).

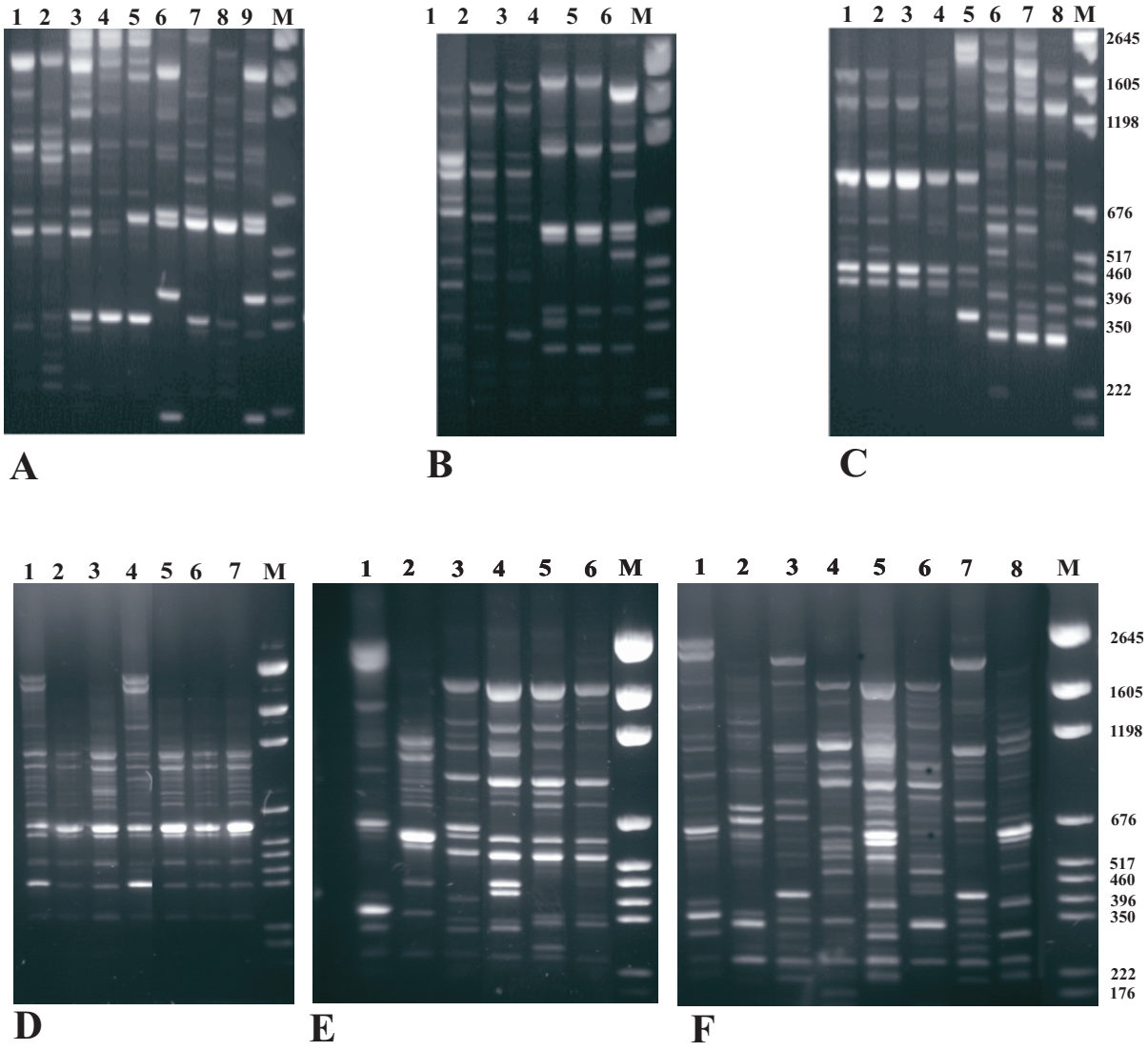

Fig. 1. RAPD fingerprints of the studied parental strains and inter-strain hybrids belonging to species of the *Paramecium aurelia* complex as revealed by RAPD-fingerprints with primer Ro 460-04. M - molecular pGEM marker, molecular weight of the marker DNA bands are given in bp. A. Strains of *P. primaurelia*: 1 – 90, 2 – RM, 3 – GA, 4 – IJ, 5 – VH, 6 – 90 x GA, 7 – GA x VH, 8 – RM x VH, 9 – IJ x VH; B. Strains of *P. pentaurelia*: 1 – 87, 2 – RAZ , 3 – 87 x RAZ; strains of *P. septaurelia*: 4 –38, 5 – RA , 6 – 38 x RA; C. Strains of *P. tredecaurelia*: 1 – 209, 2 – 321, 3 – IKM, 4 – 209 x 321, 5 – 209 x IKM; strains of *P. quadecaurelia* : 6 – 328, 7 – AN, 8 – 328 x AN; D. Strains of *P. tetraurelia*: 1 – S, 2 – J, 3 – PK, 4 – SM, 5 – J x S, 6 – PK x S, 7 – SM x sincerely; E. Strains of *P. octaurelia*: 1 – 138, 2 – IEE , 3 –138 x IEE; strains of *P. decaurelia*: 4 – 223, 5 – JN, 6 – 223 x JN; F. Strains of *P. dodecaurelia*: 1 – 246, 2 – HHS, 3 – JU, 4 – IE, 5 – HHS x 246, 6 – JU x 246, 7 – IE x 246, 8 – JU x HHS.

Their hybrid (38 x RA) shows a different band pattern from the parental ones with 67% similarity of band pattern to strain 38 and 74% similarity to strain RA (Fig. 3B).

Paramecium octaurelia

P. octaurelia strains from the USA (138, Florida) and Israel (IEE, Ein Efek) showed different band patterns (Figs 1E, 2E), the similarity of their band patterns is low. Hybrids of the strains reveal 40% similarity to the parental strain IEE and very low similarity to the strain 38 (Fig. 3E1).

Paramecium decaurelia

The basic band pattern characteristic for the species comprises several bands seen in both studied strains, 223 (standard strain of the species, from USA) and in the JN strain (Japan) (Figs 1E, 2E). The diagram presents the close relationship of both strains (80% similarity).

The hybrid of strains 223 x JN shows 88% similarity of band pattern to the strain from Japan and 80% similarity to the band pattern of strain 223 (diagram) (Fig. 3 E2).

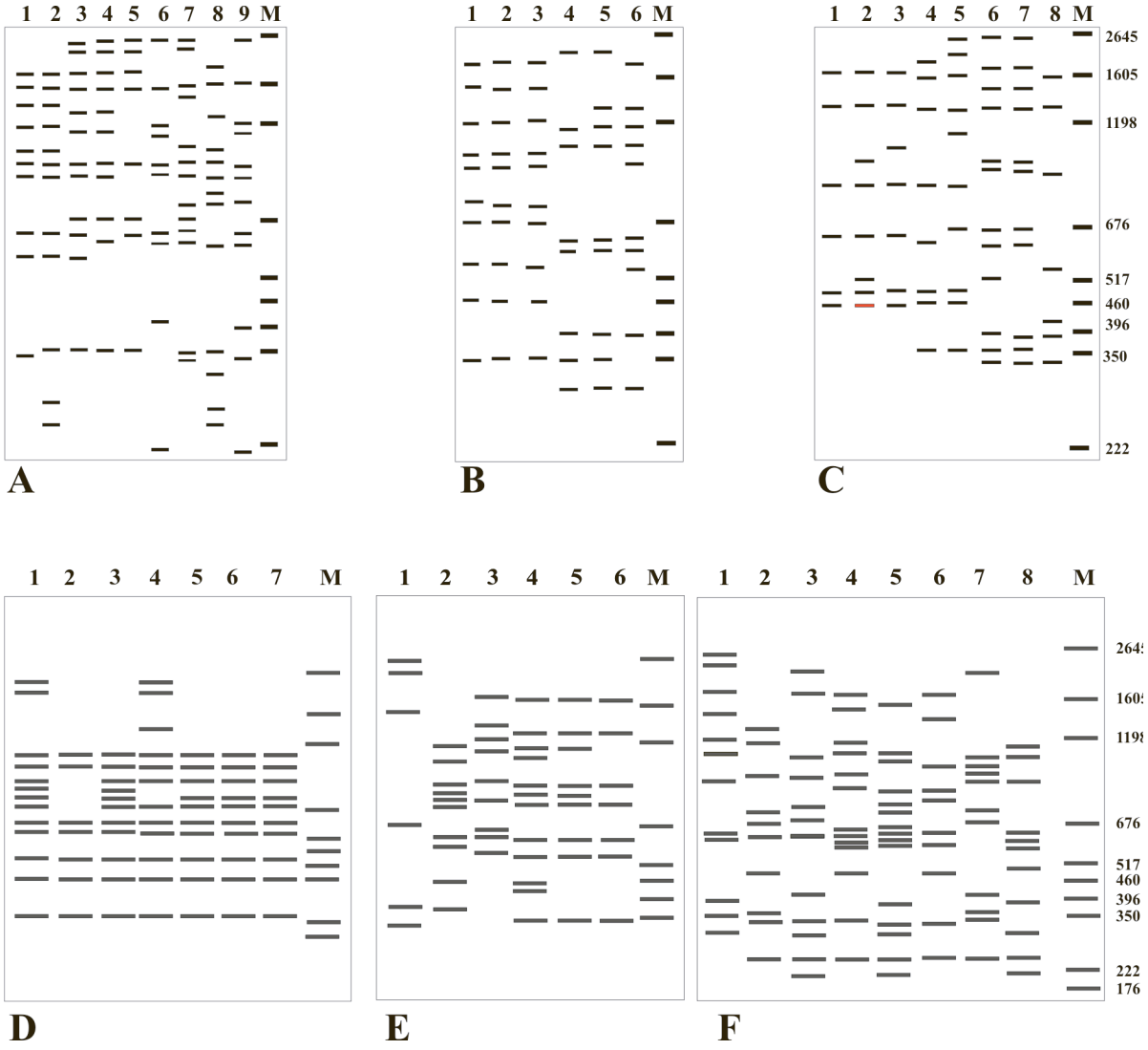

Fig. 2. Schematic representation of Fig. 1. showing specific band patterns representing different genotypes as revealed by RAPD-fingerprints with primer Ro 460-04.

Paramecium dodecaurelia

Substantial polymorphism of band patterns from different collecting sites was revealed in *P. dodecaurelia* (Figs 1F, 2F). Each strain represents a different genotype; the first appears in strain 246 from USA, the second in strain HHS from Hawaii, third in strain JU from Japan, and the forth in strain IE from Italy. Hybrids of *P. dodecaurelia* strains HHS x 246 (Hawaii x USA), JU x 246 (Japan x USA), IE x 246 (Italy x USA), and JU x HHS (Japan x Hawaii) possess different band patterns from those characteristic for parental strains and different (low) values of similarity (Fig. 3F), only the band pattern of hybrid JU x HHS is similar to the pattern of parental strain JU (similarity of 70%). Patterns of hybrids HHS x 246 and JU x 246 are similar to each other in 50%, and patterns of hybrids IE x 246 and JU x HHS are 60% similar.

Paramecium tredecaurelia

P. tredecaurelia strains 209 (France, Paris), 321 (Mexico, Taxco) and IKM (Israel, Kinet Motzkin) showed generally similar band patterns (Figs 1C, 2C). The hybrid 209 x 321 (strain from France x strain from Mexico) as well as hybrid 209 x IKM (strain from France x strain from Israel) showed some extra bands in comparison to the paternal strains. Similarity of their band patterns to the parentals is about 50%, and 55% when band patterns of both hybrids are compared (Fig. 3C1).

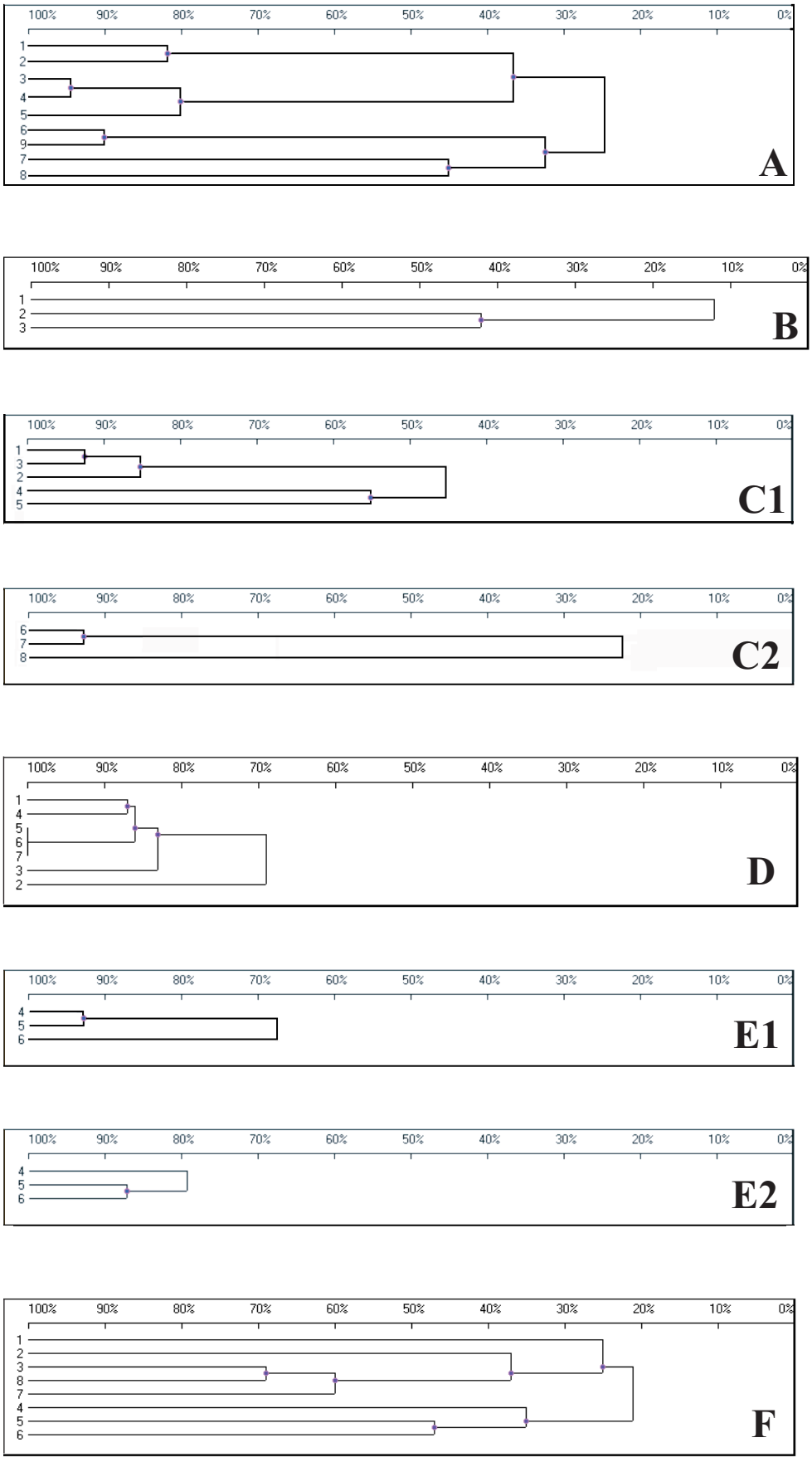

Fig. 3. Intra-species dendrograms of *P. primaurelia* (A), *P. septaurelia* (B), *P. tredecaurelia* (C1), *P. quadecaurelia* (C2), *P. tetraurelia* (D), *P. octaurelia* (E1), *P. decaurelia* (E2), *P. dodecaurelia* (F) based on RAPD fingerprinting. Designation of strains within species as in Figs 1 and 2.

Paramecium quadecaurelia

P. quadecaurelia strains 328 (Australia, Emily Gap) and AN (Africa, Namibia) revealed 86 % similarity of band patterns (Figs 1C, 2C).

The hybrid genotype is however different from the parental ones, the similarity of band pattern of the hybrid to patterns of parental strains is 24% (Fig. 3C2).

In general, a different percentage of similarity of hybrid and parental band patterns was observed in the studied species, This does not seem to be connected with the breeding system characteristic for the particular species.

Discussion

Recently, DNA-based molecular marker techniques have been widely applied in many studies revealing the genetic diversity of species. Among these the RAPD technique is frequently used, disclosing polymorphism in numerous numbers of copies.

The method was applied in several studies dealing with polymorphism of populations. It was applied in multicellular and unicellular organisms, among these ciliates (*Tetrahymena thermophila*, by LYNCH 1995 and BRICKNER *et al.* 1996; *Euplotes* sp. by KUSCH & HECKMANN 1996 and CHEN *et al.* 2000; *Stentor coeruleus* by KUSCH 1998; *Gonostomum affine* by FOISSNER *et al*. 2001).

In *Paramecium* RAPD analysis was applied in studies concerning intra-specific differentiation which revealed different genotypes within the *P. aurelia* complex, i.e. *P. triaurelia*, *P. pentaurelia*, *P. sexaurelia* and *P. novaurelia* (STOECK *et al*. 1998, 2000a), and the other species of the complex (PRZYBOŚ *et al*. 2005). The method proved useful in identification of species of the *P. aurelia* complex (STOECK & SCHMIDT 1998) and was also applied in other species of *Paramecium*, i.e. *P. nephridiatum*, *P. calkinsi*, *P. dubosqui*, *P. woodruffi* (FOKIN *et al*. 1999a,b), and *P. scheviakoffi* sp. nov. (FOKIN *et al*. 2004) or in studies concerning the existence of sibling species as in the case of *P. jenningsi* (PRZYBOŚ *et al*. 1999, 2003; SKOTARCZAK *et al*. 2004 a,b) and *P. caudatum* (STOECK *et al*. 2000b).

Intraspecific polymorphism was observed in RAPD analysis in the majority of species of the *P. aurelia* complex, being correlated with the specific degree of inbreeding of the particular species (PRZYBOŚ *et al*. 2006). SONNEBORN in 1957 had already associated several features of *Paramecium aurelia* spp. life history (type of mating type determination, occurrence of autogamy, selfing, and the length interval of sexual immaturity after conjugation) with the degree of inbreeding. The present investigations based on a comparison of inter-strain hybrid band patterns within *P. primaurelia*, *P. tetraurelia*, *P. pentaurelia*, *P. septaurelia*, *P. octaurelia*, *P. decaurelia*, *P. dodecaurelia*, *P. tredecaurelia*, and *P. quadecaurelia* to band patterns characteristic for the parental strains, however, did not reveal a close correlation between the degree of inbreeding characteristic for the species and similarity of genotypes. A low similarity of hybrid and parental band patterns was observed in case of *P. dodecaurelia*, *P. primaurelia*, and *P. octaurelia*, the species characterized by inbreeding but also in species such as *P. quadecaurelia*, characterized by moderate inbreeding. A high similarity of band patterns of hybrid and parental strains was found in *P. decaurelia* and *P. tetraurelia* – inbreeders, and also in *P. septaurelia* and *P. tredecaurelia* characterized by moderate inbreeding.

Genetic analysis of hybrids based on RAPD molecular markers was also carried out in *Chrysanthemum* sp. cultivars (HUANG *et al*. 2000). It was found that in some hybrid combinations, the parents were more similar to each other than either was to the offspring.

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