Doubly Uniparental Inheritance of Mitochondrial DNA in the Freshwater Bivalve *Anodonta woodiana* (Bivalvia: Unionidae)

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Unlike the vast majority of organisms in which mitochondrial DNA is transmitted maternally (standard mitochondrial inheritance, SMI), some marine or freshwater bivalves exhibit a different pattern of mtDNA transmission, named doubly uniparental inheritance (DUI). In this case there are two types of mtDNA, i.e. the female-transmitted (F-type) and the male-transmitted (M-type), the latter being present only in the male gonads of Unionidae bivalves. Current knowledge on DUI does not cover any freshwater mussels that are found in Poland. This study confirms DUI of mtDNA in *A. woodiana*, a Chinese mussel discovered in Poland in 1993. The sequence divergence in the *COI* gene region for the F-type ranged between 0% (separately for Polish and Japanese mussels) and 8.1% (between Polish and Japanese specimens). On the other hand, this parameter was higher for the M-type, reaching 9.7% between Polish and Japanese specimens. Sequence divergence between the F- and M-types reached 34-35% and, although very high, was still characteristic for the bivalves in which DUI had been found.

Key words: Mitochondrial DNA, doubly uniparental inheritance (DUI), COI gene, genetic variation, Unionidae, *Anodonta woodiana*.

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Mitochondrial DNA (mtDNA), contrary to nuclear DNA, is inherited only maternally. This standard maternal inheritance (SMI) occurs in the majority of eukaryotic organisms (BIRKY 1995). However in marine and freshwater bivalves, another pattern of mtDNA inheritance takes place, termed *doubly uniparental inheritance* (DUI) (FISHER & SKIBINSKI 1990; SKIBINSKI *et al.* 1994a,b; ZOUROS *et al.* 1994a, b; RAWSON & HILBISH 1995; HOEH *et al.* 1996, 1997; LIU *et al.* 1996a; CUROLE & KOCHER 2002; WALKER *et al.* 2006). In this case, two types of mitochondrial DNA are observed: the F-type, which is inherited from the mother, and the M-type, inherited from the father. A male has both types, however, the mitochondrial M-type genome located in male gonads is transmitted to male progeny. In somatic cells, the male has only the F-type of mtDNA inherited from the mother. The females usually have maternally inherited F-type mtDNA in all cells.

In freshwater bivalve species (Bivalvia: Unionidae), the divergence between the F- and M-types ranges from 28% to 34% and can be lower in the genus *Mytilus* (HOEH *et al.* 2002). In freshwater bivalves no recombination takes place and the role reversal of mitochondrial genomes is also absent; thus in phylogenetic analyses they exhibit separate clades, F and M, which can be used independently for evaluation of evolutionary history. In unionid species, M-type mtDNA occurs in male gonads only and has not been found in other tissues (HOEH *et al.* 2002; MOCK *et al.* 2004).

DUI is probably an old phenomenon. Analyses based on mitochondrial F- and M-types in freshwater bivalves suggest that they have been evolving separately for at least 100 million years with the DUI effect being present in Unionidae for 200 million years (HOEH *et al.* 2002). According to COUROLE and KOCHER (2002) the unionid male and female lineages diverged 450 MYA.

Two hypotheses pertain to the origins and occurrence of DUI in marine and freshwater bivalves (HOEH *et al.* 1996, 2002). One hypothesis states that DUI appeared independently in three ancestral lineages leading to the Unionidae family, *Mytilus*, and *Geukensia*. According to the other hypothesis, DUI appeared once in the ancestral lineage of bivalves and was gradually modified or lost in some descendant lineages.

The presence of this unusual pattern of mtDNA inheritance (i.e. DUI) in phylogenetically distant families (i.e. Mytilidae, Veneridae, and Unionidae) suggests that it may be a commonplace pattern among bivalves (HOEH *et al.* 2002). The mechanism of DUI has been well studied in Mytilidae species, referring to four marine species of the genus *Mytilus* and one freshwater species, *Geukensia demissa* (HOEH *et al.* 1996, 1997, 2002; QUESADA *et al.* 1999; ZBAWICKA *et al.* 2003). On the other hand, DUI in Veneridae was described only in *Tapes philippinarum*, based on the 16S rRNA gene (PASSAMONTI *et al*. 2003). In more than 600 freshwater unionid bivalves, DUI was discovered based on single genes (*COI*, *COII*, or cytochrome b) in 18 species of bivalves (CUROLE & KOCHER 2002; HOEH *et al.* 2002; MOCK *et al.* 2004; WALKER *et al.* 2006).

The current taxonomic limits of DUI are poorly understood and in need of study. Many researchers of freshwater bivalves use somatic tissues as sources of DNA for their evolutionary studies, from which only the F-haplotype of mtDNA is obtained (GRAFF & FOIGHIL 2000; GIRIBET & WHEELER 2002; STEPIEN *et al.* 2002; LEE & FOIGHIL 2004; ARAUJO *et al.* 2005). If the M-type is needed, DNA should be isolated from the gonads of male unionid specimens (HOEH *et al.* 2002; MOCK *et al.* 2004; WALKER *et al.* 2006).

Current knowledge on DUI does not include any freshwater bivalves that inhabit Poland's waters, except for sporadic reports on the widely distributed Chinese clam *Anodonta woodiana*. These reports are by the Japanese author, M. Okazaki (unpublished data, F and M haplotypes sequences submitted to the GenBank database, www.ncbi.nlm.nih.gov) or the author of this study, M. SOROKA (SOROKA 2005).

The Chinese mussel *Anodonta woodiana* has been reported from three sites in Poland already. Since the early 1990s, the species has massively colonised the first site of occurrence in Poland, i.e. the heated lakes near Konin in central Poland (PROTASOV *et al.* 1994; ZDANOWSKI 1994). Since 2002 and 2003, the mussel has also been found in fish ponds near Sieraków (Greater Poland) and the heated channel of the Dolna Odra power station near Szczecin, north-western Poland, respectively (DOMAGAŁA *et al.* 2003). This species originates from the Far East, from the Amur and the Yangtze river basins (KISS 1995; WATTERS 1997). It was brought to Europe mainly from China with the introduction of Chinese carp, which had started in 1963 (KISS 1995; WATTERS 1997). The mussel *A. woodiana* reached Poland most probably from Hungary in the mid 1980s, together with an import of silver carp and bighead carp stocking material (AFANASJEV *et al.* 1997; KRASZEWSKI & ZDANOWSKI 2001).

The aim of this study was to evaluate the level of mtDNA variation and to search for DUI through the characteristics of the male (M) and female (F) haplotypes in the freshwater Chinese mussel, *Anodonta woodiana*. These objectives were achieved through sequence analyses of the mitochondrial cytochrome oxidase subunit I (*COI*) gene from the somatic tissues and the gonads of male and female specimens.

Material and Methods

A. woodiana bivalves were collected from the heated lake Ślesińskie near Konin, Poland, in 1997 and 2005. In all, 12 specimens were collected, including 9 females and 3 males.

Some gonad follicles were used to produce wet slides for viewing under the light microscope for sex determination. Visible large ovary cells meant that the given specimen was a female (otherwise the specimen was classified as a male). Moreover, the external gills of the *A. woodiana* females were inspected for glochidia, which are present during summer and autumn.

Total DNA was isolated from the gills and gonads of the males and females using the standard phenol/chloroform method. PCR was carried out in order to detect the F and M haplotypes for the region of the mitochondrial cytochrome oxidase subunit I (*COI*) gene using LCO1490 and HCO2198 universal primers (FOLMER *et al.* 1994). Details of DNA extraction and conditions of PCR have been described by SOROKA & GRYGIEŃCZO-RAŹNIEWSKA (2005). After 2-% agarose gel electrophoresis, the products of *COI* gene amplification were viewed under UV light. The results were saved and the sizes of the PCR products were analysed with the BioCapt and Bio1D programs (Vilbert Lourmat, France).

The sequencing of PCR products was carried out in the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, in Warsaw (www.oligo.pl).

A comparative analysis of the obtained sequences was carried out using DNAMAN 5.2.9 software (Lynnon Corporation, Canada). Sequences available in GenBank for the analysed bivalve species were used in the analyses. In the molecular analyses, the parameters of genetic similarity and distances of the compared sequences were estimated according to the observed variation and Kimura's two parameter models (KIMURA 1980).

Level of genetic variation within the *COI* gene region in *A. woodiana* using the observed genetic variation method (below diagonal) and Kimura's 2-parameter model (above diagonal). Sequences obtained in this study underlined

	AB055627F	AB040832F	AF468683F	AB055626M	AB040831M	DQ340804M
AB055627F		0.000	0.059	0.467	0.467	0.462
AB040832F	0.000		0.087	0.467	0.467	0.460
AF468683F	0.056	0.081		0.448	0.448	0.460
AB055626M	0.346	0.346	0.337		0.000	0.105
AB040831M	0.346	0.346	0.337	0.000		0.105
DQ340804M	0.344	0.344	0.343	0.097	0.097	

Results

The sequence analysis of the *COI* gene region revealed the same sequence of nucleotides within 644 bp belonging to the F haplotype of nine *A. woodiana* females. Identical sequences were observed both in the somatic tissues (DNA isolated from gills) and the generative tissues (DNA isolated from gonads) of the females. The F haplotype obtained from the somatic tissues (gills) of the males was identical compared with the females (100% genetic similarity). The M haplotype of 576 bp was obtained from the gonad of a male specimen of this species. These sequences were submitted to GenBank under the following accession numbers: AF468683, for the F haplotype, and DQ340804, for the M haplotype.

The obtained sequences were compared with other sequences available in GenBank for this species (AB040831, M-type, AB040832, F-type, AB055626, M-type, and AB055627, F-type, unpublished data M. Okazaki). A comparative analysis revealed strong genetic variation of the *COI* gene region between the *A. woodiana* specimens from Poland and Japan in relation to the F- and M-type of mitochondrial DNA. For the F-type of mtDNA, the level of genetic variation ranged from 0 (within Polish and Japanese specimens separately) to 8.1% (between Polish and Japanese specimens). For the M-type, on the other hand, the parameter was higher, up to 9.7% between Polish and Japanese specimens. Genetic variation between the M- and F-types within the *COI* gene region reached 34%-35% (Table 1). Kimura's 2-parameter model (KIMURA 1980), which assumes that the level of transition substitutions is different from that of transversion substitutions, gave higher values of parameters within the F- and M-types as well as between them (Table 1).

Furthermore, according to literature data, the two mitotypes define separate clades, F and M (Fig. 1). Although the aforementioned genetic variation within the *COI* gene region of *A. woodiana* is very high, it does not exceed the level characteristic for bivalves in which DUI was discovered (HOEH *et al.* 1996, 1997).

Discussion

Despite the fact that mtDNA DUI occurs mainly in some marine (Mytilidae, Veneridae) and freshwater (Unionidae) bivalve species, ample differences occur in the character of this inheritance pattern between these groups of bivalves.

Genetic variation between the F and M types in the marine species of the genus *Mytilus* ranged between 2% and 21%. There is also evidence that recombination takes place between these mtDNA molecules as well as the effect of role take-over (masculinisation) and, therefore, in phylogenetic analyses these two mtDNA molecules do not create separate clades (HOEH *et al.* 1996, 1997; QUESADA *et al.* 1999; BURZYŃSKI *et al.* 2003). Furthermore, the M haplotype, besides being in male gonads, was also present in small quantities in male somatic tissues and in some female tissues
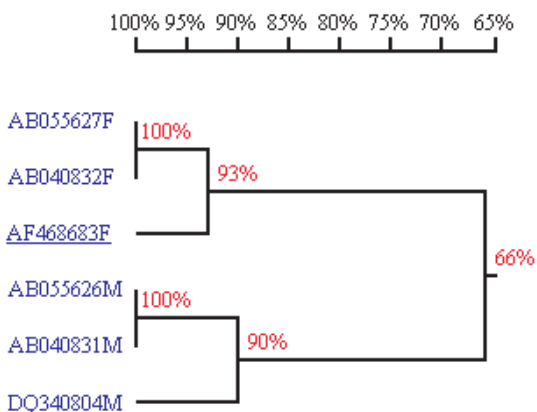

Fig. 1. Observed genetic similarity of mitochondrial F- and M-haplotypes in *Anodonta woodiana*. Sequences obtained in this study underlined.

of such species as *M. edulis*, *M. trossulus* and *Tapes philippinarum* (GARRIDO-RAMOS *et al.* 1998; DALZIEL & STEWART 2002; PASSAMONTI *et al.* 2003).

On the other hand, in freshwater bivalves (Unionidae) the observed variation between the F and M types is characteristically higher and ranges between 28% and 34% with a lower within-type variation compared to the genus *Mytilus*. The freshwater bivalves, however, lack recombination and the effect of mutual role take-over between two mtDNA molecules and hence determine separate clades in phylogenetic analyses, which can be used independently for the evaluation of the evolutionary history. The literature published so far report on the M-type present only in male gonads of freshwater Unionidae bivalves (HOEH *et al.* 2002; MOCK *et al.* 2004; SOROKA 2005).

Among the bulk of sequences submitted to GenBank (www.ncbi.nlm.nih.gov), F and M haplotypes for the mitochondrial cytochrome oxidase subunit I (*COI*) gene are not distinguished in Unionidae species, except for Anodontinae (3 *Anodonta* species from the USA, i.e. *A. woodiana*, *Pyganodon grandis*, *P. fragilis*) and Ambleminae (*Fusconaia flava*) (LIU *et al.* 1996; HOEH *et al.* 2002; MOCK *et al.* 2004). From the aforementioned species, only *A. woodiana* is found in Poland.

In the four *Unio* species available in GenBank, F and M haplotypes have not been distinguished for the *COI* gene region, which does not mean that DUI does not occur in these species; furthermore, DUI is expected here due to the historical origin of this pattern. A comparative analysis of these sequences and the species *Inversidens japanensis* (synonym *Unio japonensis*) revealed that the sequences available in GenBank are most probably F-mitotypes and exhibit 34% variation in relation to the M-mitotype. In order to confirm whether DUI (mainly of the M-mitotype) takes place and, if so, what its character is in various freshwater bivalves, comprehensive studies are necessary which would include precise DNA isolation from both somatic and generative cells of both males and females.

In this study, DNA was isolated from somatic tissues (gills of males and females) as well as from male gonads, therefore the sequence analysis of the *COI* gene region was performed for both the M- and the F-haplotypes.

The results obtained for a number of Polish *A. woodiana* specimens reveal an identical sequence within the *COI* gene region for the F-haplotype and 6-8-% divergence in relation to Japanese specimens. On the other hand, Polish and Japanese M-haplotypes were characterised by a higher level of genetic variation, which reached nearly 10% with no variation among Japanese specimens of this species. Furthermore, according to literature data (HOEH *et al.* 1996, 2002), the sequences of both haplotypes clearly form separate clades, F and M (Fig. 1). Although the described genetic variation within the *COI* gene region in *A. woodiana* is very high, it does not exceed the level characteristic for bivalves in which DUI was discovered and described (HOEH *et al.* 1996, 1997, 2002).

The variation of mitochondrial haplotypes described by LIU *et al.* (1996a, b) in *Pyganodon grandis* ranges between 0.5% and 12% within, respectively, the F- and the M-haplotypes, and between 6.1% and 8.9% between F and M. This value is lower than that described earlier in Unionidae, since it was obtained using restriction analysis of the entire mtDNA molecule (mtDNA RFLP) and not by *COI* gene region sequencing. This technique, however, reflects the general relationship of higher nucleotide substitution rates within the M genome compared with the F in Unionidae bivalves (SKIBINSKI *et al.* 1994a; ZOUROS *et al.* 1994a; HOEH *et al.* 2002; MOCK *et al.* 2004).

However, a comparison of sequences of the *COI* gene regions available in GenBank for *P. grandis* (AF2314734F, AF406801M, and AF156504) reveals 0.2% variation within the F-mitotype and 33% between the F and M mitotypes, which remains within the range that is characteristic for DUI in freshwater bivalves (HOEH *et al.* 1996, 2002).

The results of this study describe the genetic variation of mitochondrial haplotypes F and M in *A. woodiana* and, at least partially, fill the gap in the knowledge on mtDNA DUI in freshwater bivalves. Why DUI is limited to some taxa is still poorly understood and clarifying this requires comprehensive studies covering a range of genes.

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