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Phenotype and Genotype Differentiation between Flathead Grey Mullet, *Mugil Cephalus* And Thinlip Grey Mullet, *Liza Ramada* (Risso 1827) (Pisces: *Mugilidae*)

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ABSTRACT

This work aimed to study the phenotype and genotype differentiation and to compare the amount of differences in phenotype based on morphometric character indices and meristic counts with the amount of differences in genotype based on random amplified polymorphic DNA (RAPD) fingerprinting between two *Mugilidae*, Flathead grey mullet, *Mugil cephalus* and Thinlip grey mullet, *Liza ramada*. The results showed that, there were significant differences in the most of morphometric character indices but no significant differences were detected in the most of meristic counts between *Mugil cephalus* and *Liza ramada*. In addition, the euclidean distance between *Mugil cephalus* and *Liza ramada* using hierarchical cluster analysis of quantitative phenotype based on morphometric character indices and meristic counts and condition factor was (0.421). Genotype analysis based on RAPD fingerprint showed highly genetic dissimilarity (0.437) between *Mugil cephalus* and *Liza ramada*. These results confirmed that the amount of differences in genotype reflected the same amount of differences in phenotype between *Mugil cephalus* and *Liza ramada*. Therefore, it can be use either phenotype analysis based on a large number of morphometric character indices and meristic counts or genotype analysis based on RAPD fingerprinting to discriminate *Mugil cephalus* and *Liza ramada* with the same results.

Keywords: Phenotype, Genotype, *Mugil cephalus* and *Liza ramada*

INTRODUCTION

Mullets are euryhaline fish widely distributed in tropical and subtrobical and estuaries. Mullets are catadromous spawning migrating fish, the young life before maturity remains predominantly in the system of rivers and lakes (Lee

and Tamaru, 1988; El-Deeb *et al*., 1996). Five mullet species occur in Egyptian water. According to the different regions, the most commonly-cultured of mullets are *Mugil cephalus* and *Liza ramada* (Lee and Tamaru, 1988; El-Sayed, 1991). It is considered an important source of animal protein for the peoples of the Pacific Basin, Southeast Asia, India, the Mediterranean, Eastern Europe and, many parts of central and south America (Lee and Tamaru, 1988). The identification of the fry to be used for stocking purposes is of practical interest and often causes problems for farmers who want to stock the ponds with specific species (Menezes *et al*., 1992).

Different methods are used for identification but phenotype based on morphometric and meristic are considered as earliest and authentic methods for the identification of fish species in fish biology to measure discreteness and relationships among various taxonomic categories. There are many well documented morphometric studies which provide evidence for stock discreteness (Avsar, 1994; Haddon and Willis, 1995; Bembo *et al*., 1996; Anene, 1999; Turan, 1999; Zafar *et al*., 2002; Barriga-Sosa *et al*., 2004; Doherty and McCarthy, 2004; Naesje *et al*., 2004). Morphometric is the external measurments of an organism, while meristic counts means serial counts of body elements (Talwar and Jhingran, 1992). Morphological characters including meristic counts and body proportions, ofen vary clinically (i.e. along a geographic gradient) (Lindsey, 1988).

Ihssen *et al*. (1981); Allendorf, (1988); Swaine *et al*. (1991) and Turan, (1999) reported that, phenotypic adaptations do not necessarily result in genetic changes in the population and thus the detection of such phenotypic differences among populations cannot usually be taken as evidence of genetic differentiation. Studies of morphological character variation are, therefore, vital in order to elucidate patterns observed in phenotypic and genetic character variation among fish populations (Beheregaray and Levy, 2000).

The technique of random amplified polymorphic DNA (RAPD) marker (Welsh and McCelland, 1990; Williams *et al*.,

1990) has been successfully exploited for stock identification and population analysis in fish (Partis and Wells, 1996; Dong and Zhou, 1998; Bartfai *et al*., 2003; Ahmed *et al*., 2004; El-Zaeem *et al*., 2006; El-Zaeem and Ahmed, 2006).

Therefore, the aim of the present work was to study the phenotype and genotype differentiation and to compare the amount of differences in phenotype based on morphometric character indices and meristic counts with the amount of differences in genotype based on random amplified polymorphic DNA (RAPD) fingerprinting between two *Mugilidae*, Flathead grey mullet, *Mugil cephalus* and Thinlip grey mullet, *Liza ramada*

MATERIALS AND METHODS

The present study was carried out at Fish breeding and production laboratory, Animal and Fish Production Department, Faculty of Agriculture (Saba-Bacha), Alexandria University & Nucleic Acids Research Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City for Scientific Research & Technology Applications, Alexandria, Egypt.

Specimen collection

Larvae of Flathead grey mullet, *Mugil cephalus* and Thinlip grey mullet, *Liza ramada* were collected from Mediterranean sea and transferred to culture into earthen ponds at Faculty of agriculture (Saba-Bacha) fish farm for 12 month. From both sex, a total of 216 specimens of Flathead grey mullet, *Mugil cephalus* and 234 of Thinlip grey mullet, *Liza ramada* were randomly collected. Their weight and length ranged from (32.10- 50.00 g and 16.50- 19.80 cm) and (28.00-45.50 g and 15.60-18.60 cm), respectively.

Quantitative phenotype analysis

A total of 34 morphometric characters and 9 meristic counts were recorded within each species as descriped by (Bagenal, 1978; Ezzat *et al*., 1979; Taniguchi *et al*., 1996; Sahu *et al*., 2000; Costa *et al*., 2003 and Doherty and McCarty, 2004). Condition factor of each species were measured using the following equation:

$K = (W/L^3)\ 100$ (Lagler, 1956),

Where: W=body weight (g)
L= total length (cm).

All morphometric characters were transformed by dividing the measurment by either total or standard length or head length of each fish to minimize the effect of fish size (Tables 1 and 3).

Phenotype differentiation between Flathead grey mullet, *Mugil cephalus* and Thinlip grey mullet, *Liza ramada* based on morphometric character indices and meristic counts was analysed by means of the hierarchical cluster analysis of the STATISTICA package, version 5.00 (StatSoft, 1995). The cluster analysis using unweighted pair group average method (UPGMA: Sneath and Sokal, 1973) was performed on the matrix of euclidean distance in order to depict hierarchically the shape differences between Flathead grey mullet, *M. cephalus* and Thinlip grey mullet, *L. ramada.*

Statistical analysis

Data significance of the morphometric character indices and meristic counts were analyzed using unpaired Student's t-test ($P<0.05$) according to Snedecor and Cochran (1980).

Genotype analysis

Genotype analysis was performed based on random amplified polymorphic DNA (RAPD) fingerprinting. DNA was extracted from liver tissues of each Flathead grey mullet, *Mugil cephalus* and Thinlip grey mullet, *Liza ramada* according the method described by (Bardakci and Skibinski, 1994).

In the present work ten and twenty base long oligonucleiotide primers were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplification (Table 2).

PCR amplifications were performed according to the procedure of Williams *et al*., 1990 and 1993. The reaction (25 µl) was carried out in a mixture consisting of 0.8 U of Taq DNA polymerase (Fanzyme), 25 pmol dNTPs, and 25 pmol of random primer, 2.5 µl 10X Taq DNA polymerase buffer and 40 ng of genomic DNA.

Table (1): ***Quantitative phenotype traits based on morphometric characters and meristic counts used for differentiation analysis between Mugil cephalus and Liza ramada.***

Characters	Acronyms
Morphometric analysis	
Total length	**TL**
Standard length	**SL**
Head length	**HL**
Body depth	**BD**
Body width	**BW**
Head width	**HW**
Abdomen length	**AL**
Caudal peduncle length	**CPL**
Caudal peduncle depth	**CPD**
Caudal peduncle width	**CPW**
Upper jaw length	**UJL**
Lower jaw length	**LJL**
Snout length	**SnL**
Orbit diameter	**OD**
Pre-orbital length	**Pr-OL**
Post-orbital length	**Po-OL**
Inter dorsal distance	**IDD**
Snout to first dorsal	**Sn-FD**
Trunk length	**TrL**
First dorsal fin base length	**FDFBL**
Second dorsal fin base length	**SDFBL**
Pelvic fin base length	**Pel FBL**
Anal fin base length	**AFBL**
Caudal fin length	**CFL**
Pectoral fin length	**Pec FL**
Length of longest first dorsal fin spine	**LLoFDFS**
Length of last first dorsal fin spine	**LLaFDFS**
Length of longest second dorsal fin ray	**LLoSDFR**
Length of last second dorsal fin ray	**LLaSDFR**
Length of longest anal fin spine	**LLoAFS**
Length of longest anal fin ray	**LLoAFR**
Length of last anal fin ray	**LLaAFR**
Length of longest caudal fin ray	**LLoCFR**
Length of shortest caudal fin ray	**LShCFR**
Meristic analysis	
First dorsal fin spines count	**FDFSC**
Second dorsal fin rays count	**SDFRC**
Pelvic fin spines count	**Pel FSC**
Pelvic fin rays count	**Pel FRC**
Pectoral fin rays count	**Pec FRC**
Anal fin spines count	**AFSC**
Anal fin rays count	**AFRC**
Caudal fin rays count	**CFRC**
Total number of vertebrae	**TNN**

Table (2): ***The sequences, GC% and the annealing temperatures of the primers used.***

Primers	Sequence 5`- 3`	GC%	Annealing Tm °C /Sec
1	**GAA TGC GAC G**	**60**	**34/30**
2	**ATG ACG TTG A**	**40**	**34/30**
3	**GGA CTG GAG TGT GAT CGC AG**	**60**	**58/30**
4	**GGA CTG GAG TGG TGA CGC AG**	**65**	**58/30**
5	**CAG GCC CTT CCA GCA CCC AC**	**70**	**52/30**
6	**GAA ACG GGT GGT GAT CGC AG**	**60**	**52/30**
7	**TGG TGG ACC A**	**60**	**34/30**
8	**AGC AGG TGG A**	**60**	**34/30**
9	**CTG AGG AGT G**	**60**	**34/30**
10	**GGG CTA GGG T**	**70**	**34/30**

The final reaction mixture was placed in a DNA thermal cycler (Eppendorf). The PCR program included an initial denaturation step at 94°C for 2 minutes followed by 45 cycles with 94°C for 30 seconds for DNA denaturation, annealing as mentioned with each primer (Table 2), extension at 72°C for 30 seconds and final extension at 72 °C for 10 minutes were carried out. Samples were cooled at 4°C.

The amplified DNA fragments were separated on 2.5% agarose gel and stained with ethidium bromide. ΦX174 DNA Ladder marker (bp 1335, 1078, 872, ……72) was used in this study. The amplified patterns were visualized on an UV transilluminator and photographed by Gel Documentation system.

RAPD patterns were analyzed and scored from photographs. For the analysis and comparison of the patterns, a set of distinct, well-separated bands were selected. The genotypes were determined by recording the presence (1) or absence (0) in the RAPD profiles. Genetic similarity (GS) between Flathead grey mullet, *Mugil cephalus* and Thinlip grey mullet, *Liza ramada* was calculated according the formula given by Nei and Li (1979): $B_{ij}=2\ N_{ij}/(N_i + N_j)$, where N_{ij} is the number of common bands observed in individuals i and j, and N_i and N_j are the total number of bands scored in individuals i and j respectively, with regard to all assay units. Thus, GS reflects the

proportion of bands shared between two individuals and ranges from zero (no common bands) to one (all bands identical). Genetic dissimilarity (GD) was calculated as: GD = 1- GS (Bartfai *et al*., 2003).

RESULTS

Quantitative phenotype analysis

Mean values of morphometric character indices were compared between *M. cephalus* and *L. ramada*. The highest means indices of BD / SL, BW / SL, AL / SL, CPD / SL, CPW / SL, UJL/HL, LJL/HL, HW/HL, SnL / HL, TrL/SL, IDD/SL, LLaSDFR / SL, LLaFDFS / SL, FDFBL / SL, SDFBL / SL, Pel FBL / SL, LLaAFR / SL, AFBL / SL, LLoCFR / TL and Pec FL / SL of *M. cephalus* were differ significantly from those of *L. ramada*. While, the means indices of HL / SL, Sn-FD / SL, LLoFDFS / SL and LLoAFS / SL, showed significant superiority for *L. ramada* compared with those of *M. cephalus*. The results showed also that, no significant differences were detected in the indices of CPL/SL, OD / HL, Pr-OL / HL, Po-OL / HL, LLoSDFR / SL, LLoAFR / SL, LshCFR / TL and CFL / TL, between *M. cephalus* and *L. ramada* (Table 3).

Moreover, the results of meristic counts showed that the highest and lowest mean value of Pec FRC and AFRC were obtained by *M. cephalus* and differ significantly from that of *L. ramada*. While, the other meristic counts records of FDFSC, SDFRC, Pel FSC, Pel FRC, AFSC, CFRC and TNN, showed no significant differences between *M. cephalus* and *L. ramada*. In addition, *M. cephalus* had significant superiority of condition factor (K) compared with *L. ramada* (Table 3).

The euclidean distance between *Mugil cephalus* and *Liza ramada* using hierarchical cluster analysis of quantitative phenotype based on morphometric character indices, meristic counts and condition factor was 0.421.

Genotype analysis

All the ten different primers used in this work, produced different RAPD band patterns (Table 4). The number of amplified bands detected varied, depending

Table (3): ***Means and standard error of quantitative phenotype traits based on morphometric character indices and meristic counts used for differentiation analysis between Mugil cephalus and Liza ramada.***

Characters	*Mugil cephalus* Mean±S.E.	*Liza ramada* Mean±S.E.	*t*-test
Morphometric analysis			
BD/SL	23.87±0.21	21.55±0.21	*
BW/SL	14.98±0.13	11.75±0.09	*
HL/SL	24.68±0.21	27.60±0.29	*
HW/HL	67.55±0.57	54.68±0.60	*
AL/SL	45.55±0.24	43.37±0.26	*
CPL/SL	16.91±0.24	17.36±0.18	NS
CPD/SL	11.45±0.12	10.37±0.14	*
CPW/SL	6.56±0.09	5.51±0.10	*
UJL/HL	21.87±0.42	16.94±0.31	*
LJL/HL	19.70±0.38	15.46±0.27	*
SnL/HL	21.97±0.39	16.90±0.35	*
OD/HL	22.59±0.48	23.02±0.34	NS
Pr-OL/HL	21.89±0.45	22.56±0.30	NS
Po-OL/HL	58.99±0.52	57.96±0.58	NS
IDD/SL	15.23±0.35	13.00±0.23	*
Sn-FD/SL	47.98±0.30	51.56±0.23	*
TrL/SL	58.84±0.23	56.55±0.27	*
FDFBL/SL	11.06±0.29	10.19±0.18	*
SDFBL/SL	10.80±0.19	8.33±0.08	*
Pel FBL/SL	11.00±0.22	9.81±0.08	*
AFBL/SL	12.38±0.22	11.85±0.11	*
CFL/TL	20.79±0.16	20.68±0.16	NS
Pec FL/SL	17.69±0.20	17.05±0.22	*
LLoFDFS/SL	13.23±0.23	15.48±0.16	*
LLaFDFS/SL	4.92±0.14	4.79±0.12	*
LLoSDFR/SL	13.43±0.26	13.39±0.16	NS
LLaSDFR/SL	6.67±0.10	5.62±0.12	*
LLoAFS/SL	7.76±0.14	10.28±0.23	*
LLoAFR/SL	13.26±0.26	13.76±0.19	NS
LLaAFR/SL	6.65±0.12	5.96±0.12	*
LLoCFR/TL	19.66±0.19	18.95±0.17	*
LShCFR/TL	8.82±0.12	8.79±0.16	NS
Meristic analysis			
FDFSC	4.00±0.00	4.00±0.00	NS
SDFRC	9.00±0.00	9.00±0.00	NS
Pel FSC	1.00±0.00	1.00±0.00	NS
Pel FRC	5.00±0.00	5.00±0.00	NS
Pec FRC	16.28±0.11	16.00±0.00	*
AFSC	3.00±0.00	3.00±0.00	NS
AFRC	7.94±0.06	9.04±0.04	*
CFRC	16.00±0.00	16.00±0.00	NS
TNN	24.00±0.00	24.00±0.00	NS
Length-weight relationship			
K	0.92±0.01	0.72±0.01	*

t-test: * P<0.05 NS: not significant.

Table (4): ***Total number of band, polymorphic bands and genetic dissimilarity between Mugil cephalus and Liza ramada using different random sequence of primers.***

Primer No.	Total bands	Polymorphic bands	Genetic dissimilarity
1	**8.00**	**2.00**	**0.25**
2	**7.00**	**5.00**	**0.71**
3	**8.00**	**4.00**	**0.50**
4	**6.00**	**2.00**	**0.33**
5	**8.00**	**0.00**	**0.00**
6	**5.00**	**3.00**	**0.60**
7	**11.00**	**5.00**	**0.45**
8	**6.00**	**2.00**	**0.33**
9	**5.00**	**1.00**	**0.20**
10	**2.00**	**2.00**	**1.00**
Average	**-**	**-**	**0.437**

on the primers and species. In addition, to ensure that the amplified DNA bands originated from genomic DNA, and not from primer artifacts. Also, negative control was done for each primer/species combination. No amplification was detected in the control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions (Table 4 and Figure 1).

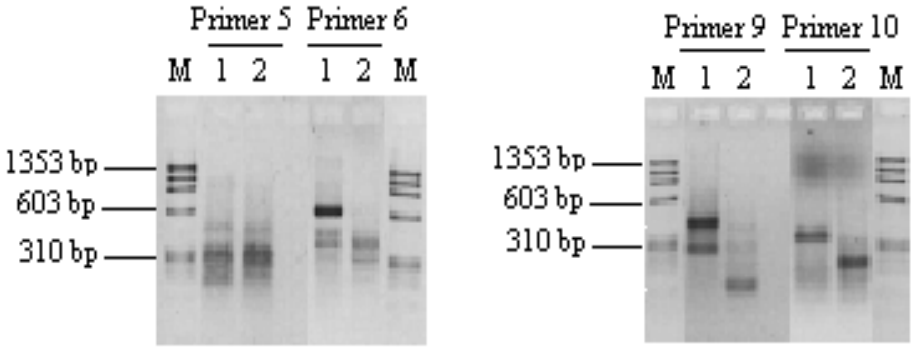

Figure (1): ***Example of RAPD amplification products. Lane M: ΦX174 DNA marker, the lanes 1 and 2 of each primer are Mugil cephalus and Liza ramada, respectively.***

The Random amplified polymorphic DNA (RAPD) fingerprint was used for detection the genetic diversity between *M. cephalus* and *L. ramada*. The results showed highly genetic dissimilarity range (0.00 to 1.00) with an average 0.437 using different random primers (Figure 1 and Table 4).

DISCUSSION

Morphometric characters and meristic counts have been the most widely used tool in racial studies in fish taxonomy (Anene, 1999). Meristic counts were much easier to evaluate and seem to be advantageous because most counts can be collected from live fish. However, meristic data alone may not provide the detail necessary to discern dissimilarities between different populations at the same species and sometimes at the same genus (North *et al*., 2002). The results of the present work are consistent with these findings as the most of morphometric character indices showed significant differences between *M. cephalus* and *L. ramada*. While the most of meristic counts showed insignificant differences between *M. cephalus* and *L. ramada*.

The main advantages of RAPD markers are the possibility of working with anonymous DNA and the relatively low expense, also fast and simple to produce RAPD marker (Hadrys *et al*., 1992; Elo *et al*., 1997; Ali *et al*., 2004). Moreover, RAPD analysis might be useful for systematic investigation at the level of species and subspecies (Bardakci and Skibinski, 1994), and more sensitive and technically easier to perform and produced results with low statistical error, whereas DNA fingerprinting detected greater genetic differentiation between Nile tilapia stains than other molecular techniques such as multilocus minisatellite marker (Naish *et al*., 1995).

The results of the present work are consistent with the findings reported by (Papasotiropoulos *et al*., 2001, 2002 and 2007; Blel *et al*., 2008; Semina *et al*., 2007), they applied several techniques for genetic analysis among *Mugilidae* family and reported that, *Mugil cephalus* is the most distinct species compared with

Liza genus. Also, the results confirmed that the amount of differences in genotype reflected the same amount of differences in phenotype between *Mugil cephalus* and *Liza ramada.* In addition, these results stated that, genetic analysis based on RAPD fingerprinting confirm the existing taxonomic system based on morphometric character indices and meristic counts and confirmed the results reported by Li *et al*. (1993) ; Louis and Barlow, (1987).

The major limitation of morphological characters at the intra-specific level is that phenotypic variation is not directly under genetic control but subjected to environmental modification (Clayton, 1981).

Therefore, it can be use either phenotype analysis based on a large number of morphometric character indices and meristic counts or genotype analysis based on RAPD fingerprinting to discriminate *Mugil cephalus* and *Liza ramada* with the same results.

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الاختلافات المظهرية والوراثية بين أسماك البورى والطوبار (العائلة البورية)

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تهدف الدراسة الحالية الى دراسة وتقييم الاختلافات المظهرية اعتمادا على دلائل الخواص المورفومترية وتعداد الميرستيك ومقارنتها بحجم الاختلافات الوراثية اعتمادا على البصمة الوراثية للتكبير العشوائى لجزئ DNA بين اثنين من أسماك العائلة البورية (البورى والطوبار). وقد أوضحت النتائج المتحصل عليها وجود اختلافات ذات دلالة احصائية فى معظم دلائل الخواص المورفومترية بينما لم تظهر النتائج أى اختلافات ذات دلالة احصائية فى معظم صفات تعداد الميرستيك بين كل من أسماك البورى والطوبار. بالاضافة الى ذلك فان تحليل الاختلافات المظهرية بين النوعين باستعمال hierarchical cluster analysis اعتمادا على دلائل الخواص المورفومترية وتعداد الميرستيك ومعامل الحالة أظهر مسافة euclidean distance قدرت بالقيمة (0.421). بينما تحليل الاختلافات الوراثية اعتمادا على البصمة الوراثية للتكبير العشوائى لجزئ DNA أظهر اختلاف وراثى بين النوعين قدر بالقيمة (0.437). يستخلص من تلك النتائج ان مقدار الاختلافات الوراثية قد يعكس نفس مقدار الاختلافات المظهرية بين النوعين. بناء على ذلك توجد امكانية اما لأستخدام تحليل الشكل المظهرى اعتمادا على تسجيل عدد كبير من دلائل الخواص المورفومترية وتعداد الميرستيك أو لأستخدام تحليل التركيب الوراثى اعتمادا على البصمة الوراثية للتكبير العشوائى لجزئ DNA للتفريق بين أسماك البورى والطوبار بنفس النتائج المتحصل عليها.