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Relationships between heterozygosity, growth parameters and age in the common pandora *Pagellus erythrinus* (Sparidae) in the Gabes Gulf (Tunisia)

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ABSTRACT: The relationship between heterozygosity and age, as well as growth-related traits, was explored in the common pandora *Pagellus erythrinus*, a sparid fish subjected to commercial exploitation in the Mediterranean Sea. Allozyme electrophoresis was used to quantify the levels of heterozygosity of 238 adult fish from 3 to 7 yr old, aged by means of otoliths and captured in the Gabes Gulf, located along the south-eastern coast of Tunisia. Analyses were conducted by using individual single-locus and multi-locus heterozygosity and 4 growth-related parameters — standard length, total body weight, otolith length and otolith weight — as well as 2 measures of condition (condition factor and relative condition factor). For the 4 growth-related parameters and 2 condition parameters analyzed at 8 polymorphic loci, heterozygotes had higher average values than homozygotes in 36 out of 48 comparisons. However, since these 6 biometric parameters are highly correlated, this proportion should not be considered globally as strong evidence of a heterozygosity–fitness correlation (HFC). Interpreting these data on a per parameter basis, only otolith weight appeared significantly and positively correlated with multi-locus heterozygosity. Thus, although there was evidence for HFC, it appeared relatively weak for the growth parameters analyzed and may have arisen by chance. The results we obtained relative to survival are more convincing. Although cohorts did not appear genetically differentiated and the whole sample did not display any internal structure after a Bayesian analysis with the STRUCTURE software, multi-locus individual heterozygosity was significantly and positively correlated with fish age. This suggests better survival of heterozygotes and establishes the presence of a heterozygosity–fitness correlation in the Tunisian population of the common pandora.

KEY WORDS: Allozymes · Heterozygosity–fitness correlation · Survival · Morphological measurements · Otoliths · Condition indices

INTRODUCTION

The ability of populations to adapt to selection pressures or to resist an extinction threat depends partly on the genetic variability available in the population (Frankham et al. 2002, Frankham 2005). The role of genetic diversity loss in extinction has been well discussed and has led to a contentious debate between ecologists and geneticists (Lande 1988, Caro & Laurenson 1994). Over the last couple of decades, several studies have demonstrated that inbreeding, reduction in effective population size and loss of genetic diversity could endanger certain populations (Westemeier et al. 1998, Frankham et al.

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1999, Bijlsma et al. 2000). Positive correlations between traits related to fitness and heterozygosity in several organisms support this point of view (Britten 1996, Reed & Frankham 2001, 2003, Coltman & Slate 2003). This link between fitness and heterozygosity is important in conservation biology given that many threatened species or populations suffer from a low level of genetic variation (Gottelli et al. 1994, Gibbs et al. 1997, Grueber et al. 2008).

Using various genetic markers, positive correlations have been reported between heterozygosity and several components of fitness in natural populations of many species (Britten 1996, Coltman et al. 1998, Bierne et al. 2000, Thelen & Allendorf 2001, Markert et al. 2004, Pujolar et al. 2005, Chapman et al. 2009, Da Silva et al. 2009, Hoffman et al. 2010). These studies focused, in particular, on the viability, growth rate, fecundity and developmental stability as traits related to fitness. However, the underlying mechanisms responsible for such positive heterozygosity–fitness correlations (HFC) remain under debate (Hansson & Westerberg 2008).

Alternative scenarios can be proposed to account for the correlation. At a given locus, fitness can be either higher in heterozygotes (overdominance) or lower in one of the homozygote genotypes. The major distinction among possible causes of HFC is between single-locus effects (each of which usually arises through linkage disequilibrium between one marker and a nearby gene) and genome-wide effects due to inbreeding, where there is a cumulative effect of many loci distributed across the whole genome (David 1997, 1998, Hansson & Westerberg 2002, Szulkin et al. 2010).

Determining whether the observed HFC is due to direct (overdominance), local (associative overdominance) or general effects (associative inbreeding depression) theoretically requires testing 3 different but related models (David 1997, 1998). This requires data sets that are qualitatively and quantitatively much stronger than those commonly available. However, the mechanism causing the observation of positive HFC is commonly interpreted empirically. For instance, correlations between microsatellite heterozygosity and fitness-related traits are considered unlikely to be due to direct effects since microsatellites are generally not part of coding sequences (Ellegren 2004). When multi-locus heterozygosity (MLH), but not single-locus heterozygosity (SLH), displays a significant association with fitness, a global effect seems more likely than a direct or local effect.

The biological model studied here is the common pandora *Pagellus erythrinus* Linnaeus, 1758 (family Sparidae), a benthic fish constituting a valuable component of the commercial fishery in Tunisia. Although *P. erythrinus* plays an important role in local microeconomics through its capture volume and its high commercial value (Ghorbel 1996), its existence may be threatened because the species is heavily exploited compared with other Mediterranean sparids. A study conducted in the Gabes Gulf on the south-eastern coast of Tunisia has shown that the stock of *P. erythrinus* is overfished and that the youngest specimens are the most overexploited (Jarboui et al. 1998). The choice of *P. erythrinus* for this study was, therefore, based on criteria of both biological and commercial interest.

Pagellus erythrinus is a common species covering a wide distribution in the Mediterranean and Black Sea, as well as the eastern Atlantic Ocean from Scandinavia to Cape Verde (Fischer et al. 1987). *P. erythrinus* is found in depths up to 320 m but most commonly does not exceed 10 to 100 m (Spedicato et al. 2002). *P. erythrinus* is mainly a protogynous hermaphroditic species (Buxton & Garratt 1990). Individuals are primarily female and become male during the third or fourth year of life. The age of first sexual maturity occurs between the second and third year. Reproduction in *P. erythrinus* may extend from spring to early autumn and varies according to regions and hydrological conditions (Ghorbel 1996, Pajuelo & Lorenzo 1998, Valdés et al. 2004, Coelho et al. 2010).

At 2 locations in Tunisia, expected heterozygosity (H_e) increased with age compared among all year classes in *Pagellus erythrinus*, and cohorts were not genetically differentiated (Fassatoui et al. 2011). This pattern may occur when individuals displaying greater heterozygosity live longer, but it may also be the simple consequence of admixture increasing with age (which may occur if the older individuals have dispersed farther from their birth place), in which case the older individuals are not expected to display a higher multi-locus heterozygosity. This could be tested thoroughly considering the individual index of heterozygosity (multi-locus heterozygosity, MLH). We thus focused, for the present study, on one of these locations from the Gabes Gulf and decided to investigate the possibility of HFC more thoroughly by exploring the relationship between individual heterozygosity, detected by allozyme electrophoresis techniques, and several fitness components related to growth or survival (age) in a natural population of *P. erythrinus*. Such data will provide guidelines for the management and conservation of the species.

MATERIALS AND METHODS

Study area and population

The study was conducted in the Gabes Gulf, located on the south-eastern Tunisian coast. The Gulf of Gabes occupies a crucial location in the center of the Mediterranean Sea, at the junction of the eastern and western basins. One of its main features is the amplitude of the tides, the highest in Tunisia, due to the very wide and gently sloping continental shelf (Sammari et al. 2006).

A total of 238 adult *Pagellus erythrinus* were collected quarterly over a year between December 2006 and December 2007. Sample collection was performed at the commercial fishing landing of Zarzis City by using a stratified random method (Fassatoui et al. 2011). A preliminary investigation was carried out at the collection site with fishers to verify the geographic origin of fish and capture techniques employed. Individuals were transported frozen in dry ice directly to the laboratory and stored at −25°C until dissection.

In the laboratory, morphological measurements were taken from each individual. Fish were then dissected to isolate the liver and a piece of the right dorsal muscle (400 mg each) for electrophoresis. Otoliths (sagittae) were removed, cleaned and stored in sterile, numbered microcentrifuge tubes (Eppendorf) until further analysis. Sex was determined macroscopically during dissection by direct observation of the gonads. The males have white gonads with a smooth surface, while the females have red brick-coloured gonads with a rough surface. The age of each fish was estimated by directly counting the number of slow-growing winter zones (annuli) from otoliths according to the method of Bagenal & Tesch (1978). Each otolith was counted at least twice by independent observers and a third count was made in the event of disagreement.

Fitness measures

Four parameters related to growth were measured for each specimen: Standard length (L_s, length from the tip of the snout to the posterior end of the last vertebra, measured to the nearest millimeter with an ichthyometer), total body weight (W_b, measured on a top-loading Mettler balance to the nearest 0.01 g), otolith length (L_o, length of otolith from rostrum to postrostrum, measured to the nearest micrometer under a binocular microscope (Wild Heerbrugg M20) with micrometer grids) and otolith weight (W_o, measured with a precision balance to the nearest 0.0001 g). In addition, we calculated the condition factor (CF) and the relative condition factor (RCF) for each fish, according to Ricker (1975) and Le Cren (1951), respectively. The condition factor is based on the analysis of length–weight data and is used for comparing the condition, fitness or well-being of fish, based on the assumption that heavier fish of a given length are in better condition. Ricker's (1975) condition factor was calculated as $CF = 1000 \times (W_b/L_s^{b_1})$, where L_s is in centimeters, W_b in grams and b_1 is the slope from the logarithmic transformation of the length–weight regression for all samples. The relative condition factor, as described in Le Cren (1951), was calculated as $RCF = W_b/\hat{W}_b$. The observed body weight (g) of each specimen is compared with its expected body weight ($\hat{W}_b = aL_s^b$) based on its observed length (cm). The expected weight was estimated by using a logarithmic transformation of length–weight regression (determined by a and b) of all specimens. RCF indicates whether a specimen is in better (RCF > 1) or worse (RCF < 1) condition than an average specimen of the same length.

Allozyme electrophoresis

Liver and muscle tissue were homogenized separately in an equal volume of Tris/EDTA/NADP buffer (pH 6.8). The homogenates were centrifuged at 10 000 × g for 10 min at 4°C. The supernatants containing soluble enzymes were stored at −25°C. Electrophoresis was performed in starch gel at a concentration of 11% using 4 buffers: Tris/borate/EDTA at pH 9 (TBE 9), Tris/borate/EDTA at pH 8.6 (TBE 8.6), Tris/citrate at pH 8 (TC 8) and Tris/citrate at pH 7 (TC 7) according to Ayala et al. (1972) and Pasteur et al. (1987).

The enzyme systems used for analysis were determined based on the results of a few studies on the genetics of sparid fish, particularly those of Reina et al. (1994) and Alarcón & Alvarez (1999). These studies have been used as a reference for the interpretation of zymograms in *Pagellus erythrinus*. The level of polymorphism was not considered as a criterion for the selection of enzyme systems, and 14 enzyme systems were retained for their clear profiles and thus screened (Table 1). The staining protocols followed that of Pasteur et al. (1987). Allele assignment was carried out comparing the relative distance with the most common allele (**100*).

Table 1. Enzyme systems chosen for heterozygosity-fitness correlation analysis in common pandora *Pagellus erythrinus* from the Gabes Gulf. Enzyme Commission (EC) numbers follow the International Union of Biochemistry Nomenclature Committee. Gene locus nomenclature follows Shaklee et al. (1990). TBE: Tris/borate/EDTA; TC: Tris/citrate

Enzyme system	EC no.	Gene locus	Source tissue	Electrophoresis conditions Buffer	pH
Alcohol dehydrogenase	1.1.1.1	*ADH**	Liver	TBE	8
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6PDH**	Liver	TC	8
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-1**	Muscle	TC	8
		*GPI-2**	Muscle	TC	8
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH**	Muscle	TC	8
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*IDHP-1**	Muscle	TC	7
		*IDHP-2**	Liver	TC	8
Lactate dehydrogenase	1.1.1.27	*LDH-1**	Muscle	TC	8
		*LDH-2**	Muscle	TC	8
Leucine aminopeptidase	*.*.*.*	*LAP**	Muscle	TC	7
Malate dehydrogenase	1.1.1.37	*MDH-1**	Muscle	TC	8
		*MDH-2**	Muscle	TC	8
Malic enzyme (NADP+)	1.1.1.40	*MEP-1**	Muscle	TC	8
		*MEP-2**	Muscle	TC	8
Phosphoglucomutase	5.4.2.1	*PGM-1**	Liver	TC	8
		*PGM-2**	Muscle	TC	8
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	Liver	TC	8
Octanol dehydrogenase	1.1.1.73	*ODH**	Liver	TBE	9
Superoxide dimutase	1.15.1.1	*SOD-1**	Liver	TBE	8.6
		*SOD-2**	Liver	TBE	8.6
Xanthine dehydrogenase	1.2.1.37	*XDH**	Liver	TBE	9.6

Statistical and genetic analyses

Genotypes were scored directly from the gels. Allele frequencies, multi-locus and single-locus genetic diversity indices (number of alleles, observed heterozygosity [H_o] and H_e) were calculated with the GENETIX v. 4.05.2 software package (Belkhir et al. 2004). Individual MLH was measured as the number of allozyme loci for which the sampled individuals were heterozygous. This measure was used as the main indicator for studying correlation between fitness and heterozygosity in *Pagellus erythrinus*. Departures from Hardy-Weinberg equilibrium expectations were investigated with Wright's (1951) F_{IS} index as described in Weir & Cockerham (1984). Significance of divergence from equilibrium was evaluated by an exact test as performed by Raymond & Rousset (1995a) in the STRUC program of the GENEPOP v. 3.4 software package (Raymond & Rousset 1995b). GENEPOP was used for the estimation of F_{IS} values and for testing the null hypothesis of Hardy-Weinberg proportions. The potential occurrence of null alleles over loci was tested with the MICRO-CHECKER v. 2.2.3 software package (Van Oosterhout et al. 2004).

A Bayesian clustering analysis for inferring population structure was performed with the STRUCTURE v. 2.2 software package (Pritchard et al. 2000, Falush et al. 2003, 2007). The model with admixture and correlated allele frequencies was chosen to estimate the number of genetic clusters (K) in all samples of *Pagellus erythrinus* without any *a priori* grouping. Analyses for each K from 1 to 10 were replicated 10 times with 100 000 Markov Chain Monte Carlo (MCMC) iterations and 50 000 burn-in period steps to estimate the posterior probability that the data fit the hypothesis of K clusters [P(X/K)]. The optimal value of K was selected as the one that maximized the probability of the data (averaged across different runs).

Prior to the analysis of the relationship between individual heterozygosity and fitness related traits, data obtained for fitness-related parameters (length, weight and otolith dimensions) were tested for normality with a Shapiro-Wilk's test (Shapiro & Wilk 1965). When there was a significant deviation from the normal distribution, a logarithmic transformation of data was conducted to improve normality. After this logarithmic transformation, a second test for normal distribution was performed.

Principal component analyses (PCA) were carried out for 2 purposes. The first one was to uncover possible relationships between morphological measurements (including CF and RCF), in addition to age, gender and MLH classes. The relationships between these variables were presented graphically in the form of a circle of correlations. Bartlett's test of sphericity (Bartlett 1950) was used to test the hypothesis that the correlation matrix was an identity matrix in which all diagonal terms are 1 and all off-diagonal terms are null. Second, a PCA was performed on the 4 growth-related parameters exclusively (L_s, W_b, L_o and W_o) to replace these correlated variables by a single synthetic variable, the coordinate of the specimens on the first axis of the PCA, noted as PCA-1 hereafter.

To determine whether there was an association between single-locus heterozygosity (SLH) and growth estimators (L_s, W_b, L_o, W_o, CF and RCF), we used the unilateral 2-sample Student's *t*-test and Fisher's *F*-test to compare means and variances between homozygotes and heterozygotes (we used the unilateral test since the alternative hypothesis is that the heterozygotes have higher growth related traits than homozygotes). The polymorphism of the examined loci was fixed at the level of 99% for these analyses.

To assess relationships between MLH values and age or growth estimators, Spearman's (nonparametric) correlation analyses were performed, and their regression coefficients and associated p-values were calculated. We also studied the relationship between MLH and growth estimators (including the synthetic variable PCA-1) by using analyses of covariance (ANCOVA), considering age as a factor in addition to sex, or by restricting the analyses to a single gender (males and females separately). All these statistical analyses were performed in XLSTAT v. 2010.5 (Addinsoft). Significance for all statistical tests was taken as $p < 0.05$.

RESULTS

Electrophoretic screening of 14 enzyme systems in 238 specimens of *Pagellus erythrinus* revealed 21 inferred loci. Only the *GPI-2** locus was not scored owing to difficulty in interpreting the electrophoretic patterns. The genetic parameters for each locus are displayed in Table 2. The number of alleles per locus ranged from 1 to 5 and the mean was 2.15 (SE = 1.22). The most polymorphic locus was *PGM-1** with 5 alleles and 12 observed genotypes. Mean observed heterozygosity was low (H_o = 0.047), although this parameter varied greatly from one locus to another (between 0 and 0.341, SE = 0.085). Eight loci were found to be polymorphic at the level of 99%, of which only 5 were polymorphic at the level of 95% (*GPI-1**, *IDHP-1**, *MDH-1**, *PGDH** and *PGM-1**). The former 8 loci were retained for further analysis concerning the effect of SLH on growth estimators.

Table 2. *Pagellus erythrinus*. Locus-specific parameters in samples of common pandora from the Gabes Gulf. N: sample size for each locus; N_A; number of alleles per locus; N_G; number of observed genotypes per locus; $f_{(100)}$; frequency of the common allele for each locus; H_o: observed heterozygosity; H_e: expected heterozygosity; F_{IS}: Wright's (1951) inbreeding coefficient, calculated according to Weir & Cockerham's (1984) estimators; for F_{IS} values: *p < 0.05, **p < 0.01 and ***p < 0.001, determined by exact test

Locus	N	N_A	N_G	$f_{(100)}$	H_o	H_e	F_{IS}
*ADH**	237	3	3	0.981	0.038	0.037	−0.013
*G6PDH**	237	3	3	0.994	0.012	0.012	
*GPI-1**	235	4	5	0.947	0.089	0.101	0.122
*G3PDH**	238	1	1	1	0	0	
*IDHP-1**	232	2	3	0.877	0.159	0.215	0.262***
*IDHP-2**	179	3	3	0.989	0.022	0.022	−0.006
*LAP**	238	1	1	1	0	0	
*LDH-1**	238	1	1	1	0	0	
*LDH-2**	238	1	1	1	0	0	
*MDH-1**	237	3	5	0.939	0.105	0.116	0.094
*MDH-2**	237	1	1	1	0	0	
*MEP-1**	237	3	3	0.992	0.008	0.016	
*MEP-2**	237	3	4	0.986	0.021	0.029	0.280*
*ODH**	237	1	1	1	0	0	
*PGDH**	236	3	4	0.924	0.135	0.143	0.058
*PGM-1**	234	5	12	0.748	0.341	0.415	0.178**
*PGM-2**	238	2	2	0.998	0.004	0.004	
*SOD-1**	237	1	1	1	0	0	
*SOD-2**	237	1	1	1	0	0	
*XDH**	237	1	1	1	0	0	
Mean		2.15	2.8		0.047	0.055	0.160***

Allozyme genotypes showed significant deviations from Hardy-Weinberg equilibrium expectations. In the total sample, F_{IS} values varied considerably between loci and the average multi-locus value was positive, suggesting heterozygote deficiency (mean F_{IS} = 0.160, $p < 0.001$). This deficiency was not significant ($p > 0.05$) for *GPI-1**, *MDH-1** and *PGDH** loci. An analysis of the data with MICRO-CHECKER v. 2.2.3 showed that the homozygote excess was most likely due to the presence of null alleles at locus *IDHP-1**. Therefore, subsequent analyses that involved MLH were performed twice, either including or excluding.

The assignment test in STRUCTURE v. 2.2 detected a single cluster. The log likelihood was maximized at $K = 1$, where $\ln[P(X/K)] = -1136.8 \pm 3.39$, indicating that no subdivision was detected within our samples (Fig. 1).

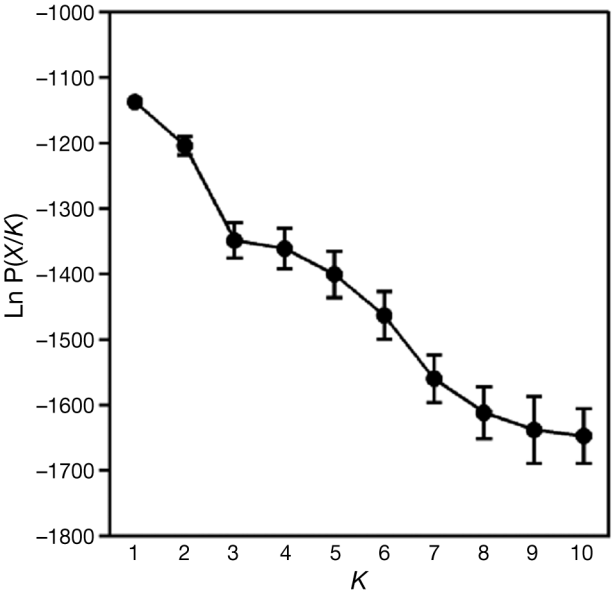

Fig. 1. STRUCTURE v. 2.2 results. Ln[P(*X*/*K*)] is the log likelihood of each value of *K*, which is the number of simulated clusters. Data are mean (±SD) values of Ln[P(*X*/*K*)]

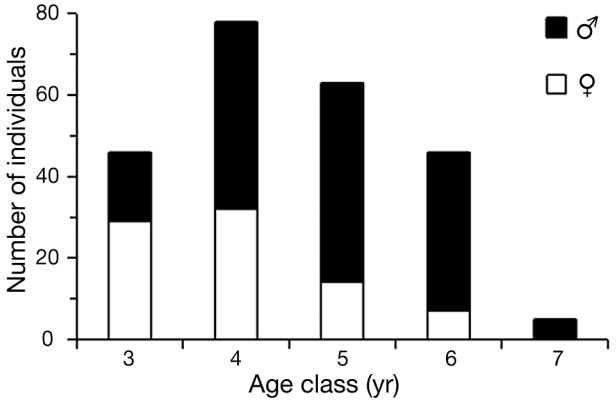

Fig. 2. *Pagellus erythrinus*. Distribution of the number of individuals sampled from the Gabes Gulf according to sex and age class

Reading of the growth bands from otoliths was performed for all 238 individuals. The otoliths of *Pagellus erythrinus* were relatively large and generally not difficult to interpret. This reading allowed us to distinguish 5 age classes in the commercial fishery population from the Gabes Gulf, which corresponded to individuals aged from 3 to 7 yr. The distribution of individuals grouped according to age and sex is shown in Fig. 2. Most individuals were found to belong to the Age 4 and 5 year classes, and males were more abundant than females in all classes above the Age 3 year class.

Normal distribution was demonstrated only for L_s and L_o. W_b and W_o were not normally distributed in males and in the total sample of *Pagellus erythrinus*. Thus, all morphological data underwent logarithmic transformation and statistical testing was done with parametric tests. The normality distribution was verified for all parameters after logarithmic transformation, except for L_o in the total sample (Shapiro-Wilk's normality test: $W = 0.988$, $p = 0.044$).

Ricker's (1975) CF was calculated as 1000 ($W_b/L_s^{2.634}$) for each individual and the estimated body weight used to calculate Le Cren's (1951) RCF was calculated with the length–weight relationship formula: $\ln(\hat{W}_b) = -2.681 + 2.645 \ln(L_s)$, ($r = 0.971$, $p < 0.001$).

To investigate the effect of single loci, the growth parameters of homozygous and heterozygous individuals were compared for each of the 8 loci polymorphic at the level of 99% (Table 3). For the least polymorphic loci (*IDHP-2**, *MEP-2**, and *ADH**) the numbers of heterozygous individuals were low (4, 5 and 9, respectively), which reduced the power of the SLH comparisons; however, we retained these loci to avoid a possible bias favoring loci under balancing selection inherent to selection of most polymorphic loci (Table 3). In most cases, heterozygous individuals had greater growth parameter means than homozygous individuals, but no significant (i.e. $p < 0.05$) differences were observed. Likewise, for each sex examined separately, no significant associations were detected between SLH and growth estimators (results not shown). For condition indices, 4 loci displayed higher mean values for heterozygotes, 4 other loci displayed the opposite pattern and the only significant differences were found at *PGDH** locus (Table 3), and variances appeared higher in heterozygotes than in homozygotes. Given the number of different tests, these may have appeared as significant as a result of multiple testing since they lose significance after multiple test correction.

From the PCA based on growth parameters, age and gender, as well as MLH classes, the loading of each variable along the 2 first axes, which summarize 72.77% of the total variance, is shown schematically in Fig. 3. PCA revealed significant correlation amongst the input variables (Bartlett's sphericity test: $p < 0.0001$). Contributions of morphological measurements and age appeared to be strong and that for gender was moderate, while the signal of MLH classes was weak. The first axis resulting from the PCA on the 4 growth-related traits provides a good synthetic variable (PCA-1) since it explained 89.3% of the variance.

The correlation analysis between age and MLH indicates that there is a statistically significant posi-

Table 3. *Pagellus erythrinus*. Mean (SD) values of standard length (L_s), body weight (W_b), otolith length (L_o), otolith weight (W_o), Condition Factor (CF) and Relative Condition Factor (RFC) for homozygous and heterozygous individuals at the 8 polymorphic loci in common pandora from the Gabes Gulf. N: sample size for each locus; Δ: the sign of difference in each parameter between heterozygotes and homozygotes. The difference in means and variances versus zero was evaluated by unilateral test of Student's *t*-test and Fisher's *F*-test, respectively. Significant p-values are shown in **bold** (p-values were obtained after logarithmic transformation of the growth parameters)

Locus		N	Parameter					
			L_s (mm)	W_b (g)	L_o (mm)	W_o (g)	FC	RFC
ADH*								
Homozygotes		228	161.272 (12.871)	108.743 (23.768)	8.680 (0.726)	0.103 (0.023)	70.741 (3.706)	1.002 (0.053)
Heterozygotes		9	167.000 (11.203)	116.630 (21.214)	8.892 (0.915)	0.111 (0.024)	69.511 (2.485)	0.985 (0.035)
Δ			+	+	+	+	–	–
p	*t*-test		0.094	0.147	0.22	0.153	0.837	0.843
	F-test		0.653	0.692	0.203	0.873	0.22	0.215
GPI-1*								
Homozygotes		214	161.224 (12.974)	108.516 (23.855)	8.670 (0.727)	0.102 (0.023)	70.644 (3.728)	1.001 (0.053)
Heterozygotes		21	163.286 (11.744)	112.660 (22.484)	8.836 (0.764)	0.108 (0.023)	71.075 (3.291)	1.007 (0.046)
Δ			+	+	+	+	+	+
p	*t*-test		0.233	0.202	0.167	0.125	0.305	0.31
	F-test		0.554	0.712	0.827	0.935	0.526	0.514
IDHP-1*								
Homozygotes		195	161.185 (12.920)	108.286 (24.007)	8.652 (0.739)	0.102 (0.023)	70.527 (3.696)	0.999 (0.052)
Heterozygotes		37	161.946 (13.028)	110.068 (22.499)	8.760 (0.673)	0.106 (0.021)	70.936 (3.168)	1.005 (0.045)
Δ			+	+	+	+	+	+
p	*t*-test		0.321	0.256	0.203	0.135	0.204	0.206
	F-test		0.774	0.789	0.685	0.619	0.245	0.266
IDHP-2*								
Homozygotes		175	162.183 (13.546)	110.542 (25.041)	8.700 (0.760)	0.104 (0.024)	70.755 (3.773)	1.003 (0.053)
Heterozygotes		4	167.250 (7.274)	119.950 (11.922)	8.548 (0.287)	0.105 (0.015)	71.830 (3.727)	1.017 (0.053)
Δ			+	+	–	+	+	+
p	*t*-test		0.216	0.186	0.626	0.396	0.287	0.292
	F-test		0.295	0.188	0.134	0.465	0.811	0.803
MDH-1*								
Homozygotes		212	161.217 (12.910)	108.611 (23.910)	8.680 (0.739)	0.103 (0.023)	70.706 (3.654)	1.002 (0.052)
Heterozygotes		25	163.200 (12.003)	111.204 (21.304)	8.727 (0.652)	0.104 (0.022)	70.362 (3.917)	0.997 (0.056)
Δ			+	+	+	+	–	–
p	*t*-test		0.226	0.268	0.368	0.377	0.671	0.675
	F-test		0.648	0.616	0.442	0.834	0.586	0.58
MEP-2*								
Homozygotes		232	161.272 (12.734)	108.639 (23.387)	8.679 (0.730)	0.103 (0.022)	70.701 (3.692)	1.002 (0.052)
Heterozygotes		5	168.600 (15.758)	120.288 (34.104)	8.921 (0.725)	0.113 (0.039)	69.232 (2.649)	0.980 (0.037)
Δ			+	+	+	+	–	–
p	*t*-test		0.112	0.165	0.23	0.221	0.811	0.817
	F-test		0.551	0.446	0.946	0.172	0.55	0.529
PGDH*								
Homozygotes		204	161.515 (13.067)	109.092 (24.209)	8.695 (0.741)	0.103 (0.023)	70.645 (3.517)	1.001 (0.050)
Heterozygotes		32	161.094 (11.602)	108.501 (20.749)	8.658 (0.696)	0.103 (0.020)	71.098 (4.600)	1.007 (0.065)
Δ			–	–	–	+	+	+
p	*t*-test		0.549	0.494	0.593	0.434	0.299	0.298
	F-test		0.494	0.404	0.814	0.336	**0.031**	**0.029**
PGM-1*								
Homozygotes		154	161.214 (12.258)	108.581 (22.767)	8.669 (0.692)	0.103 (0.022)	70.757 (3.640)	1.003 (0.052)
Heterozygotes		80	162.100 (14.038)	110.105 (25.764)	8.735 (0.812)	0.104 (0.025)	70.561 (3.770)	1.000 (0.053)
Δ			+	+	+	+	–	–
p	*t*-test		0.337	0.377	0.296	0.366	0.65	0.652
	F-test		0.209	0.361	0.089	0.197	0.704	0.689

tive correlation for both data sets, including or excluding locus *IDHP-1** (Spearman's correlation coefficient: r_S = 0.150 or 0.144, p = 0.02 or 0.026) (Table 4). Examination of the effects of individual MLH on growth parameters revealed low positive correlations with L_s, W_b, L_o and W_o and negative correlations with CF and RCF (Table 4). None of these correlations was significant, except for otolith weight

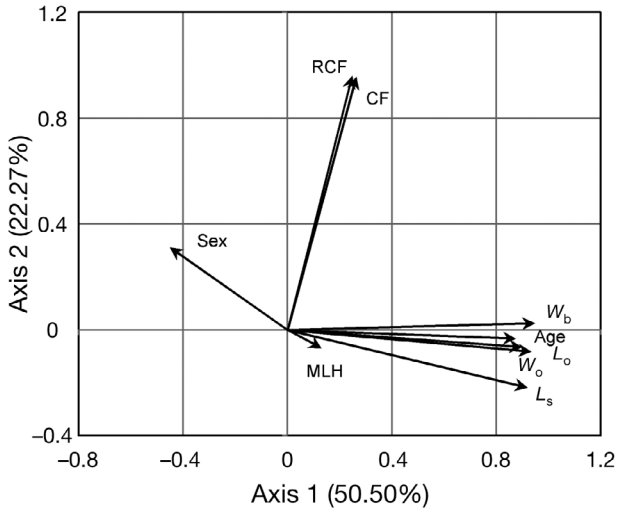

Fig. 3. *Pagellus erythrinus*. Principal component analysis plot of the loading of different variables measured in common pandora samples from the Gabes Gulf. MLH: multi-loci heterozygosity; L_s: standard length (mm); W_b: body weight (g); L_o: otolith length (µm); W_o: otolith weight (g); CF: condition factor; RCF: relative condition factor

(Spearman's correlation coefficient: r_S = 0.128, p = 0.048) for the data set with locus *IDHP-1**, though numerous p-values were lower than 0.1 (Table 3). The synthetic growth-related variable PCA-1 was positively correlated with MLH (Spearman's correlation coefficient: r_S = 0.121 or 0.113, p = 0.061 or 0.081). Additionally, results from ANCOVA with age as a covariate showed no statistical tendency for the effect of MLH on growth estimators in the total sample or in sexes taken separately, except for a significant effect of MLH on L_o in males (F = 3.301, df = 3, p = 0.022) for the MLH without locus *IDHP-1** (Appendix 1). The effect of age on growth estimators was found to be highly significant, but not for CF in females. Also, the effect of sex on growth estimators appeared significant, with the exception of W_b.

Table 4. *Pagellus erythrinus*. Results of the tests of the non-parametric Spearman's rank correlation (r_S) between multi-locus heterozygosity (MLH) and age, growth-related parameters (or the first coordinate of the PCA analysis made on those 4 parameters, PCA-1) and condition factors in common pandora samples from the Gabes Gulf. The sign of the regression coefficient is given with the p-value of the null hypothesis of an absence of correlation. L_s: standard length; W_b: body weight; L_o: otolith length; W_o: otolith weight; CF: condition factor; RCF: relative condition factor. Two analyses were performed in each case (with 20 loci or with 19 loci after removal of *IDHP-1**). Results are similar despite changes in p-values but not in the signs of the regression. *Significant p-values are in **bold** (p < 0.05); #p-values between 0.05 and 0.10

Parameter	MLH		
	r_S sign	p	
		20 loci	19 loci
Age (yr)	+	**0.020***	**0.026***
PCA-1	+	0.061#	0.081#
Ln(L_s) (cm)	+	0.065#	0.075#
Ln(W_b) (g)	+	0.078#	0.093#
Ln(L_o) (µm)	+	0.137	0.173
Ln(W_o) (g)	+	**0.048***	0.083#
Ln(CF)	–	0.721	0.494
Ln(RCF)	–	0.681	0.458

DISCUSSION

Genetic diversity in *Pagellus erythrinus* samples from the Gabes Gulf was relatively low at the protein level. The presence of fixed alleles, the low number of alleles per locus and the high frequency of common alleles (greater than 0.74), as well as the low mean observed heterozygosity (heterozygous individuals represent 4.7% of total population), showed that *P. erythrinus* maintains low levels of polymorphism at the genetic markers studied.

Comparisons of observed versus expected numbers of heterozygotes predicted at each locus under Hardy-Weinberg expectations indicate that heterozygote deficiencies are common (highly significant multi-locus F_{IS} value, and 3 loci out of 8 gave significant mono-locus F_{IS} values; one of them appeared to be affected by null alleles). These deficiencies can in theory be attributed to several causes: technical causes (sampling bias or artifacts such as null alleles), eco-biological causes (direct selection against heterozygous individuals), population causes such as spatial or temporal Wahlund effects (mixing of differentiated populations or cohorts, respectively) or inbreeding. The results of the Bayesian clustering (STRUCTURE) analysis suggested a lack of genetic differentiation in our samples of *Pagellus erythrinus* of the Gabes Gulf. A previous study (Fassatoui et al. 2011) conducted by using the same individuals, but grouped by age, also revealed no significant genetic differentiation (mean F_{ST} between age groups = 0.0002 ± 0.0041, p = 0.350) with no evidence of recent genetic bottlenecks. Accordingly, the contribution of temporal Wahlund effects to these deficits seems to be low, particularly in this population from the Gabes Gulf. The significant positive correlation between age and MLH therefore suggests increased survival of individuals with higher MLH. The fact that no clear HFC was found by using SLH compared with MLH seems to support the hypothesis that a global effect rather than direct or local overdominance causes the

observed HFC. However, appreciably inbred individuals are extremely rare in anything but the smallest of populations or cases with large variance in reproductive success or strong population structure. The common pandora is unlikely to have a small enough effective population size for this hypothesis to be reliable. To distinguish among causes of HFC is not realistic with our data set (cf. Introduction).

This is in agreement with the heterozygote deficiency that we found, which suggests inbreeding. Furthermore, *Pagellus erythrinus* is subject to commercial exploitation, which may lead to the increased effects of certain evolutionary pressures, particularly selection and inbreeding. Thus, inbreeding avoidance mechanisms should be favored by natural selection in *P. erythrinus.* The following are some mechanisms that predate commercial exploitation and are known in this species: (1) protogynous hermaphroditism limits the chances of mating between related individuals, at least between siblings; (2) external fertilization in an open marine environment favors the random meeting of gametes (dispersion by currents); (3) recruitment by which the annual integration of juvenile fish to benthic populations occurs after the post-settlement stage (addition of new genetic variants into a population by migration) may help to limit the effects of inbreeding.

No significant relationship was found between the growth traits measured and individual heterozygosity in *Pagellus erythrinus* of the Gabes Gulf, except for otolith weight (with all loci) or otolith length in males (without locus *IDHP-1**) (Table A1). Considering that multiple tests were used these 2 significant results are probably statistical artifacts. Several studies based on allozyme markers, particularly in molluscs, have indicated an absence of a significant correlation between growth rate and individual heterozygosity (Gosling 1989, Slattery et al. 1991, Saavedra & Guerra 1996, Marsic-Lucic & David 2003). In fishes, the heterozygosity–growth rate relationship was explored mainly in rainbow trout *Oncorhynchus mykiss* (Thelen & Allendorf 2001) and European eel *Anguilla anguilla* (Pujolar et al. 2005). Both studies revealed significant correlations with heterozygosity at allozyme loci, but no evidence was found for microsatellite markers.

Several reasons (under the hypothesis that there is an advantage of being heterozygote) may explain the absence of HFCs with some growth parameters: (1) growth is not the major factor related to heterozygotes' advantage, (2) there is a lack of sufficient statistical power and (3) MLH across allozyme markers is a poor indicator and unable to reflect such correlations.

Indeed, levels of allozyme variation were low. Moreover, the correlations were performed only with 4 heterozygosity classes (all the analyzed individuals ranged from 0 to 4 heterozygous loci) owing to the low proportion of polymorphic loci present in this species. In addition, according to Coltman & Slate (2003), a minimum of 600 individuals, or even 1000 according to David (1998), are required for a reasonable statistical detection of a positive correlation between heterozygosity and fitness. Chapman et al. (2009) in a recent meta-analysis publication has shown that small sample sizes can produce large fluctuations around the predicted true mean effect sizes of HFCs.

MLH explained less than 1.6% (all loci) or 1.4% (without *IDHP-1**) of the total variance in growth estimators (Spearman's coefficients of determination R_S^2 ranged from 0.00784 to 0.01334 in the total sample [without *IDHP-1**]). This means that the large fraction of variation in growth is partitioned between environmental effects, genetic factors other than the loci studied and epistatic interactions. In other words, the genetic component related to heterozygosity for the studied loci that explains the variation in growth is minimal with respect to the other components cited above.

HFCs in natural populations are usually weak, as has been shown by Britten (1996), Coltman & Slate (2003) and Chapman et al. (2009) in published meta-analyses. The fact that survival results from a combination of numerous components may explain why detection of age–MLH correlations requires smaller sample sizes than detection of single growth parameter–MLH correlations.

In conclusion, although our results strongly suggested that survival of individuals with higher multilocus heterozygosity is greater, they did not clearly highlight correlations between growth-related traits and single-locus heterozygosity in *Pagellus erythrinus* from the Gabes Gulf, except for 1 out of 4 measures (which may be due to statistical artifacts). Heterozygote advantage may have a determinant influence on survival in *P. erythrinus*. Consequently, any future management aimed at ensuring the viability of this species should be focused on maximizing genetic diversity within population. We provided all raw data and p-values (Table A1) so that this study can eventually be validated in meta-analyses. Additional work that uses non-coding markers, such as microsatellites, or numerous markers, such as next-generation sequencing or genotyping technology, should provide important information on multiple factors underlying heterozygosity–growth relationships in this species.

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Appendix 1. *Pagellus erythrinus*. ANCOVA results, based on the number of heterozygous allozyme loci and growth parameters with age as a cofactor, in (a) female, (b) male and (c) combined sexes of common pandora from the Gabes Gulf. L_s: standard length; W_b: body weight; L_o: otolith length; W_o: otolith weight; CF: condition factor; RFC: relative condition factor. Significant p-values are shown in **bold**. MLH were computed without locus *IDHP-1** in this analysis. When this locus was included in MLH computation, no significant effect of MLH was observed in males (see 'Results')

Source	df	Growth parameter											
		Ln(L_s) (cm)		Ln(W_b) (g)		Ln(L_o) (µm)		Ln(W_o) (g)		Ln(CF)		Ln(RCF)	
		F	p	*F*	p	*F*	p	*F*	p	*F*	p	*F*	p
(a) Female (N = 82)													
Age	1	91.095	**<0.001**	95.012	**<0.001**	70.691	**<0.001**	112.148	**<0.001**	0.724	0.398	0.529	0.469
MLH	4	0.269	0.897	0.408	0.802	0.912	0.462	0.373	0.827	0.162	0.957	0.156	0.960
(b) Male (N = 156)													
Age	1	143.911	**<0.001**	181.422	**<0.001**	126.538	**<0.001**	181.875	**<0.001**	16.495	**<0.001**	15.402	**<0.001**
MLH	3	0.445	0.721	0.423	0.737	3.301	**0.022**	0.867	0.460	1.259	0.291	1.260	0.290
(c) Combined sexes (N = 238)													
Sex	1	5.781	**0.017**	2.079	0.151	9.695	**0.002**	9.800	**0.002**	7.036	**0.009**	7.156	**0.008**
Age	1	228.591	**<0.001**	271.392	**<0.001**	200.914	**<0.001**	293.050	**<0.001**	14.781	**<0.001**	13.400	**<0.001**
MLH	4	0.149	0.963	0.295	0.881	2.020	0.092	0.478	0.752	0.856	0.491	0.851	0.494

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