Genetic Structure and Gene Flow of *Octopus maya* (Mollusca: Cephalopoda) in the States of Yucatan and Campeche, Mexico

Estrutura genética e fluxo gênico do polvo maya (Mollusca: Cephalopoda) nos Estados de Yucatan e Campeche, México

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ABSTRACT
It was determined the population genetic structure of the red octopus Octopus maya in the states of Campeche and Yucatan in Yucatan Peninsula, Mexico, through the expression of isozymes in polyacrylamide gels. Mantle samples from 25 octopuses, captured in nine sites of the Yucatan Peninsula, were used to characterize the genotypic expression revealed by the expression of 26 loci in thirty enzyme systems. Program TFPGA version 1.3 (Tools for Population Genetic Analyses), was used to process data of allozyme gene frequencies of the studied populations. The parameters determined were: descriptive statistics, F statistics, genetic distances, Hardy - Weinberg, UPGMA and the number of migrants as an indicator of gene flow. The average number of alleles per locus, percentage of polymorphic loci, average and direct and direct heterozygosity were: 1.08 ± 0.05 to 1.15 ± 0.04. P95 26.9231% to 34.6154, Have 0.1142 to 0.1390 and Hdir = 0.0354 to 0.0938 respectively. Heterozygosity values in a range of 0.3506 to 0.4793 and G6PDH for ARGK with an average heterozygosity value of 0.1824, Fisher average value of 0.5313 and 0.0140 Fst indicates a heterozygous deficiency but it is within the ranges reported for marine invertebrate species. The number of migrants derived from the Slatkin equation is 1824 per generation, globally indicates some degree of variability between sites and is consistent with the low values of Nei genetic distance found, particularly the node showing the separation of the population of Lagartos River and Dzilam Bravo from the other locations with an obtained value of 0.0004. From the results of this study, it is concluded that locations of Octopus maya have a certain level of interpopulation genetic variability that does not reflect its fragility.

Keywords: Octopus maya, genetic structure, genetic variation, gene flow, isozymes.

INTRODUCTION
The characterization of the population genetic structure constitutes a very useful criterion for the preservation of species with commercial and ecological importance since it is an indicator.
of the heterogeneity or homogeneity of populations over large geographical regions (Utter 1991; Casu et al., 2002). This heterogeneity, as established by Maltagliati et al. (2004) has demonstrated that it is a reflection of the genetic variation of the individuals that make up the population, since genetic variation is one of the fundamental parameters in the evolutionary process. This could through genetic erosion result in a phenomenon such as genetic drift, inbreeding, prolonged bottlenecks or a low adjustment resulting in the opportunity to fix harmful alleles. (Mitton, 1994).

Understanding the genetic structure of the population is essential for effective management of a species/fishery, as it provides information on connectivity patterns and isolation between populations (Utter, 1991; Maltagliati et al., 2002a, Maltagliati et al., 2002b, Tello et al., 2005), in order to identify populations of a unique genetic character, as well as populations at higher risk of extinction, and thus provide a basis for defining management units.

The ecology of larvae strongly influences the structuring of a population and there is much speculation about the evolutionary advantages that develop larvae, in whether the panmixia that is maintained in widely dispersed marine species, is due to the movement of larvae by currents and events related to them.

Cephalopods are important from an ecological point of view, a very successful group, as shown by their global geographic distribution, trophic importance and the great contribution to global biomass and productivity in all marine habitats (Boyle & Boletzky, 1996; Lipinski, 1998). Cephalopods have a unique life history, unique characteristics, including rapid non-asymptotic growth, short lives, and high individual variability (Clarke, 1996; Moltschaniwski, 2004). However, this makes them sensitive to ecological disturbances, caused by fishing activity or environmental change, which can trigger rapid inter-annual fluctuations in cephalopod abundance (Boyle & Boletzky, 1996). Several methods have been adopted to examine the movement patterns of cephalopods, including recapture marking methods, electronic tags, genetic markers, and morphometric markers (Doubleday 2009). However, there is little information about the population structure and dispersal patterns of most cephalopod species. Genetic markers have been used to investigate the population structure of various species, but a common problem is that cephalopods tend to have a low level of genetic variability. Only a handful of studies investigating population structure in octopus populations have been carried out and restricted to Octopus vulgaris and based on the development of genetic work (Casu et al., 2002; Maltagliati et al., 2002a; Maltagliati et al., 2002b; Murphy et al., 2002; Cabranes et al., 2007; Fernandez-Rueda & Martínez, 2008; Moreira et al., 2011) and morphometry have also been used in the study of this cephalopod (Hermosilla et al., 2011).
The *Octopus maya* Voss y Solís, (1966) marine cephalopod of the *Mollusca Phylum*, is a coastal species typical of the Yucatan Peninsula, which lives in cracks of rocks and holes in the bottom and sometimes occupying gastropod shells and various submerged objects that serve as burrows. The *Octopus maya*, commonly called red octopus, fishery is of great importance on the coasts of the state of Yucatan where it competes due its large volume of catch with the grouper fishery (*Epinephelus morio*) and in Campeche with the shrimp fishery (*Penaeus sp.*) (Solis-Ramirez & Chavez, 1986).

Currently, no different fishing units have been identified throughout the range of this species using alloenzymes (Tello et al., 2007). However, two heterologous microsatellite loci have already been identified that show significant differences between Seybaplaya south of Campeche and El Cuyo, in the eastern tip of Yucatan, but it was not possible to define different fishing units because the analysis of four microsatellites together indicates that the differences between the sampled localities are not significant (Juárez et al., 2012).

The main objective of this work was to obtain information about the genetic structure of the *Octopus maya* population and to quantify the levels of enzymatic variability within and between the different localities of the Yucatan peninsula, which together with biological data and ecological attributes such as its dispersion potential could be related to its level of genetic variability, the flow of genes and the subsequent implementation of resource management measures.

### 2 MATERIALS AND METHODS

**Collection of organisms:** A total of 25 octopuses were collected in nine localities of the Yucatan Peninsula: Progreso, 21°10'21"N - 89°34'89"W; Sisal, 21°89'32"N - 90°05'15"W; Celestún, 20°86'26"N - 90°86'26"W; Dzilam de Bravo, 21°19'01"N - 88°35'88"W; Lagartos River, 21°60'02"N - 88°16'41"W in Yucatan and Campeche, 19°63'66"N - 90°67'76"W; Sabancuy, 19°03'54"N - 91°17'69"W; Champotón, 17°49'25"N - 88°35'88"W; Arena Island, 20°62'08"N - 90°44'98"W, in Campeche. The mantle of each octopus was dissected approximately 1 g and preserved in liquid nitrogen.

**Extraction of isoenzymes and electrophoresis:** The sample was macerated until its total dissolution, using TEB extraction buffer, adjusted to pH 7 (Shaklee & Keenan, 1986).

Mantle samples were homogenized into an equal volume of extraction buffer consisting of Tris HCl 12.1 g, EDTA 336mg, NAD+ 20 mg and adjusted to a pH of 7 (Shaklee and Keenan, 1986), centrifuged at 10 000rpm for 10 min. at 7°C in a refrigerated centrifuge. 7.7 % polyacrylamide gels, prepared for use with the native system (Brewer, 1970), were used to perform the electrophoretic run, histochemical development and phenotypic determination of the samples.
The phenotypic presence was determined following the procedures of Shaw and Prasad (1970), Brewer (1970) and Shaal and Anderson (1974). Presumed loci and alleles were designated by means of the nomenclature system used by Shaklee and Keenan (1986). Multiple loci of a particular enzyme were designated numerically (1, 2, 3, etc.) considering the fastest to lowest anodal mobility. Alleles of a particular locus were designated due to their relative anodic mobility and naming the most frequent allele as 100 and the others above and below it with the respective values. Loci and alleles were designated according to the nomenclature system proposed by Shaklee and Keenan (1986). A locus was considered polymorphic if the most frequent allele has a probability of less than 95 %. (Towsend and Shing, 1984) and the level of heterozygosis was determined in relation to the Hardy-Weinberg Equilibrium law. The program called TFPGA version 1.3 (Tools for population genetic analyses), was used to carry out the analysis of genetic data of population alloenzymes (Miller, 2000).

Two alternatives were used to test the balance of the Hardy - Weinberg's law, the so-called Chi-Square fit goodness tests, and the exact Haldane tests (Miller, 2000), considering the alternative of grouping the genotype analysis into the homozygote categories for the most common allele, heterozygote for the most common allele and all other genotypes. To evaluate genetic differentiation between populations, the methods of F statistic developed by Wright (Sokal & Rohlf, 1995; Weir, 1990), the measure of variation between individuals within populations, \( f = F_{is} \), \( f = F_{is} \) or fixation measure and the measure of variation between populations, \( Q = F_{st} \) or coancestrity coefficient are applied. The LIPINSKI resampling alternatives known as Jacknifing and Bootstrapping were used to remove the possible bias obtained with the sampling and to establish more reliable estimators when carrying out the statistical analysis. The results for each locus and for each allele were estimated at a 95 % confidence level with about 1000 repetitions for Bootstrapping on all loci. The similarity measures used by this program are given for Nei's distance options and Nei's insesgada (1978), Wright's modified distance from Rogers' and Reynolds et al.'s distance and coancestridad (1983), all these measures in Weir, (1990). This method was used by means of Bootstrapping analysis to have a graphical representation or dendogram of the results of the genetic distances and from them to make inferences of the possible relations between the analyzed sites (Sokal & Rohlf, 1995). The estimation of gene flow was determined by the number of migrants between populations (Slatkin, 1987).
3 RESULTS

The isoenzymatic analysis of Octopus maya showed that, of the 30 enzymatic systems used for this study, 19 showed enzymatic activity and of these 8 showed levels of heterozygosis. The average results of the heterozygosis and polymorphism values are presented in Table 1.

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>SAMPLE SIZE</th>
<th>AVERAGE HETEROZYGOSIS</th>
<th>AVERAGE UNBIASED HETEROZYGOSIS</th>
<th>DIRECT AVERAGE HETEROZYGOSIS</th>
<th>% POLYMORPHIC LOCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROGRESO</td>
<td>25</td>
<td>0.1321</td>
<td>0.1348</td>
<td>0.0938</td>
<td>30.7962</td>
</tr>
<tr>
<td>SISAL</td>
<td>25</td>
<td>0.1355</td>
<td>0.1382</td>
<td>0.0785</td>
<td>30.7962</td>
</tr>
<tr>
<td>ARENA ISLAND</td>
<td>25</td>
<td>0.1374</td>
<td>0.1403</td>
<td>0.0569</td>
<td>30.7962</td>
</tr>
<tr>
<td>CAMPECHE</td>
<td>25</td>
<td>0.1280</td>
<td>0.1306</td>
<td>0.0585</td>
<td>34.6154</td>
</tr>
<tr>
<td>CELESTÚN</td>
<td>25</td>
<td>0.1390</td>
<td>0.1418</td>
<td>0.0523</td>
<td>30.7962</td>
</tr>
<tr>
<td>SABANCUY</td>
<td>25</td>
<td>0.1142</td>
<td>0.1165</td>
<td>0.0415</td>
<td>26.9231</td>
</tr>
<tr>
<td>DZILAM DE BRAVO</td>
<td>25</td>
<td>0.1182</td>
<td>0.1207</td>
<td>0.0354</td>
<td>26.9231</td>
</tr>
<tr>
<td>CHAMPOTÓN</td>
<td>25</td>
<td>0.1145</td>
<td>0.1168</td>
<td>0.0585</td>
<td>26.9231</td>
</tr>
<tr>
<td>LAGARTOS RIVER</td>
<td>25</td>
<td>0.1270</td>
<td>0.1296</td>
<td>0.0736</td>
<td>28.0000</td>
</tr>
</tbody>
</table>

The value of 0.1142 heterozygosis is the lowest obtained. The results of heterozygosis in this work do not indicate an appreciable diversity and a clear heterogeneity of the populations. Polymorphism values are shown, 34.6154 % for Celestún and 26.9231 % for Sabancuy with a global average value of 29.6190 % in all populations. These values indicated intrapopulation diversity with little interpopulation heterogeneity.

It was established that EST1, G6PDH, OCTDH2 and PGM1, presented significance, to the Chi Square test (p < 0.05), which means that they depart from equilibrium, however, these same loci appeared as non-significant when performing the Haldane test to the same sites (data not shown), considering all loci, only EST1 continued with this pattern of behavior. However, considering the fact that for genotypes in which only two alleles are present, the Haldane test is the most recommended (Miller, 2000), it can be established that of the 8 loci that showed variation in the mantle of the 9 sites analyzed, only one of them deviates from the equilibrium condition. The heterogeneity of the analyzed loci, especially those that presented differences, was not homogeneous among the nine localities, just as it were not the same loci that presented variability in each site, being only EST1 the locus that presented more variability in the set of localities. The
The highest value of Fis was presented in EST1 with 0.3898 and the index of Fst fixation in ARGK with 0.0988 (Table 2).

The value of Fst or Ø, presented a value of 0.1205, which is within the range of values of similar species and indicates that 12.05% of genetic variation results from differences between populations and 87.95% resulting from variation within populations.

The level of gene flow was determined based on the equation of Reynolds et al. (Slatkin, 1987) \( Nm = \frac{1}{Fst - 1}/4 \) and the index of fixation, Fst, resulting that the number of migrants per generation is: 1,824, which means a low level of gene flow between populations.

### TABLE 2 Genetic structure of *Octopus Maya* population in the Yucatan Peninsula.

<table>
<thead>
<tr>
<th>Loci</th>
<th>F</th>
<th>Θ</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARGK</td>
<td>0.1795</td>
<td>0.0988</td>
<td>0.2020</td>
</tr>
<tr>
<td>EST1</td>
<td>0.2419</td>
<td>-0.0255</td>
<td>0.3898</td>
</tr>
<tr>
<td>G6PDH1</td>
<td>0.2686</td>
<td>-0.0213</td>
<td>0.1542</td>
</tr>
<tr>
<td>MDH1</td>
<td>0.0977</td>
<td>0.0698</td>
<td>0.2505</td>
</tr>
<tr>
<td>ME</td>
<td>0.1688</td>
<td>0.0898</td>
<td>0.1525</td>
</tr>
<tr>
<td>OCTDH1</td>
<td>0.2372</td>
<td>-0.0269</td>
<td>0.2020</td>
</tr>
<tr>
<td>OCTDH2</td>
<td>0.2140</td>
<td>-0.0388</td>
<td>0.1956</td>
</tr>
<tr>
<td>PGM1</td>
<td>0.0968</td>
<td>-0.0254</td>
<td>0.1255</td>
</tr>
<tr>
<td>Average</td>
<td>0.1880</td>
<td>0.1205</td>
<td>0.2091</td>
</tr>
</tbody>
</table>

Of all the distance values obtained in the analyses, those established by Nei's original model were in the range of 0.0006 the lowest and 0.0090 the highest, in Roger's analysis the lowest value was 0.0077 and the highest was 0.0385 and in Reynolds' test, the lowest distance value was -0.0163 and the highest was 0.0406 (data not shown). Although it must be said that regardless of the test used, the relationships between the populations were maintained, such is the case of the population of Progreso and Sisal that had the lowest value of distance between them in all the tests and the populations of and Champotón, which presented the highest value in all the analyses performed.

Hierarchical relationships between all the populations based on the different models of determination of genetic distances were carried out by means of a Cluster UPGMA or Dendogram analysis (Sokal & Rohlf, 1995). Figure 1 presents the Cluster analysis and the Dendogram corresponding to Nei's statistic, corroborating what was established in the distance test.
It was determined that moderate genetic variability was detected within the *Octopus maya* samples. The percentage of polymorphism, from 26.9231 to 34.6154 %, was within the normal values detected in marine invertebrates, while the mean heterozygosis was below the range of 0.15 reported for the same group of organisms. The presence of a number of alleles at low frequencies is an indication of the stability of the species, that is, that the population of *Octopus maya* is demographically stable, indicating that this population has not been subject to recent genetic events, as it may have been subject to a founding effect or recurrent bottlenecks; in fact, one of the aspects that makes this species desirable to be exploited is its high population size. Considering the care that must be taken when comparing allozimic data, the heterozygosis values detected in this study are relatively higher than those detected by the determination of isoenzymes in other cephalopod species, but still within the established range for invertebrate organisms, where a generalized occurrence of heterozygosis deficiency relative to Hardy-Weinberg law expectations has been fully reported in studies of isoenzymes in marine mollusks and other invertebrates (Maltagliati *et al.*, 2002a, Maltagliati *et al.*, 2002b; Doubleday 2009; Quiang *et al.*, 2009; Fuentealba *et al.*, 2010; Hmida *et al.*, 2012). The average heterozygosis values for the populations in *O. maya* are between 0.1142 and 0.1390 (Table 1) and the outputs of the Hardy-Weinberg equilibrium law are mainly supported by some loci such as EST1 and G6PDH1 (data not shown) in most populations. The deficiency of heterozygotes in some loci of some localities analyzed in this study allow us to establish the exclusion of some phenomena similar to endogamy and the Wahlund effect because they should affect in a similar way all the loci examined and if on the contrary the idea is that the causes could be a limited capacity of dispersion of gametes or larvae or endogamy, including self-fertilization. Arguments such as fertility and mortality of *O. maya* may be compelling reasons to think about the
role of selection in determining the genetic structure and thus selective pressures in the first life stage of the mollusk could contribute or be responsible for deviations from the Hardy-Weinberg equilibrium (Sen & Burmeister, 2008).

The mean Fst value of 0.1205 or value of standardized variance in the frequency of alleles does not deviate significantly from zero and from it the evidence of a certain degree of structuring, implying that 12.05 % of the total genetic variation results from differences between populations and the remaining 87.95 % is the reflection of the variation within populations and a relative cohesiveness and specificity of the same (Camilli et al., 2001; Casu et al., 2002).

Positive and significant values for Fis indicate a deviation from the Hardy-Weinberg equilibrium due to a deficit of heterozygotes, which may be due to a fertilization between relatives; however, selective pressures against heterozygotes, the presence of null alleles and sampling errors cannot be totally excluded. In fact, the occurrence of selective forces could cause the post-settlement of genetic divergence even at microgeographic scales, distances of less than 150 km, and it could be assumed that natural selection would act against heterozygote genotypes, even if functional differences relative to the genotype or unfavorable to the levels of exhibited enzyme activity occur later (Camilli et al., 2001; Casu et al., 2002; Neigel, 2002).

The estimate of the average number of effective migration Nm was 1,824. A value of Nm > 1 is interpreted as evidence of sufficient genetic flow to prevent differentiation of populations due to genetic drift (Constantino, 1968).

In the case of species where larvae occupy the same physical niche as adults, larval dispersion may be limited, and in the case of many cephalopods once thought to be cosmopolitans, they now appear as geographically segmented, with Octopus maya being a species where adults are relatively sedentary, the differentiation of populations at relatively close geographic scales is mainly due to the movement of their larvae (Doubleday et al., 2009, Allcock et al., 2010; Rodríguez & Tello, 2011).

Analyses for genetic differentiation of the Octopus maya population were conducted using Nei’s, Roger’s and Reynold’s estimators. UPGMA Cluster analyses reflect the degree of structuring of the population and do not express a clear geographic pattern in the distribution of the genetic variation of this species. Distance and identity values obtained for Octopus maya indicate that they are typical values for species or populations that are well mixed (Goodman, 1973; Maltagliati et al., 2003).

Despite the relatively low values of genetic distance, 0.0006, 0.0077 and -0.0163 (data not shown), determined by the aforementioned estimators, there are signs of a certain substructuring as demonstrated by the values of Fst and corroborate the idea of seeing the population from the
perspective of being a simple genetic unit. From a taxonomic perspective it is generally assumed that Identity values above 0.9 or distance below 0.1 indicate specificity and with Identity values below 0.8 and distance above 0.22 correspond to an interspecific differentiation with a dark zone between these values (Maltagliati et al., 2003). The dendrogram generated by Nei distance measurements and UPGMA analysis (Figure 2) was similar but not identical when grouping localities. The distance values were different in all cases, however, none of them changed the distribution of the populations in the Cluster analysis.

The fact that a homogeneous catch quota is being managed in the range of the species, when in reality abundance is heterogeneous and fluctuating, suggests that possibly the current fishing activity does not favor the genetic variability of the species. It has been recommended that, in order to improve the fishery, the catch quota should be maintained at 30% of the estimated biomass for purposes of conservation of the resource, however, in recent years the actual catch has exceeded this level, reaching over 70% of the biomass estimated for 2006. Another factor that could be acting synergistically together with the high catch quotas, negatively affecting the genetic variability are the events of red tide; as an example, in 2008 was obtained the minimum production of this resource in recent years (5800 tons in Yucatan), the same year in which an important red tide occurred. Through this study it is not possible to determine which factor (the current exploitation regime or red tide events) is affecting the population to a greater extent, causing the deficit of heterozygotes in the loci mentioned, as well as significant endogamy, even these indicators could be affected since much older times in the evolutionary past of the population by various factors alien to human activity.

In this sense, the knowledge of the evolutionary history of the population (Tsangridis et al., 2002) is also of great relevance in order to know how its genetic variability was in the evolutionary past, and to be able to compare it with the current genetic variability, which will help to determine if it is actually being affected by fishing activity.

Facing the discussion generated here, another point to consider for the future management of the fishery is to know to what extent the changes in the temperature of the ocean would be affecting the patterns of distribution, of local abundance and possible migration between localities, to solve questions in relation to the existing risks for the species in case of a gradual warming of the ocean in the near future.

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