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Short Communication

Ecological significance indicators for an estimation of DNA quantity and purity by the use of RAPD technique for fish (S. aurita) at Libyan coastal areas

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Abstract

The paper presents findings of a study conducted in the western part of the coast in the Libyan cities of Tripoli (polluted with organic pollutants) and Sabratha. It deals with the molecular markers that require the extraction of genus and the genetic DNA extraction of genetic DNA in pure and sufficient concentration, compared with free genetic, i.e. free of contaminants, especially sugars Polysaccharides and phenolic compounds Polyphenols and proteins, which keep these molecules inherent to DNA during the process of extraction deposited with DNA. 24 samples of fish (S. aurita), 6 samples per month with 6 samples of muscle tissue of sardine's fish as reference are collected. The random amplification multiple image RAPDs technique of DNA is used, to identify The effect of pollutants on sardine's fish exposed to pollutants. Ecological Significance Indicators results are compared to the one obtained from reference samples, using polymerase chain reaction enzyme (PCR). The results show that there is a clear difference in the group. In addition, according to the molecular weight, there is disappearance of three of the packages respectively pack 2 (1000bp) & Pack 4 (300bp) & pack 5 (100bp). Finally, the paper shows that how the RAPD technique can easily assessed the estimation of DNA quantity and purity.

Keywords: Genetic, Ecological significance indicators, Isolation, Packages

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1. Introduction

Most pollutants in the environment are able to affect genetic structure of various organisms. It is found that, exposure to contaminants generally leads to reductions in genetic diversity in a variety of species. The proposed mechanism is that the toxicity of the contaminants leads to population crashes, resulting in genetic bottlenecks. Population genetic structure could also be influenced by selection, in which certain genotypes confer a selective advantage over others in the population.

Assessment of differences in allele frequencies between contaminated and reference sites that occur in many species (Guttman, 1994), indicates a selective advantage of certain genotypes over others in contaminated populations. However, many possible contributing factors to differences in allele frequencies (*e.g.,* neutral mutation and genetic drift, founder effects, gene flow), show that this in and of itself is not conclusive evidence that contaminant-induced selection influences population genetic structure.

Further, the individuals with the alleles which are more prevalent in contaminated populations have higher survival than others when exposed to copper in the laboratory. Also enzymatic activity of these particular alleles is less inhibited by copper *in vitro* than the less prevalent alleles in non-contaminated populations. In a similar study Miura et al. (1995) exposes *Gambusia holbrooki* females to mercury and finds differential survival of glucose-phosphate isomerase genotypes. These differences in survival are found to be consistent with differences in reproductive performance, in terms of number of gravid females and number of developing embryos per female. Such studies provide compelling evidence that the differences in population genetic structure are influenced by contamination-mediated natural selection (Wurgler and Kramer, 1992).

In particular, a class of genetic effects resulting from exposure to environmental pollutants exists that is not necessarily due to direct alterations of the DNA molecule. Rather, these genetic effects are the result of organisms adapting to a polluted environment. Subsequent ecological phenomena, such as bottlenecks, inbreeding, and selection, could result in changes in allele frequencies of populations (Bickham and Smolen, 1994).

There is ample evidence in the literature that pollution exposure can alter population genetic structure *via* genetic bottlenecks and selection. However, determination of ecological affects, such as calculation of Indices of Biotic Integrity or community diversity in aquatic ecosystems are very time consuming and require intensive sampling of organisms from multiple taxonomic levels. For instance, determination of population density usually requires multiple sampling efforts, or assumptions (often difficult to verify) about capture success rate or efficiency of sampling methods.

In addition, when measuring such parameters as population dynamics/ demographics, community structure, or ecosystem productivity, allowances have to be made for such abiotic variables as habitat structure and complexity, nutrient levels, temperature, *etc.,* which entails further time- and labor-intensive sampling efforts. Furthermore, the onset of ecological effects may be latent to the exposure, further complicating determination of causality. Consequently, demonstration of differences in ecological parameters between two or more sites is often problematic, and assignment of a xenobiotic etiology even

more so. By contrast, collecting samples of small fish for population genetic analysis is easily accomplished by one or two people (depending on the species type) (Capuzzo, l985).

Furthermore, many contemporary assays used in population genetics are PCR-based. This technique only requires minute amounts of DNA, so that extremely small tissue samples can be used, such as from very small organisms or tissue biopsies. Also, once in the laboratory, every aspect of PCR-based assays, from DNA extraction to agarose gel analysis, can be automated, eliminating operator subjectivity and allowing low-cost analysis of large numbers of samples necessary for environmental monitoring. Also, certain molecular characteristics of these bands must be known (*e.g.,* copy number, molecular basis of band presence or absence) must be known in order to use these markers in tests of population genetic theory (Lynch, 1994). Molecular identification of these bands is important also because specific RAPD bands may or may not be present at all geographic locations, or in all species (Clark, 2001). In cases where RAPDs were used with other methods of DNA fingerprinting, comparable results were obtained (Naish et al., 1995).

The most useful approach to use these techniques is a tired strategy. Wherein, the lower cost and less labour-intensive population genetic assays are used to help assess the ecological risk at a number of contaminated sites. It could serve to prioritize sites for clean up or monitoring. It should be noted that the use of RAPDs and STRs DNA-based markers is not a substitute for ecological monitoring. It is an efficient way to screen a large number of field sites in order to determine on which sites to concentrate the more intensive ecological monitoring efforts. Few studies use DNA-based assays to examine population genetics of contaminated population. These types of issues are addressed at a recent workshop on genetic and molecular ecotoxicology sponsored by the National Institute of Environmental Health Sciences, USA (Anderson et al., 1994). The emerged consensus states that new research endeavors are needed to test hypotheses focused on mechanisms and effects of genotoxicants across levels of biological organization (from the molecular to the ecosystem). Furthermore Nadig et al. (1998) compared a population of redbreast living in a mercury-contaminated stream with two reference streams, and found that the contaminated population was more genetically distinct from the reference populations than would be predicted by geographic distance.

In Libya, different waste products and certain chemical wastes found at Tripoli beach contain many substances that are considered toxic. Environmental contaminants can produce irreversible and often deleterious effects in organisms under controlled laboratory conditions. However, very little is known about the effects of chronic exposure to low levels of multiple contaminants pervasive and persistent in the environment. The study of the molecular markets , require the extraction of genetic DNA, in pure and in sufficient concentration, compared with free genetic martials , i.e. free of contaminants, especially sugars Polysaccharides and phenolic compounds Polyphenols and proteins, which keep these molecules inherent to DNA during the process of extraction deposited with DNA. This study involves the use of some chemical solution that raises the ability to get rid of these pollutants.

This study is conducted in the western part of the coast in the Libyan cities of Tripoli and Sabratha. Tripoli coast is polluted mostly with organic pollutants, and the Sabratha is the most cleaner part of Libyan coast.

24 samples of fish (S. aurita), 6 samples per month with 6 samples as reference samples of muscle tissue, of sardine's fish are collected. The random amplification multiple image RAPDs technique of DNA is used, to

identify the effect of exposure of sardine fish to pollutants. It is compared to the reference samples, using polymerase chain reaction enzyme (PCR).

The results show that there is a clear divergence in the group of fish exposed, compared to those in the control group. There is disappearance of three of the packages i.e., pack 2 (1000bp) & Pack 4 (300bp) & pack 5 (100bp), respectively, according to the molecular weight.

Consequently, the fish samples, are collected on second and third month. They equal in packets number by (66.6%), which indicates the percentage of genetic similarity between samples of two months. The samples of February and March, are more homogeneous in terms of both percentage of similarity between them (94.3%), while samples of measurement rate (91.2%), and different samples of the first month samples for measurement by (8.7%) and the similarity between samples of measurement increased by (93.3%).

2. Methods and materials

24 samples of fish (S. aurita), 6 samples per month with 6 samples as reference samples of muscle tissue, of sardine's fish are collected. The random amplification multiple image RAPDs technique of DNA, to identify the effect of exposure of sardine fish to pollutants is used, compared to the reference samples, using polymerase chain reaction enzyme (PCR).

2.1. DNA Isolations and Purification

The study of molecular markers requires the analysis of DNA. The extraction of genetic material for the fabric of the object in pure and in sufficient concentrations is used to obtain genetic material free of the contents of the other cell and free of contaminants. These compounds, during the extraction of DNA, are deposited. These are inherent to DNA, thereby hindering, the operation of extraction of pure genetic material. Therefore, some solution is used, which have the ability to get rid of these pollutants, to launch and to liberalize the cellular compounds.

For this reason, chelex method is used with a concentration of 10%, to extract the genetic material of the fabric of a fish. About 0.2mm of sardines, is placed in bandrof tube (1.5ml), added 500 µl of chelex, and mixed it in mixing device (called vortex), and from the top layer of tubes take 50µl. By using spectrum spectrophotometer instrument, polymerase chain reaction , for enzymatic reaction of pure and free sample of contaminants (see Table 1) shows the materials used in the chain reaction of polymerase enzyme to assess the prefixes, for one reaction.

The used primer is evaluated by using electrophoresis device. Later the products of the sequence reaction are studied for enzyme polymerization. This is done by the gel on ultra violet (UV light), which connects with a computer. So, the cut glass in the genetic material bands i.e dark, and the prefixes are evaluated. Table 2 shows thermal interaction of the sequential polymerization of PCR enzyme used in the study.

Table 1. Materials Used in the Chain Reaction of Polymerase Enzyme to Assess the Prefixes of the reaction

Final Concentration	The required quantity	The substances
----------	5.45µl	Distilled water ddH2o
10 x	1.5 µl	PCR ((1X PCR Buffer)
5Pmol	2 µl	5POML/µL)Primer
0.54 µl	0.15 µl	Tag-polymerase enzyme (0.054/µl)
1mM	0.9 µl	Dioxy acid triphosphates dntp,s 5mm
20-30ng	2 µl	DNA Template
5x	3 µl	Q Solution 1x

Table 2. Thermal Interaction of the Sequential Polymerization of PCR Enzyme used in the study

Primary cycle to break the hydro genic bonds for three minutes at a temperature of 94 °C (Denaturing)	
Hydrogen bonds break for a period of one minute at 94 °C	
Started link for one minute at 37 °C	
DNA elongation for two minutes at 72 °C	
Final elongation cycle for seven minutes at a temperature of 72 °C	

3. Discussion

This study shows that, the pollutants are accumulated in fish tissues, which cause severe diseases in kidneys and in liver tissues function. As a result this could inhibit the efficiency of this tissue in the process of blood

manufacturing. In terms of sequences the study shows a clear divergence in the group of fish exposed, compared to those in the control group. It has been disappearance of three of the packages, according to the molecular weight pack 2 (1000bp) & Pack 4 (300bp) & pack 5 (100bp). Table 3 shows the recurrence of the packages or the disappearance in contaminated and reference sardines samples by RAPDs technique. The diagram (1) shows the degree of genetic similarity between samples of the fish under study.

To estimate the degree of genetic diversity in the genotypes of the fish samples under study, by using (1) and (0) to determine the presence and absence of glass piece (Amplified Fragment) respectively.

The genetic distance between each sample is calculated with all the samples on the basis of the difference (Percentage Difference) (PD), according to the following equation:

$$PD = 1 - [2Nij / Ni+Nj]$$

This ratio (percentage difference) lies between 0.000 and 1,000, where 0.000 indicates that all packets formed a joint between two samples i and j, (i.e. full resemblance) and 1,000 points to the lack of joint packages between the two samples, (i.e., the entire difference).

The development of genotypes of the samples under study in groups and genetic (Clustering Analysis) is used by a computer program "Analysis of the arithmetic average of the totals for bilateral non-weighted", shortly known (UPGMA). The genetic similarity between the genotypes under study is estimated by using the following equation:

$$(G.S)= 2Nij / Ni+Nj= 1-PD$$

Table 3. The Recurrence of the Packages or the Disappearance in Contaminated and Reference Sardines Samples by RAPDs Technique

Packages	Sample1	sample2	sample3	sample4	sample5	sample6	sample7	sample8	sample9
1. 1200bp	+	+	+	+	+	+	+	+	+
2. 1000bp	-	+	+	-	-	+	+	-	-
3. 700bp	+	(+)	(+)	+	+	+	+	+	+
4. 300bp	-	-	+	-	-	+	-	-	-
5. 100bp	-	-	+	-	-	+	+	-	-

Table 4. An average Asymmetry (Genetic Difference) of the Fish under the Study

The mean	Sample No.
0.1305	1
0.1199	2
0.1199	3
0.2222	4
0.1199	5
0.1199	6
0.3448	7
0.1199	8
0.1199	9
0.2222	10
0	11
0	12
0.3379	13
0	14
0.2033	15
0.2033	16
0	17
0	18
0	19
0.3012	20

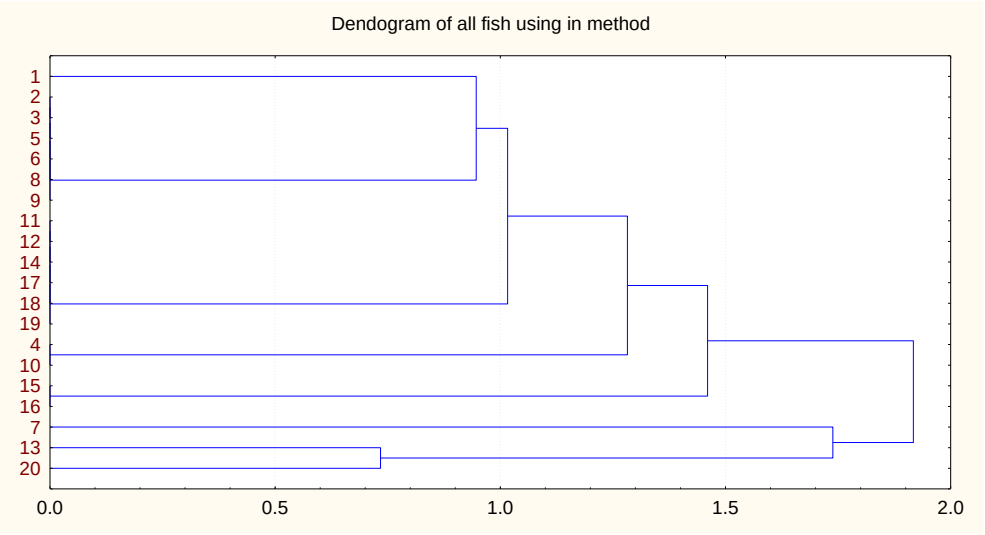

Figure 1. Degree of Genetic Similarity between Samples of the Fish under Study

4. Conclusion

Based on the results of the study the paper shows that how the use of random amplification technique of DNA molecules, and multiple image (RAPDs), to identify the effect of exposure to pollutants on Sardine are capable of determining the sensitivity of fish muscle tissues, compared with reference one's, by using polymerase chain reaction enzyme PCR.

By integrating population genetic approaches into the current program, the Ecological Significance Indicators lead to advancements in assessing ecological risk at contaminated waste sites. One particular useful application in this regard is the development of population-based biomarkers. This is a reflection of perturbations at higher levels of biological organization. It can be used as early-warning indicators of such perturbations. The other beneficial use is the characterization of previously unidentified loci that could be used as novel individual- and population-level biomarkers of genotoxicant exposure and effects. This demonstrates that how Ecological Significance Indicators for the Estimation of DNA Quantity and Purity can be assessed in aq robust manner by the Use of RAPD Technique for Fish (S. aurita) at case study cities in Libyan coastal areas.

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