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EFFECT OF GLOBAL WARMING ON DNA CONTENT AND COI GENE EXPRESSION IN BUTTERFLY

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ABSTRACT

Global Warming is an average increase in the temperature of the atmosphere near the Earth's surface and in the Troposphere, which can contribute to changes in global climate patterns. Changing climate may promote harmful physical impacts such as effects on weather and radioactive forcing. Global warming causes the rise in the sea level and ocean temperature. This change affects the ecosystem and lifestyle of living organism. The butterfly (Tawny Coster) was collected from Chennai,chengalpattu and Tanjore, Tamil Nadu .The Genomic DNA was isolated from the samples and the DNA was amplified in the polymerase chain reaction (PCR). Gene specific PCR was used to find the presence of COI gene. RNA levels are determined by reverse transcriptase polymerase chain reaction. The purpose of this research work is helpful to find out how Global Warming is affecting small creatures. If this Global Warming is continuously increasing it leads to extinction of small creatures.

KEYWORDS: Global warming, Genomic DNA, Butterfly, COI gene

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INTRODUCTION

The Tawny Coster (*Acraea terpsicore*) is a small leathery winged butterfly which is common in grassland and scrub habitats. It belongs to the Nymphalidae or brush-footed butterfly family. It has a weak fluttery flight. It is avoided by most insect predators. The butterfly is found in India and Sri Lanka. It is common all the year round and is equally at home in forest clearings and open country. Though mainly seen at low elevations it has been recorded at heights of up to 7,000 feet (2,100 m) in south India and sometimes in the North (Swihart et.al,1964). It is plentiful in the pre-monsoon and monsoon period and becomes scarce later on. The butterfly exudes an oily and smelly yellow liquid when handled and is unpalatable to birds and most insects. They are well protected and have a slow and weak flight, frequently visiting flowers and are easily netted (Ehrlich et.al,1964). The term global warming refers to the increase in the average temperature of global surface air and oceans since 1950, and to continuing increases in those temperatures. Every year, almost 7 billion tons of carbon dioxide is released into the atmosphere by human activity. That is the equivalent of 107,700,000 jet airplanes being in the air at once! This harrowing figure is the main cause of global warming, and has been increasing for the last 50 years(Thornth waite ,2001).Global warming is caused by the increase of greenhouse gasses, carbon dioxide and methane primarily, in the earth's upper atmosphere directly caused by human burning of fossil fuels, industrial, farming, and deforestation activities(Ledley et.al,1999).

Some of the sources that expose the causes of global warming include tree-ring research, ice-core analysis, and sediment research. For example, research from looking at sediment layering shows that the water level of the ocean is 60 ft lower than 400,000 years ago. Contributing is tree-ring research, which shows the amount of precipitation in the atmosphere. By looking at this kind of data, scientists can help evaluate the amount of change that we have might have inflicted compared to the thousands of years prior to our modern existence. One of the main effects global warming is causing now is the melting of the north and south polar caps. The deforestation that is occurring around the globe is a key factor to causes of global warming(Das et.al,2010). Studies are underway that have a goal to reduce global warming with genetically engineered plants that will be completely efficient in recycling carbon dioxide. The result of these plants would be less carbon dioxide in the atmosphere. Another solution to the problem is replenishing the oceans with algae. Algae are also one of nature's carbon dioxide recyclers. There are plans to grow trillions of algae at farms and release them into the oceans around the world. Renewable types of energy, like hydroelectric, geothermal, wind, and solar energy, are all barely in use in America. By switching to these more efficient types of energy, the amount of carbon dioxide released into the atmosphere would be drastically decreased (Sascha et.al,2012). A fragment of the mitochondrial cytochrome c oxidase gene has been proposed as standard DNA bar-coding marker for the identification of organisms (Kresset.al,2005).The enzyme cytochromecoxidase or ComplexIV (PDB 2OCC, EC1.9.3.1) is a large transmembrane protein complex found in bacteria and the mitochondrion. It is the last enzyme in the respiratory electron transport chain of mitochondria (or bacteria) located in the mitochondrial (or bacterial) membrane(Liqq et.al,2011). Complex IV receives an electron from each of four cytochrome c molecules, and transfers them to one oxygen molecule, converting molecular oxygen to two molecules of water. In the process, it binds four protons from the inner aqueous phase to make water, and in addition of proton electrochemical potential that the ATP synthase then uses to synthesize ATP (Rubinoff et.al, 2006).

MATERIALS AND METHODS

For isolation of genomic DNA, segment of butterfly was placed in a centrifuge tube containing 300µl of homogenization buffer and 75µl of lysis buffer and ground using a thin glass rod. The whole content was incubated at 65°C for 30min (under a controlled environment).To this 17µl of 8M Potassium acetate was added. The tubes were incubated in ice for 30min to overnight. The incubated samples were centrifuged at 15000 rpm for 15min at 4°C. The aqueous phase was transformed to a new tube and 400µl of absolute ethanol was added (to precipitate the nucleic acid content from the aqueous phase).Precipitated nucleic acid was subjected to centrifugation at 12000rpm for 15min at 4°C. Air dried Pellet was dissolved in 20µl of T.E buffer and Stored at -20°C for further use. The extracted samples were analyzed in 0.8 % agarose gel electrophoresis stained with Ethidium bromide(Cox,1968). A solution of nucleic acids strongly absorbs UV with an absorbance maximum of 260nm and proteins at 280nm which is linearly related with the concentration of DNA and RNA and the amount of contamination in the solution. The concentration of nucleic acid in a solution can be calculated if one knows the value of A_{260} of the solution. A solution of double-stranded DNA at a concentration of 50ug/ml in a 1cm quartz cuvette will give A_{260} reading of 1.A solution of single-stranded DNA/RNA that has A_{260} of 1 in a cuvette with a 1cm path length has a concentration of 40ug/ml. The ratio of absorbance at 260 and 280nm hence provides a clear idea about the extent of contamination in the preparation. A ratio between 1.7 and 1.9 is indicative of fairly pure DNA preparation. But values less than 1.8 signify the presence of proteins as impurities. The values greater than 1.8 signify the presence of organic solvent in the DNA preparations. A ratio of 1.8 determines the purity of DNA (Wilfinger et.al,1987). To exponential increase accumulation of the specific DNA fragments by PCR technique was carried out (Kainz et.al,2000).

Total RNA from tissue samples of butterfly was isolated using one step reagent (Bibasic Inc.). It is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent, a mono-phasic solution of phenol and guanidine isothiocyanate, is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi (2006) and its concentration was measured by spectrophotometer. The optimal value for RNA purity is accepted to be between 1.9 and 2.2 (Rutter 1979). The total RNA content was visualized by agarose gel electrophoresis. After RNA isolation, RNA was immediately reverse transcribed with EasyScript Plus™ Reverse Transcriptase to produce cDNA. The cDNA obtained was amplified by PCR (Sambrook et.al,1989). A constitutively expressed gene, namely GAPDH, was chosen in order to assess the quality of PCR.A positive control and a negative control for Reverse Transcriptase PCR were maintained(Robert et.al,2005).

POSITIVE CONTROL – House Keeping gene – GAPDH
NEGATIVE CONTROL – RNA with Taq Polymerase.

PRIMERS USED IN THE STUDY

GAPDH Fwd	5`CAGTGCCAGCCTCGTCTCAT 3`
GAPDH Rev	5`AGGGGCCATCCACAGTCTTC 3`

CO1 fwd 5' – GGT CAA CAA ATC ATA AAG ATA TTG G – 3'
CO1 rev 5' – TAA ACT TCA GGG TGA CCA AAA AAT CA – 3'

AGAROSE GEL ELECTROPHORESIS OF PCR PRODUCTS

In a total volume of 25 ml, 1.5% agarose and 1X TBE buffer were prepared and poured onto a gel tray. To the first well, 4µl of DNA Ladder (100ng/µl) was loaded. PCR product was mixed with the

loading dye. The mixture was loaded to each well. The gel was run at 50 V for 90 min and visualized in a uv transilluminator (Diffenbach et.al,2003).

POSITIVE CONTROL FOR RT REACTION

GAPDH -Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is one of the most commonly used housekeeping genes used in comparisons of gene expression data (Robert D. Barber *et al* 2005). GAPDH was simultaneously amplified along with the respective genes to confirm uniformity of RNA concentration taken for the expression studies.

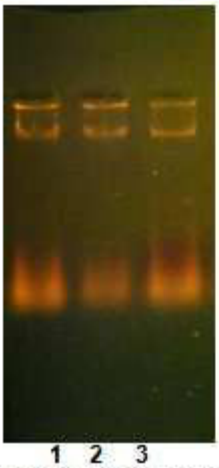
RESULTS

Genomic DNA were isolated from butterfly samples which were collected from high temperature polluted and medium temperature nonpolluted areas (Fig 1). The maximum DNA concentration was observed in Tanjore sample and found to be 6950µg and its purity 1.6. The minimum DNA conte nt was found to be 1850µg in which sample collected from chennai (Table 1). The DNA content was rapidly decreased due to global warming. The purity of DNA was highly indicated in chennai sample. This was due to the denaturation of proteins and rapid mutation of gene. The COI gene of three different samples were amplified and compared with 1kb ladder (Fig 2). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is one of the most commonly used housekeeping genes used in comparisons of gene expression data. GAPDH was simultaneously amplified along with the respective genes to confirm uniformity of RNA concentration taken for the expression studies.

DISCUSSION

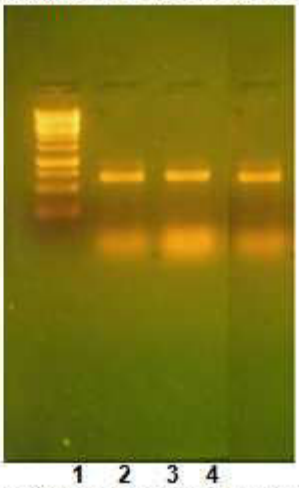
The present study attempts at elucidating the harmful effects of the increasing global temperature on the survival of varied species of insects. In the present research work, the common butterfly (*Tawny coaster*) has been used as a model insect to study the effect of climate change on the organism

Figure 1:GENOMIC DNA



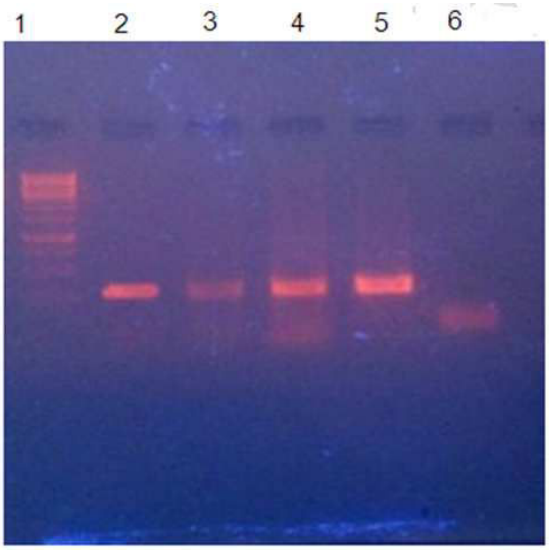
Lane1: chengalpattu sample
Lane 2:Tanjore sample
Lane 3:Chennai sample

Figure 2:AMPLIFICATION OF COI GENE



Lane1: 1kb DNA Ladder(Marker)
Lane2: Amplified product of COI gene of chengelpattu sample
Lane3: COI gene of Tanjore sample
Lane4 : COI gene of Chennai sample

Figure 3 RT-PCR OF COI GENE



Lane 1– 1kb Ladder, Lane 2 – GAPDH, Lane3 –Chennai sample
Lane 4 - Chengalpattu sample, Lane 5 – Tanjore sample, Lane 6 – Negative control

Table 1
DNA concentration of three different samples and its purity

	O.D AT 260nm	O.DAT 280nm	CONCENTRATION(µg)	PURITY
Tanjore sample	0.056	0.036	2800	1.55
Chengelpattu sample	0.139	0.087	6950	1.6
Chennai sample	0.037	0.021	1850	1.76

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