VI. 1. Introduction

The environmental temperature on insects caused dramatic changes in behaviour, physiological activities and biochemical changes (Agrell and Landquist, 1973) particularly haemolymph lipoprotein in *Triatoma infestens* (Maria *et al*., 1991; Rolf *et al*., 1999). Jeffrey and Jesusa (2006) assessed the biochemical fitness of a predator, *Podisus maculiventris* in relation to food quality and effect of five preys. The information about the influence of temperature on various biochemical entities were available in the literature (Himano, 1979, Maa, 1987). Proteins constitute the basic entities in the living being and undergo both qualitative and quantitative changes during development (Engelman, 1979). The fat body is the principal store house of lipid in insects. Most of the lipid is present as a triglycerol which commonly constitutes more than 70% of the dry weight of the insect fat body (Chapman, 1998). Lipids are synthesised from the fat body and secreted into haemolymph through physical activities (Brooks, 1969; Beenakkers *et al*., 1985). Its importance along with protein was also available for heteropteran insects (Kunkel and Nordin, 1985; Helosia *et al*., 1997). Further more Beenakkers *et al*., 1985 viewed that the whole body content is an important carbohydrate reserve in many insects (Beenakkers *et al*., 1985).

Earlier molecular profiling provides a rapid means of quantifying prey diversity within predators but when there are specific prey DNA targets with group specific primers is the principal method of choice (Symondson, 2002). This is fine
for sample laboratory studies, but when there are multiple potential target prey species (Sheppard et al., 2004) and fragments (Hoogendoorn and Heimpel, 2001) the time required to assay each predator potential target becomes limiting.

In field studies, the mean number of prey items in a generalist predator gut may be as a few separate PCR assays evaluated (Harper et al., 2005). This technique is effectively peculiar for many useful field-based ecological studies. Rapid PCR – based screening systems for the study of the prey diversity of generalist predators have been developed to expend the potential of molecular detection in to various areas of food web research (Dodd, 2004). From the published results, it was very clear that except the haematophagous reduviids such as Trypanosoma rangeli and Trypanosoma cruzi (Moser et al., 1989; Breniere et al., 1995; Russomando et al., 1996; Shiankal et al., 1996; Vallejo et al., 1999), till date, no information was available for the polyphagous reduviid predators.

The Polymerase Chain Reaction (PCR) technique was developed by Ehrlich in 1989. It is one of the simplest, fastest and least expensive molecular approaches is to use RAPD – PCR (Randomly Amplified polymorphic DNA) (Shappiro et al., 1988) is used to amplify a region of DNA that lies between two regions of known sequence (Teresa et al., 2002). It has used in many fields including to know the genetic variability in insects (review of Sheppard and Hardwood and Obrycki, 2005b).

Therefore, it was envisaged to analyse quantitatively and electrophoretically in relation to temperatures modification also on imperative one. This chapters deals with changes of whole body and egg macromolecules
(carbohydrate, protein and lipids) of two reduviid predator(s) by spectrophotometer methods, whole gut protein by electrophoresis method; gut DNA polymorphism by RAPD – PCR analysis by AGE in relation to six different constant temperatures on R. marginatus and R. fuscipes.

VI.2. Materials and Methods

VI.2.1. Total carbohydrate, protein and lipid

Eggs were incubated for 12 to 15 days (10,15, 20°C), 6 to 7 days (25 to 30°C) and 4 days (35°C) have been used for analysing the protein (Lowry et al., 1951), carbohydrate and lipids (Bragdon, 1951). 30 adult reduviids of both male and female were kept in the BOD incubator and maintained at 10, 15, 20, 25, 30 and 35°C separately untill their death. After the (one month) stipulated period 10 insects were randomly selected and their whole body total carbohydrate, protein and lipid content were estimated using the above-mentioned methods.

VI.2.2. Procedure for Electrophoresis

SDS polyacrylamide slab gel electrophoresis was carried out using Leamli (1989) method with minor modifications. A sandwich was made with two glass plates separated by spacer strips. The spacer strips are coated with vaseline for adhering mechanisms. The glass plate was kept vertically by placing it on to a stand, which can hold the plates vertically. Few ml of distilled water was poured between the plates to check leakage if any. The resolving gel of 12% (pH 7.6) was poured in to the space between the glass pates after removing distilled water. The level should be about 2cm below from the notch. It was kept for polymerization for
about 30 mts. Then made a layer of distilled water on the surface of the resolving gel, to avoid the contact between the gel surface, air and also to make an even surface.

When polymerisation was completed poured off the distilled water and stacking gel was poured (7.5% PH 7.6) over the resolving gel and the Teflon comb with fingers (each finger with 7 cm wide) was inserted into the gels, and allowed to polymerize for 30 minutes. After polymerisation, the glass plates were clipped out from the stand and also, removed the bottom Teflon spacers. Both of the slab gel was made clean with filter paper and attached the plate to the electrophoresis apparatus. The electrode buffer (TBE) was poured to the lower and upper chamber. Then the Teflon comb was carefully removed from the gel, supernatant of the previously prepared sample was added in each well in about a volume of 15 μl with the help of microtitre syringe. Marker protein of 14-100 KDa (Genei, Bangalore, India) was loaded in one well as a reference. Initially a current of 60 V was supplied with the sample entered into the separating gel and electrophoresis was continued at 120 V till the marker dye reached the bottom of the separating gel (resolved gel). At the end of electrophoresis run glass plates were gently moved apart with a spatula, by running a stream if electrode, the gel in to a solvent resistant plastic trough for staining (Coomassive brilliant blue- CBB) for over night and destaining (24 hrs) until clear band can be seen.

VI.2.3. Protein profile Studies
Under six various temperatures subjected *R. marginatus* and *R. fuscipes* whole gut was used for this present study.

**VI.2.3.1. Reagents preparation**

**VI.2.3.2. Stock solutions**

i. Acrylamide 30%-Bisacrylamide (29.2 : 0.8) prepared by mixing acrylamide (29.2 gm), and bisacrylamide (0.8 gm) in 100 ml distilled water. The solution is filtered through Whatman No.1 filter paper and stored at 40°C in a dark bottle.

ii. Separating gel buffer (resolving gel) (1.5 Tris HCl. - pH 8.8)- 18.17 g Tris was dissolved in approximately 40 ml of distilled water and adjust the pH to 8.8 with 1N HCl using pH meter. Make the final volume up to 100 ml.

iii. Stacking gel buffer (0.5M Tris HCl pH 6.8) 0.057 g of Tris was dissolved in approximately 40 ml of distilled water and pH adjusted the pH to 6.8 with 1N HCl and made the final volume up to 100 ml. The solutions i, ii, and iii were filtered through Whatman No.1 filter paper and stored at 4°C in a dark bottle.

iv. 10% SDS (Sodium Dodecyl Sulphate) - 1 gram of SDS was dissolved in 10 ml of doubled distilled water. The solution was clear and colorless and kept at room temperature.

v. Ammonium per sulphate (APS) 10% –100 mg of APS was dissolved in 1 ml of distilled water APS. The solution is unstable and
decomposes readily at room temperature and hence it should be made fresh just before use.

vi. TEMED (N, N, N, N, - Tetra methyl ethylene diamine) - This reagent was acting as a catalyst for gel formation.

vii. Electrode buffer (reservoirs Buffer) –3.028 gm of Tris, 14.45 gm Glycine, 0.5 gm SDS were mixed with 500 ml of distilled water. The solution was stored at 4°C in a dark bottle.

viii. Coomassie Brilliant blue stain – R – 250 - 50 ml methanol, 7 ml acetic acid, 200 mg coomassic blue were mixed with 43 ml distilled water. The solution is blue in color and kept at room temperature.

ix. Destaining solution - 30 ml ethanol, 67 ml of distilled water were mixed with 7 ml of a acetic acid. The solution is colorless and kept at room temperature. This solution once used can be reused. For this, pupae after destaining add a teaspoon of activated charcoal to this solution and allow settle the impurities properly. Then the blue color disappears and the solution is filtered through Whatman No.1 filter paper and this can be used for destaining again.

ix. Sample buffer (3ml) – prepared by mixing 3 ml each of 0.5 M Tris – HCl (6.8) and 10 % SDS 0.3 ml, β - mercaptoethanol 2.4 ml, 3 ml, Glycerol 3 ml, Distilled water 3 ml, Bromophenol blue 1 pinch. The solution is blue in color and is stored at 4°C.
xi. Resolving gel (125-15ml) – Prepared by mixing 4.9 ml distilled water, 6.0 ml acrylmaide, 3.8 ml Tris (8.8), 0.15 ml 10 % SDS, 0.15 ml 10 % A PS, 0.006 ml TEMED and allows 20-30 minutes for polymerisation.

xii. Stacking gel (125 – 4 ml) – Prepared by mixing 2.70 distilled water, 0.067 ml acrylmaide, 0.50 ml Tris (6.8), 0.04 ml 10 % SDS, 0.04 ml 10 % APS and 0.004 ml TEMED.

xiii. 7 % Acetic acid – mix 7 ml acetic acid and 93 ml distilled water and this solution is used for the preservation of gel.

Insect Ringer solution (IRS) – It was prepared by mixing 7.5 gm sodium chloride, 0.035 gm Potassium chloride, and 0.22 gm Calcium chloride with 1000 distilled water. This solution was kept at room temperature.

**VI.2.3.3. Protein sample preparation for Electrophoresis**

6 to 10 adult of *R. marginatus* and *R. fuscipes* were selected separately from the stock insects which maintained in different temperature including the room temperature categories. Foregut was dissected out from the predators and homogenised with homogeniser. Eppendorf tubes containing 75μl of gut sample was boiled at 50-60°C for 3 mts and allowed to cool at room temperature. The sample was then centrifuged at 10,000 rpm supernatant was collected and used as the protein sample for electrophoresis.
VI.3. DNA Extraction and amplification

Six to ten reduviid adults predators were reared at different temperature regimes for more than a month were randomly selected and homogenized with 0.5 ml extraction buffer (8% DTAB, 1.5M Nacl, 100mM Tris, 50M EDTA 10% SDS and proteinase K) and grind further. The extract was incubated for 2-3 hrs at 50°C–60°C to allow the separation of DNA also for the denaturation of proteins. The mixture was centrifuged for 5 minutes at 10,000 rpm. The supernatent was cleaned away from the protein and lipids by phase extraction with an equal volume of phenol, chloroform and iso amyl alcohol (25:24:1). DNA was precipitated by adding one-tenth volume of 3M NaCl + two volume ice-cold 95% absolute ethanol and incubated for one hr at -20°C. The precipitated DNA was centrifuged, then washed with 70% ethanol, DNA was vacuum dried and resuspended in 100μl TE buffer, pH 8.0). The concentration and purity of extracted DNA was determined spectrophotometricaly (UV- instrument) at 260 nm and 280 nm. Samples showing the one OD (optical density) equivalent to 50 μg and purity (determined by the ratio of 260 nm and 280 nm) 1.5 to 1.8 alone were taken for further analysis. Template DNA extracts were stored at -20°C and thawed at room temperature for further amplification.

VI. 3.1. PCR Amplification

The extracted DNA from the experimental predators were subjected to PCR analysis using 6 universal primers among six, further proceed for amplification 3 primers were selected such as KTG-3-(5′-GTAGACCGCT-3′), KTG-5 (5′-AACGCGCAAC-3′) and OPE 8-(5′-AACGGCGACA-3′) (GENEI
scientific supplies, Bangalore). PCR reactions were performed in 25 μl of reaction mixtures contained 1 mM dNTP mix (5.0 μl), 1.0 μl template DNA (50 ng / μl), 10 mM RAPD primer (2.5 μl), 10X Reaction buffer, 25 mM Mg Cl₂ (1.5 μl) 2.5 units of Taq polymerase enzyme (5U / μl) (Bangalore Genei, India) and sterile de-ionised water. Above-mentioned 25 μl of reaction mixtures was placed in PCR tubes in two layers. The bottom layer consists of all reagents except Thermus aquaticus (Taq), sterile distilled water and sample DNA. The upper layer consists of Amplification was performed with thermocycler (Master cycler ep’s eppendorf, India) for 40 cycles. Thermal cycles were programmed for initial denaturation at 94°C for 2 minutes. Each cycle consisted of 40 seconds annealing at 94°C and also with 1 minute annealing at 48°C, followed by 72°C for 5 minutes as final extension. Amplified, samples were stored at 4°C prior to electrophoresis. PCR amplified products were separated on 1.4% agarose gels submerged in 1X TBE, and the banding profiles was visualised with ethidium bromide. Gels were documented using Biotech documentation and analysed with Gel Del TM software (Bangalore) (Carezza Booto et al., (2005). Genetic similarity and Dissimilarity dendrogram was made from the similarity data using UPGMA method of the programme Digital Gel Documentation (Biotech, Tamil Nadu, India)

V.3.2. Statistical analyses

Using three selected primers with randomly selected six temperatures treated both predators were comparative analysis was made. RAPD patterns and gut protein polypeptide profiles were visually analysed and scored form photographs. For the analyses and comparison of the patterns a set of distinct, well
separated bands were selected. The genotypes were determined by recording the presence (i) or absence (o) of these bands and neglecting other (weak and unsolved groups) bands. Genetic identity (GS) and genetic distance (DS) values between the total six temperatures were calculated using the data generated from the RAPD profiles using digital gel documentation (Biotech, Tamil Nadu, India). Genetic distance values were utilized to contract to dendrogram through clustering analyses (UPGMA) to determined the relationship among the six various temperatures on the predator of *R. marginatus* and *R. fuscipes*.

VI.4. Results

VI.4.1. Whole animal macromolecules

VI.4.1.1. Carbohydrate

Total carbohydrates, protein and lipid content of *R. marginatus* in relation to different temperature regimes (10 to 35°C) is presented in figure 13. From the figure it was very clear that, total carbohydrate content was lower at 10°C (22.2 µg/mg). It gradually increased when the temperature was increased (23.5, 25.11, 27.8 and 28.8, µg/mg for 15, 20, 25 and 30°C respectively) and attains its peak at 35°C (30.14µg/mg). Statistical analysis between control (25.3µg/mg) with different temperature showed that 25, 30 and 35 were significant at 5% level. Similar observation was also recorded in *R. fuscipes* too. Between the two reduviids *R. fuscipes* had maximum carbohydrate content than *R. marginatus*. 
VI.4.1.2. Lipid and Protein

In contrast to the carbohydrate, lipid content was gradually decreased from the lower temperature to the higher temperature (Figure 11). As observed for carbohydrate, the lipid content was low and high in *R. marginatus* respectively. In *R. marginatus*, the protein content the control category (291.20 µg/mg) and 20°C (290.71 µg/mg) and 25°C (293.54 µg/mg) were more or less similar. However, in *R. fuscipes*, protein level in control (272.0 µg/mg) and 25°C (273.28 µg/mg) categories were similar. Statistical analyses were made between control and 10 to 30°C reveals that all the comparisons were significant at 5 % level.

VI.4.1.3. Protein

Protein content was gradually increased from 10 to 25°C and 10 to 30°C for *R. marginatus* and *R. fuscipes* respectively.

VI.4.2. Temperature and gut protein profile of *R. marginatus* and *R. fuscipes*

*R. marginatus* adults gut protein analyses by SDS PAGE. Showed that both 35, 30°C produce 5 polypeptides where as 25, 20°C and 15°C produced seven bands with molecular weight between 6.5 to 205 kDa. Two molecular weight polypeptide such as 56.0 and 205 kDa were absent at 10°C in *R. marginatus* (Plate 4a). From the dendrogram analyses explained that gut protein profile, showed higher genetic identity (GS) at 20°C (0.83), than the lower temperatures such as 10, 15°C (GS = 0.77). (Figure 15(a)). As in the case of dissimilarity (Genetic distance-GD), minimum value was recorded (0.16) temperatures of 35, 15 and
20°C. It was further reduced when the reduviid was subjected between 25 and 30°C (0.11) (Figure 15b).

Plate 4(b) depicted gut protein profile of *R. fuscipes*. Four uniform polypeptides appeared at 35, 30, 25 and 20°C (between the molecular weight range 2.0 to 205 kDa) except at 10°C. However, at 35 and 20°C once polypeptide (<205 kDa) was peculiar, in gut protein because, absence of this higher Mw. The dengrogram analyses showed the highest genetic similarity between 25 and 30°C (0.81). Lowest genetic similarity (0.22) was noted at 10°C and 15°C. Although dissimilarity (DS) was did not possessed struck main variations, even though they ranged between 0.23 to 0.11. A dendrogram was predicated to find out the genetic relationship in *R. marginatus* and *R. fuscipes* which subjected to various temperatures. From the results it was very clear that predators reared at 30°C had closer relationship with 25°C and also another category of 10 and 15°C in *R. marginatus*, whereas in *R. fuscipes*, relationship was recorded between the temperatures 10 and 15°C.

**VI.4.2.1. Eggs macromolecular profile**

In another study biochemical composition of eggs in relation to temperature was evaluated. Regarding the total carbohydrate, maximum content was recorded at 35°C for the two reduviids. Furthermore, egg protein contents were gradually increased up to 30°C for both the reduviids. In contrast, lipid content was gradually decreased up to 35°C in *R. marginatus* and *R. fuscipes* (Figure 14).
VI.4.3. DNA Amplification of *R. marginatus* and *R. fuscipes*

PCR amplified products having 400 and 600 bp were common irrespective of the primers in *R. marginatus*. Such a similarity was not observed when *R. fuscipes* whole body DNA was amplified with OPE-8, KTG-3 and KTG-4 Primers. Interestingly OPE-8, KTG-3 and KTG-5 produced a unique bp product of 1200-150 and 50 bp in *R. marginatus*. Similarly these 3 primers produced 950, 200 and 300 and 900 bp in *R. fuscipes*. Present study reveals that RAPD markers were efficient for the assessment of genetic similarity and dissimilarity coefficient using Digital Gel Documentation between the six temperatures within the same species described in the dendrogram. Apparently the resulting data present in Tables, 19, 20 were further processed for cluster analysis using the unweighed paired group of average method (UPGMA). Totally seven primers were tested, four primers (KTG- 1, 2 and 4 and OPE-8) yielded no clear or any scorable bands, but remaining 3 primers (KTG –2, KTG – 5 and OPE – 8) were amplified, produced scorable with polymorphic bands. Primers KTG –3 and KTG – 5 amplified maximum numbers of polymorphic bands ranged about 31 to 34, in *R. marginatus*. Primers such as OPE-8 and KTG –3 produced 26 and 32 bands in *R. fuscipes*.

VI.4.4. Genetic similarities (GS) in *Rhynocoris marginatus*

4.4.1. Primer – KTG – 3

Figure 17a shown KTG –3 primer predicted dendrogram, it consists of two clusters. Cluster – 1, and this deserved higher GS value was higher (0.84) than the remaining temperatures. As in the case of another temperatures held in cluster – II,
this also again stands for only one temperature at 20°C, and it consisted estimated GS value was 0.80. Where as, cluster II again divided into II-b1 and C-II b2. These subclusters belong to the temperatures of 30 and 35°C also possessed similar GS value and 15 including 25 and 10°C respectively. Of these four temperatures of 15°C (C-II b2) consisted estimated GS value was higher (0.70) this was 10% increased away from C-II b1 consisted 30 and 35°C. Finally C-II b2 represented temperature of lower (10°C) and optimum (25°C) both the temperatures shared equal GS value of 0.40. From this result clearly showed 40.4% deviation were observed between RT and lower temperatures 10°C.

4.4.2. Primer KTG – 5

When estimating the KTG – 5 primers (Figure ), dendrogram revealed mainly two sub clusters. From this cluster – 1 had been the temperature at 30°C expressed GS value was (0.76). Then the cluster –II again divide into two sub-clusters, such as C-II-a and b. Here, cluster –II a represented at 35 and 15°C, both the temperatures shared another higher as well as similar (GS) values of 0.57, followed by cluster II – b consisted remaining temperatures and its observed GS values were RT°C (0.6b), 25°C (0.65), and following lower temperatures 20 and 10°C noted GS value of 0.29. Since the overall results clearly noted highly diverged 0.53% at 35°C (0.23). Since the overall results clearly noted highly diverged 0.47% at 10°C from the initial genetic similarity index of 0.76 at 30°C (Table 19).
4.4.3. Primer OPE – 8

Based on *R. marginatus* OPE-8 primer could be provided dendrogram. Consisted of two cluster, They were cluster I and cluster II. C-I mainly stands from temperatures (RT) expressed highest GS value of 0.84. Since cluster –II broadly divided into two subclusters namely Cluster II a and Cluster II b. At 35°C category include C-II b. Interestingly both temperatures shared GS value of 0.75. Similarly remaining adjacent temperatures such as 20 and 25°C represented C-II a and 10 and 15°C stands for C-II b. Since these four temperatures (10-25°C) had been possessed the similar value of 0.50. Here this primer predicted overall similarity that was 0.34% deviation observed form RT initial GS index of 0.84 noted 10-25°C. This result concluded closely relationship seen between similar at 10-15, 20-25 and 30-35°C.

VI.4.5. Genetic similarities (GS) in *R. fuscipes*

VI. 4.5.1. Primer KTG-3

KTG-3 primer had drawn a dendrogram predicted results clearly visible in figure 18a. From this figure expressed smaller accessions instead of clusters arranged with each temperature as a decreased manner in accord with GS estimated values. The higher genetic identity or GS observed at higher temperature at RT (0.95) followed by temperature consisted GS value was 30°C (0.94), 25°C (0.93), 20°C (0.90) and 15°C (0.85). Finally medium GS value (0.67) was denoted at lower temperature (10°C) as well as higher temperature (35°C). Each temperature reported all the Genetic Similarities were similar as soon as they had been little deviation range about 0.01% between RT-25°C and 0.05% for 20-15°C and 0.20% shows at 15-35 respectively. The overall results indicated 0.28% away from initial GS (0.95) to RT-10°C.
VI. 4.5.2. KTG-5

Apart from the primer KTG-5 revealed dendrogram see figure 18c. In this primer does not divide into clusters but they were arranged the separate accessions and it stands for lower and higher temperatures (RT-10°C) instead of cluster. As in the case of estimated higher GS value also at the RT (0.88) then the lower temperatures (35°C) secured adjacent value of 0.86. Similarly following temperatures had been little variation noted in GS value such as 30 °C (0.84) and 25 °C (0.77), 20 °C (0.66). Finally at 15 and 10 °C lower temperatures noted lowest GS value noted 0.34. The overall results shows closely related GS range 0.02% to more deviation 0.54% observed away from initial GS index value at RT (0.88) seen among the each six temperatures.

VI. 4.5.2. OPE-8

Based on OPE-8 primer showed cluster analysis (figure 18b) mainly divided into two clusters that was namely cluster I and II. Cluster I consisted only one temperature of RT and it was also secured higher GS value of 0.92. Another cluster II again broadly divided into two subclusters (cluster IIa and cluster II b). At 10 and 20 °C had been possessed another higher as well as similar estimated GS value of 0.87. These two temperatures represented Cluster II a stands for remaining temperatures of 35, 15°C and both temperatures observed GS value Cluster II a and C-II b was similar 0.75. (Table 20). At 25 °C and 10 belongs to cluster II b both temperatures also had been shared similar estimated GS value 0.75 as like that C-II a. As a resulted dendrogram showed a did not found more deviations (0.57%) made between RT-10°C, at the same time a meager variation 0.17% revealed among these six temperatures.
VI.5.1. Discussion

VI.5.2. Macromolecules

Macromolecules like carbohydrate, protein, lipid content of *R. marginatus* and *R. fuscipes* had been dearth in the life time. Previously George and Ambrose (1999b, George *et al.*, 2002) demonstrated that insecticide affect carbohydrate, protein, lipid content of *R. marginatus*. Among the three macromolecules initially the whole body carbohydrate content could be attributed to its higher utilization as well as energy releasing site were warranted by altered metabolism due to agreement observation discussed in other insects belongs to Coleoptera (Price 1965; Pigman and Horton, 1970). Such high energy demands for various endothemic biochemical reactions can be readily react on carbohydrate reserves because they are principal and immediate chief source of energy precursors (Wyatt, 1976). More over prolonged treatment of cooling as well as higher temperature affected functional either quantitatively nor qualitatively at the range of metabolical changes. It leads to reduce the synthesis of protein by deranging the protein synthetic machinery.

Appearance of some protein substances mainly affected by temperature variation says Bradford (1976). According to Ryan and Dick (2001), when ever temperature reach to peak (or) higher (or) exceeded at 35°C, adult insects were enable to ultimately adopted and also this changes caused mainly depends upon the treated temperature. This kind of opinion was also agreed by Shappiro *et al.* (1988). Temperature changes also affect the Lepidopteran pests, its may be related to heat shock proteins induced by sub-lethal temperature an other environmental
stresses (Dodd, 2004; Zhang et al., 2007), reported that major during heat shock they should be disintegrate induced by conditioning at 33 - 41°C after for 2 to 3 hr at 20°C. Further more of the energy as well as break down of food particles of insects and metabolic process, directly or indirectly controlled by biotic factors suggested by Salt, 1970.

VI.5.3. Gut Protein Pattern

Gut protein pattern shows significant reduction in the number of polypeptides when the redvuiid was subjected to difficult temperatures. Molecular weights of gut protein of two predators were range between 205 to 14.3 kDa and below 205 to 14.3 kDa in R. marginatus and R. fuscipes respectively. The electrophoretic variation of the protein bands in the whole alimentary canal of adult these predators showed Plates (6a and b). Such qualitative profiles of protein was observed during adult transformation with temperature confirms earlier findings (Tefler et al., 1983; Ryan and Dick, 2001). As in the case of whole gut of R. marginatus showed six polypeptides at room temperature. Intensity of polypeptides, position, size and shape for all 6 temperature determined slightly qualitative changes between 10-15, 30-35 and 20-25°C. In R. fuscipes higher and lower temperature had been peculiar high molecular weight polypeptides. This agreed with earlier findings in Coleopteren predators did not based upon only temperature but endepeptidases in alimentary canal (Addedire and Balogun, 1995).
VI 5.4. RAPD – PCR

The inter population profile of the six temperature combination showed a remarkable banding patterns of powerful band, high intensities of genetic variation or suggesting heterogenous as well as homogenous amplified DNA between 10 to 15, 20 - 25, 25 - 30 and finally 35°C envisaged faint banding pattern which ranged then 2000 to 200bp MW and 1000 to 300bp MW in *R. marginatus* and *R. fuscipes* respectively. In *R. fuscipes*, KTG - 5, KTG - 3 primer produced amplified products were homogenous with respect to all temperatures. This results demonstrate precisely KTG and KTG -3 primer in *R. marginatus* adults revealed that weak and powerful band were observed between the 10°C to 35°C and in OPE – 8, we recorded low molecular bp bands with uniform pattern.

This result clearly showed higher and lower temperatures caused changes IN the DNA pattern and level both in terms of qualitatively and quantitatively. The success of PCR depends on the quality of the DNA, must be free from any contaminants and from protein, nuclease that interfere the amplification process. Greenstone *et al.*, 2005, King *et al.*, 2008.

PCR based protocol shows great power for quick and simple characterisation of genetic variation within and among the population (Whittman, 2005). PCR techniques has been successfully used in studies on DNA of reduviids, *T. cruzii* (Breniere *et al.*, 1992; Carezza Botto *et al.*, 2005) and preliminary studies on the selection and activity cycles on heteroptera reduviidae (Canals *et al.*, 1997; Moser *et al.*, 1989; Russomodo *et al.*, 1992). Qualification and Quantification of DNA under such circumstance was becomes necessary to amplify the DNA.
polymerase chain reaction and then quantify the PCR product. The logic being that the amount of product would be measured and the amount as well as purity of the extracted initial DNA. Due to extreme sensitivity of the PCR reaction, however even very small variation in the reaction efficiency would result in significant differences in the amount of final product formed. RAPD analysis allowed grouping the insects in accordance to the place of capture in contrast yo the molecular analysis. It is possible that this grouping could be due to the phenotypic plasticity, the expression of different phenotypes in single genotype when subjected to different environments (Whitman, 2005)

These result indicated that high quality DNA can be isolated from both of the predators reared under range of temperatures. In addition, successful PCR is possible when the amount of DNA specifically with temperature produced amplification product was widely varied. This demonstrates that quantity of DNA was a initial process for amplification of isolated DNA molecules. The study also had been addressed the suitability of reagents used to stored the experimental adult predators for subsequent DNA isolate in Deep freeze (-20°C). Storage conditions are apparently not critical for experimental samples stored less than 6 months.

Temperature mainly determined biologically and biochemical functional activities including macromolecules of DNA and RNA content (Dodd, 2004; Carreza Booto et al., 2005). Result also indicate that when we analysed the trees, which generated from both predators with 3 various primers, KTG- 3, KTG-5 and OPE-8 denoted that the tree constructed using the KTG-5, KTG-5 were shown variable than OPE-8 and in R. marginatus. In another way in R. fuscipes predator,
KTG - 5 and OPE 8 showed similar variation but they did not observed more variation between 10°C to 35°C.

From this study we understood that when the temperature reached at 35°C or more or less than 10°C amplified DNA of the predators were less intense compared to optimum normal temperatures banding profile. Notably this similar trend was also observed by Garnoel and Barett, 1993. Some individual of the heat treated group of insects exhibited a faint band, indicating temperature fluctuation its also caused or accompany with presence of small amount of the bacterium present on the alimentary canal of an insects previously reported by Tsuchida et al., (2006).

VI. 6. Conclusion

We concluded that PCR is an excellent tool that can be applied to identity genetic polymorphism as well as change the genetic constituents depends upon the temperature variation within and among the same individual of this predators. PCR employed here is a method, which has the large applicability of RAPD but also can generate differences the banding pattern that are more information for population analysis. More ever, this result indicates prolonged temperature (highest above 35°C) stores could be reduce or denature the protein molecules. In RAPD banding profiles of difference temperature (10-35°C) differ from each other in terms of both the numbers as well as size of the amplified fragments with size and MW ranged between 2500 and 100dp and 1500 bp to 4300 bp in *R. marginatus* and *R. fuscipes* respectively.