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# ANALYSIS OF GENETIC DIVERSITY IN CONGENERIC SPECIES OF GRASSHOPPERS BY GEL ANALYSIS OF PROTEINS, ALPHA AMYLASES AND GENOMIC DNA (BY RAPD-PCR)

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## ABSTRACT

Genetic variation between the six congeneric species of grasshoppers has been analyzed by SDS-PAGE (*Sodium dodecyl sulfate polyacrylamide gel electrophoresis)* and RAPD-PCR technique. The study revealed the presence of polymorphism in muscle proteins ,alpha amylases and in genomic DNA between each congeneric species. Each species had different number of proteins and amylase electromorphs indicating as many number of genes. The RAPD –PCR generated large number of polymorphic bands by each marker applied and also revealed large number of genomic duplications, highest duplication in genome and polymorphism in genomic DNA was observed in *C.trachypterus* and *C.oxypterus* followed by *A.exaltata* and *A.gigantica* in contrast more similarity for DNA recorded in *O.abruptus* and *O.senegalensis*. These genetically divergent ,sympatric and congeneric species co-occurred without any habitat specialization.

**KEYWORDS: Congeneric Species, Morphology, Sympatric, Genetic Diversity, Polymorphism, Amylase isoform Extra bands, muscle proteins**

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# INTRODUCTION

Darwinian doctrine states that every new species is formed through Descent with modification from their ancestral form. Attaining a species status by an organism involves acquisition of evolutionary independence through development of barriers to gene exchange otherwise termed reproductive isolation, which is majorly due to genetic variations. Congeneric species retain their generic characters during speciation and how these develop new specific character was the central point of interest, rest of the research revolves around it ,often widely examined to understand the speciation process has been which reviewed by Gissi[1] . Here genetic variation between the congeneric species of grasshoppers has been analysed to know how much genetic variation these carry in between species . Six species of grasshoppers belonging to three genera taken for this study include *Acrida exaltata* and *Acrida gigantica* (subfamily acridinae), *Oedaleus abruptus* and *Oedaleus senegalensis* (subfamily oedipodinae), *Chrotogonus trachypterus* and *Chrotogonus oxypterus* (subfamily pyrgomorphidae). These congeneric species are classified in to respective taxonomic status based on the morphological differences and similarities[2,3].Majorly body color, band markings, colored spots as well nature of pronotum have been used in all these species in addition to other physical characters for classification. Wing character in Oedepodinae is used for classifying these grasshoppers in to tribes and species with certain limits[4]. Congeneric species are interesting to many workers to analyze different aspects of variations and the distribution[5,6], as well Congeneric phylogeography reviewed by D. J. Funk and K. E. Omland[7]. The six species of grasshoppers used in this study were sympatric in their occurrence. All these six species of grasshoppers have been posed for cytogenetic analysis by several workers to understand the genetic variation but due to karyotypic conservatism such studies have not revealed a clear cut genetic differences between each species[8,9,10]. So as to understand genetic variation among these congeneric grasshoppers application of molecular technique, felt, to be more suitable. Different molecular technique have been used to assess the genetic diversity between the species of insects SDS PAGE analysis of proteins[11,12,13] and enzymes [14,15] have been used , along with these RAPD PCR is also employed in different organisms by several workers.[16]Protein electrophoresis is still one of the well practiced molecular technique in systematics and population genetics.[17] RAPD technique has also been successfully employed in protozoans,[18] in grasshoppers[19,20,21,22,23] ,In other insects[24-29]in nematodes[30], mice[31] to evaluate genetic polymorphism. RAPD–PCR technique, its convenience and utility is well reviewed by a few workers .[32,33] These techniques are employed here to find extent of genetic variation in between the species of congeneric grasshoppers.

# MATERIAL AND METHODS

Six species of grasshoppers *A.exaltata, A.gigantica, O.abruptus, O.senegalensis, C.trachypterus* and *C.oxypterus* collected from their natural habitat are utilized for their genetic variation analysis. These species occurred as sympatric occupying the same habitat.

***SDS-PAGE ANALYSIS (Sodium dodecyl sulfate polyacrylamide gel electrophoresis)***

50mg of the thigh muscle was weighed in a vial and homogenized with 300ul of 1XPBS using tissue grinder and centrifuged at 6000rpm for 5mins. Supernatant was collected (crude) and stored at $20^0$c for SDS-PAGE analysis.

Proteins were separated by Laemmli[34] sample buffer, components used for SDS-PAGE analysis were 1.5M Tris Ph8.8, 1.0MTris pH6.8,30% Acrylamide ,Bisacrylamide, Potassium per sulphate, sample loading buffer(4% SDS,20% glycerol,125mM Tris-cl,ph6.8,10%β mercapto ethanol, bromophenol blue dye) crude protein samples, TEMED, Protein standard marker,1XTris-Glycine SDS buffer. Protocol[35.36] consisted of 12% resolving gel and 5% stacking gel. 6ul of each sample was boiled with SLB loaded using micro pipette (pipette man's from Gilson & Nichipet companies). Electrophoresized at 50V initially for 30mins then increased the voltage to 100V. Gels were stained with Coomassie brilliant blue stain. The results were photographed and Molecular weight of each bands was determined by Gene tools of G box gel doc system Sygene.

***ZYMOGRAPHY***

To analyse enzyme activity in grasshopper, gut samples using 1XPBS extraction buffer.20mg of samples was weighed & homogenized with 100ul 1XPBS. Later 20ul of crushed samples was used for zymogram. The technique relies on detecting enzymes, so the samples were kept live or frozen to preserve enzyme activity. (samples are not boiled during loading). SLB contains glycerol, β-Mercapto ethanol Tris buffer and Bromophenol blue essentially does not contain SDS. Resolving gel of 12% and stacking gel of 5% was prepared. The samples were loaded using microsyringe apparatus. Electrophoresized at 50V for 30min initially and then at 100V. Marker lane was stained in Coomassie blue stain, gel was rinsed in distilled water transferred to 50ml renaturation solution (1ml of triton x100 in 49ml of distilled water) and incubated on rocker for 30mins at room temperature. Gel was rinsed in distilled water and 50ml of developer (10mg of Naphthyl acetate dissolved in sodium phosphate buffer $pH^7$ with 20mg fast blue) kept in dark for overnight. Electrophoretic gels of zymograph was washed and stained with Potassium iodide solution so that amylase activity appeared as white bands on brown back ground.

***RAPD- PCR***

For isolation of DNA, hind leg femur from each of the species is used. The DNA isolation was done using lysis buffer and isopropanol, as described by Sambrook et al.[35] Ten arbitrary primers mentioned (Table-1) procured from Bangalore Genie were used for RAPD-PCR (Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction) analysis. RAPD-PCR were carried out following the procedure executed by Williams et.al[37,38] PCR cycles were performed with 5min at 94c for initial

denaturation and then 40 cycles of 30 sec, 94c and 40 cycles at 45c for 1min extension was performed at 72°C 40 cycles for 3 min and final extension of 1min at 72 °CPCR amplifications were carried out in thermal cycler (Bio Rad). The PCR products were separated on 1.5%agarose gel contained ethidium bromide in0.5xTDE buffer at 100v constant voltages. DNA ladder was loaded with each gel. The gels were photographed using Uvitech[USA] .the bands are scored for presence as [+] and absence as[-]. Each PCR preparations were repeated twice in order to ensure that RAPD banding pattern were clear.

**Table 1**
***List of DNA primers used for analysis***

| Sl. No | Primers | Sequence |
|---|---|---|
| 1 | 970-11 | Gtaaggccg |
| 2 | OPA-02 | Tgccgagctg |
| 3 | OPA-11 | Caatcgccgt |
| 4 | OPAA-09 | Agatgggcag |
| 5 | OPAI-05 | Gtcgtagcgg |
| 6 | OPB-10 | Ctgctgggac |
| 7 | OPB-18 | Ccacagcagt |
| 8 | OPC-06 | Gaacggactc |
| 9 | OPC-07 | Gtcccgacga |
| 10 | OPD-02 | Ggacccaacc |

## RESULTS

Genetic diversity between the six congeneric species of grass hoppers analyzed by SDS PAGE for proteins generated in total of 56 bands of varying numbers in between each set of congeneric grasshoppers (Table . 2 Fig .1) with molecular weight range between 10.34KDa to 116.97KDa. Ten and eight bands of varying mol.wt. between 11.KDa to 115.27KDa found in species *A. exaltata* and *A .gigantica* ,one band was monomorphic and other bands were polymorphic; 12 and 14 bands generated for species *O.abruptus* and *O.senegalensis* had mol.wt. range of 10.4KDa to 111.65KDa and 10.85KDa to 102.02 KDa respectively were all polymorphic. Like wise dissimilarity was also seen in species *C.oxypterus* and *C.trachypterus* these two had generated 08 and 11 bands respectively and the mol .wt. found to be between 18.64KDa to 166.95KDa and12..39KDa to 108.91KDa (Table 2 Fig.1) for respective species. Amylase enzyme analysis in the present study shows the number of alleles for isoforms of amylases were two each for species *A.exaltata* and A. *gigantica* as well for *O. abruptus* and *O.* senegalensis. The other species *C. oxypterus* and *C .trachypterus* had three alleles each for amylase as recognized by number of electrophoretic bands generated. The mol. wt. differences were 41.93KDa and 60.66KDa for *A.exaltata* ,42.81KDa and 64.99KDa for *A. gigantica* , 37.53KDa and 47.66 KDa for *O. abruptus* and 36.58KDa and 44.28KDa for *O.senegalensis*. Where as *C. Oxypterus* had three bands with mol.wt.57.58KDa, 59.30 KDa and 70.80KDa, *C. tracypterus* had bands with mol.wt.56.23KDa , 63.96 KDa and78.80 KDa(Table .3 Fig .2 ). The band profiles obtained from RAPD-PCR finger printing with all the ten primers generated total of 53 clearly visible bands for the congeneric species *A.exaltata* and *A.gigantica* [table 4 fig-3a], profiles of *O.abruptus* and *O.Senagalensis* had shown 63 bands [table 5 fig-3b] and 55 bands for *C.trachypterus* and *C.oxypterus. were generated* [ table 6 fig-3c] .Regard to polymorphism of band profiles- among the 53 bands of *A.exaltata* and *A.gigantica* 69.8% were monomorphic bands and 30.2% were polymorphic, species *O.abruptus* and *O.senegalensis* had 81% monomorphic and 19% polymorphic bands of the 63 bands generated. Whereas *C.trachypterus* and *C.oxpterus* showed highest polymorphism for the 68 bands, of these only 3.4% were monomorphic and remaining 96.6% were polymorphic .Most unique was no bands generated by the primer opb-18 in both these species of *Chrotogonus*, in *Acrida* this primer generated 4 monomorphic bands, in *Oedaleus* it generated 3 monomorphic in each species and 1 polymorphic bands .In both the genera *A.exaltata* and *A.gigantica.,* six of the ten primers employed produced only monomorphic bands .In these two species .opc-7 had generated highest number of polymorphic bands [six bands ] In other two congeneric species sets the primer opc-6 generated 12 bands in species of *Oedaleus* and 14 bands in both the species of *Chrotogonus*. In Chrotogonus speciesthere was greater band difference among the 14 bands generated by opc-06,of these12 bands were polymorphic. Where as in *O. abruptus* & *O.senegalensis* this primer generated 10 monomorphic & 02 polymorphic bands.

## DISCUSSION

Taxonomic classification based on morphological characters do not reveal the extent of genetic variation between the species .The genetic diversity within a species determines the adaptive potential for its survival[39]. Studies based on protein polymorphism provide an insight in to the genetic diversity in these species of grasshoppers. The femur muscle proteins used for electrophoresis had generated distinct banding pattern to represent polymorphism expressed by different genes. Electrophoresis has been used as a potential tool to differentiate cryptic species in micro organisms and other animal types[40,41]Genetic variations in natural population of Drosophila have been studied by electrophoretic analysis of proteins ,isozymes and allozymes[42,43,44],in all these electrophoretic studies whole body homogenates of adults are used. These studies have revealed that the extent of genetic variation was exhibited by certain factors like molecular weight as well its amino acid composition, even single amino acid substitution can be detected. It is a well established fact that the molecular mobility of proteins in an electric field

depends on their molecular weight, conformation and surface electrical charges. In the present study on three sets of six congeneric species clearly indicated vast difference in their muscle protein electromorphs .*A.exaltata* differed from *A.gigantica*by two extra protein bands and had one monomorphic band with molecular weight 11.42 KDa remaining bands differed in molecular weight as well mobility. The other set of congeneric species *O.abruptus* and O.*senegalensis* had more number of electrophoretic bands former had 12 bands and the latter had 14 bands all were polymorphic with difference in their molecular weights. *C.Oxypterus* and C. *trachypterus* slightly different from other grasshoppers in having least number of protein variants with difference of three bands in between, had 08 and 11 bands respectively none of the bands were monomorphic. Thus the absence of some bands in between the species is a striking feature and along with this extra bands could be used as an index of species ,with former or latter feature. Of the different amylase types, only Alpha amylases are found in insects[46] meant for digestion of carbohydrates the energy producing nutrients required for juvenile growth and to maintain adult longevity[47].Various isoforms of amylases are reported in different group of insects by several workers but amylase related studies in grass hoppers[48], compared to other insects are limited . It is assumed presence of enzyme isoforms provides an increased capability to insect to adapt to different food sources as well to overcome the amylase inhibitors of plant origin[49] thereby leading to diversification of insects in their food habit. The importance of isoenzyme data in elucidating systematic relationship is well understood[43]. The present analysis has revealed the existence of isomorphic forms of amylases with different molecular weights .*A.exaltata and A. gigantica* had two isoforms amylase different from those two isoforms of *O.abruptus* and *O .senegalensis*. remaining two species had three different iso amylases with different eletromorphic manifestation indicate the extent of variation in their genetic organization ..Variation in digestive enzymes and duplication of those enzymes may be an event of diversification of a given organism in the process of speciation . In this RAPD analysis, polymorphism was scored for the presence versus absence of particular band. The proportion of DNA fragments shared between species is correlated with the genetic divergence of DNA in terms of percentage[50].Our data demonstrates low level of similarity between *C.oxypterus* and *C.trachypterus* indicating high level of genetic divergence between these two.Whereasmore similarity exists between *O.abruptus* and *O.sensgalensis*, DNA of these two species showed only 19% divergence and it is least compared to other two congeneric set of grasshoppers. *A.exaltata* and *A.gigantica* revealed about 70% of DNA similarity and 30% polymorphism. The genetic system of *C.oxypterus* and *C.trachypterus* is unique in not responding to opb-18 is the index of the difference in its DNA from other congeneric species of genera *Acrida* and *Oedaleus* those produced varying number of bands for the same. Only opc-06 primer produced two similar bands but also generated 12 polymorphic bands reveals the number of variations for the given fragment of DNA existing within the species of *Chrotogonus*. Extra RAPD bands generated in between different congeneric species of grass hoppers for a given marker has been recorded. Species *A.gigantica* had two duplicated alleles each of opc-06,opc-and opd-o2 *,A.exaltata* has only two extra alleles of opai-05. Species *O.abruptus* had more number of duplications compared to *O.senegalensis* the former had two duplicate alleles each of opc-06,opc-07,opd-02 ,the latter had one extra copy of opb-18. Greater number of duplications were seen in *C.trachypterus* with four duplications each in opa-02,opa-09,opd-o2 ,extra alleles two each were there in opa-11, opai-05, where as opc-07 had three duplications and 970-11 had one extra gene. The congeneric species *C.xypterus* had four duplications each in opb-10 and opc -06.alleles

There fore the variation between the congeneric species of grasshoppers is not only due to polymorphism of the genomic DNA but also due to duplications to the vast extent. Such duplications help in expansion of the gene number within an organism and its further evolution This study aimed at the assessment of genetic variability between the congeneric species of grasshoppers using three different criteria has shown the usefulness of these technique to ascertain in between species variability successfully. Analysis has shown the existence of variation between the six congeneric species of grasshoppers at different percentage levels of polymorphism or monomorphism for a given marker .The existence of variation is crucial to a species or population to evolve itself in to the changes in environment around it or to any factor that brings the change in a gene; such a population genetic variability is expected to evolve in characters of morphology, physiology as well as behavior to cope with the environmental changes[50]. High level of genetic variation may arise for different reasons as observed in congenerieric species C*.Italicus, C.Wattenwijilianus, C.barbarus* have recorded higher level of genetic variation regard to state of outbreak, the species *C.barbarus* less outbreak species had higher level of genetic variation than the other two because in the non out break species the chance of breeding was more than the other two[6]. In the grasshopper *D. Pratensis* the genetic variation is found to be existing between higher and lower altitudes distribution[22]. Allozyme studies on two congeneric grasshoppers *S.lineatus* and *S. Stigmaticus*has not shown any variation in the genetic system[51].In morabine grasshoppers genetic variation as well as chromosomal variation is well documented, researchers have found that the geographical isolation leading to fixation of variations in the karyotype followed by separated contacts resulted in parapatric distribution of these[53]. Karyotypic variations associated with genetic differences in geographical population of congeneric species of Oedipodinae are recorded[4]but chromosomal variations deviating from normal karyotype are not reported by any workers for *oedaleus* species which show least polymorphism for genomicDNA in the present analysis . Though all the six congeneric species are sympatric, genetic variation is existing in between these grasshoppers at different levels and showed no evidence of hybridization in between as there were no intermediate forms found in natural population. This study exemplifies a phenomenon of occurrence of

genetically divergent sympatric species without habitat specialization within a common area accompanied by reproductive isolation. By the results ,it can be presumed these congeneric species have diverged by addition of genes and that has to be further examined. Earlier report on phylogenetic studies has shown that congeneric species *C.oxypterous* and *C.trachypterous* have taken their origin from different nodes of phylogentic tree[52] our unpublished work has revealed the other two sets of congeneric species originate from a common node in respective position of phylogenetic tree reflects the way how different congeneric species could be formed in course of their evolution.

**Table 2**

***SDS PAGE analyses of protein polymorphism in congeneric species of grasshoppers***

| SL NO | MOL WT | Rf VALUE | A1 | A2 | A9 | A10 | P21 | P22 |
|---|---|---|---|---|---|---|---|---|
| 1 | 10.34 | 0.92 | | | 1 | | | |
| 2 | 10.85 | 0.91 | | | | 1 | | |
| 3 | 11.42 | 0.86 | 1 | 1 | | | | |
| 4 | 12.39 | 0.85 | | | | | | 1 |
| 5 | 12.67 | 0.81 | | | | 1 | | |
| 6 | 13.43 | 0.80 | | | | | | 1 |
| 7 | 14.16 | 0.78 | | | | 1 | | |
| 8 | 17.35 | 0.74 | | | | | | 1 |
| 9 | 18.22 | 0.70 | | | 1 | | | |
| 10 | 18.57 | 0.69 | | | | 1 | | |
| 11 | 18.64 | 0.64 | | | | | 1 | |
| 12 | 20.66 | 0.61 | | | | 1 | | |
| 13 | 26.21 | 0.61 | | | | | | 1 |
| 14 | 27.11 | 0.60 | | | | | | 1 |
| 15 | 27.12 | 0.60 | | 1 | | | | |
| 16 | 27.23 | 0.60 | | | | 1 | | |
| 17 | 29.39 | 0.57 | | | 1 | | | |
| 18 | 29.68 | 0.57 | | | | | 1 | |
| 19 | 29.92 | 0.57 | | | | 1 | | |
| 20 | 30.88 | 0.56 | 1 | | | | | |
| 21 | 31.02 | 0.56 | | 1 | | | | |
| 22 | 32.88 | 0.55 | | | 1 | | | |
| 23 | 33.93 | 0.54 | 1 | | | | | |
| 24 | 36.29 | 0.54 | | | 1 | | | |
| 25 | 37.91 | 0.53 | | | | | | 1 |
| 26 | 38.99 | 0.46 | | | | 1 | | |
| 27 | 39.51 | 0.46 | | | 1 | | | |
| 28 | 40.27 | 0.46 | | | | | 1 | |
| 29 | 40.50 | 0.46 | | | | | | 1 |
| 30 | 40.59 | 0.43 | | 1 | | | | |
| 31 | 41.51 | 0.42 | 1 | | | | | |
| 32 | 42.83 | 0.41 | | | | 1 | | |
| 33 | 43.41 | 0.41 | | | 1 | | | |
| 34 | 43.45 | 0.41 | | | | | | 1 |
| 35 | 43.61 | 0.40 | 1 | | | | | |
| 36 | 46.03 | 0.38 | | | | 1 | | |
| 37 | 46.37 | 0.38 | | | 1 | | | |
| 38 | 47.03 | 0.36 | | | | | 1 | |
| 39 | 47.61 | 0.34 | | 1 | | | | |
| 40 | 52.98 | 0.33 | 1 | | | | | |
| 41 | 54.70 | 0.32 | | | | 1 | | |
| 42 | 63.61 | 0.30 | | | | | | 1 |
| 43 | 66.49 | 0.26 | | | | | 1 | |
| 44 | 67.00 | 0.25 | | | 1 | 1 | | |
| 45 | 67.50 | 0.23 | 1 | | | | | |
| 46 | 68.27 | 0.23 | | 1 | | | | |
| 47 | 79.03 | 0.22 | | | 1 | | | |
| 48 | 80.84 | 0.21 | | | | 1 | | |
| 49 | 86.00 | 0.16 | 1 | | | | | |
| 50 | 87.47 | 0.15 | | 1 | | | | |
| 51 | 87.32 | 0.15 | | | | | 1 | |
| 52 | 94.68 | 0.14 | | | | | | 1 |
| 53 | 95.36 | 0.13 | | | 1 | | | |
| 54 | 102.02 | 0.12 | | | | 1 | | |
| 55 | 104.15 | 0.10 | 1 | | | | | |
| 56 | 107.73 | 0.09 | | 1 | | | | |
| 57 | 108.91 | 0.08 | | | | | | 1 |
| 58 | 111.65 | 0.081 | | | 1 | | | |
| 59 | 113.83 | 0.078 | | | | | 1 | |
| 60 | 115.27 | 0.076 | 1 | | | | | |
| 61 | 166.95 | 0.01 | | | | | 1 | |

***A1- A. exaltata, A2 – A. gigantica, A9- O. abruptus, A10–O. senegalensis, P21- C. oxypterus, P22 – C. trachypteru***

**Table 3**
***Polymorphism of alpha amylase enzymes among six congeneric grasshoppers***

| SL NO | MOL WT | Rf VALUE | A1 | A2 | A9 | A10 | P21 | P22 |
|---|---|---|---|---|---|---|---|---|
| 1 | 36.58 | 0.54 | | | | 1 | | |
| 2 | 37.53 | 0.53 | | | 1 | | | |
| 3 | 41.93 | 0.4 | 1 | | | | | |
| 4 | 42.81 | 0.39 | | 1 | | | | |
| 5 | 44.28 | 0.38 | | | | 1 | | |
| 6 | 47.66 | 0.36 | | | 1 | | | |
| 7 | 56.23 | 0.33. | | | | | | 1 |
| 8 | 57.58 | 0.32 | | | | | 1 | |
| 9 | 59.30 | 0.28 | | | | | 1 | |
| 10 | 60.66 | 0.27 | 1 | | | | | |
| 11 | 63.96 | 0.26 | | | | | | 1 |
| 12 | 64.99 | 0.25 | | 1 | | | | |
| 13 | 78.80 | 0.20 | | | | | 1 | 1 |

***A1- A. exaltata, A2 – A. gigantica, A9- O. abruptus, A10–O. senegalensis, P21- C. oxypterus, P22 – C. trachypterus***

**Table 4**
***RAPD Polymorphism of genotypes of A. exaltata&A. gigantica***

| Primer | Monomorphic Bands | Polymorphic Bands | Total number Of bands | % of polymorphism |
|---|---|---|---|---|
| 970-11 | 3 | 0 | 3 | 0% |
| Opa-02 | 3 | 0 | 3 | 0% |
| Opa-11 | 2 | 0 | 2 | 0% |
| Opaa-09 | 5 | 0 | 5 | 0% |
| Opal-05 | 5 | 2 | 7 | 28% |
| Opb-10 | 7 | 0 | 7 | 0% |
| Opb-18 | 4 | 0 | 4 | 0% |
| Opc-06 | 4 | 4 | 8 | 50% |
| Opc-07 | 3 | 6 | 9 | 66% |
| Opd-02 | 1 | 4 | 5 | 80% |
| | 37 | 16 | 53 | |

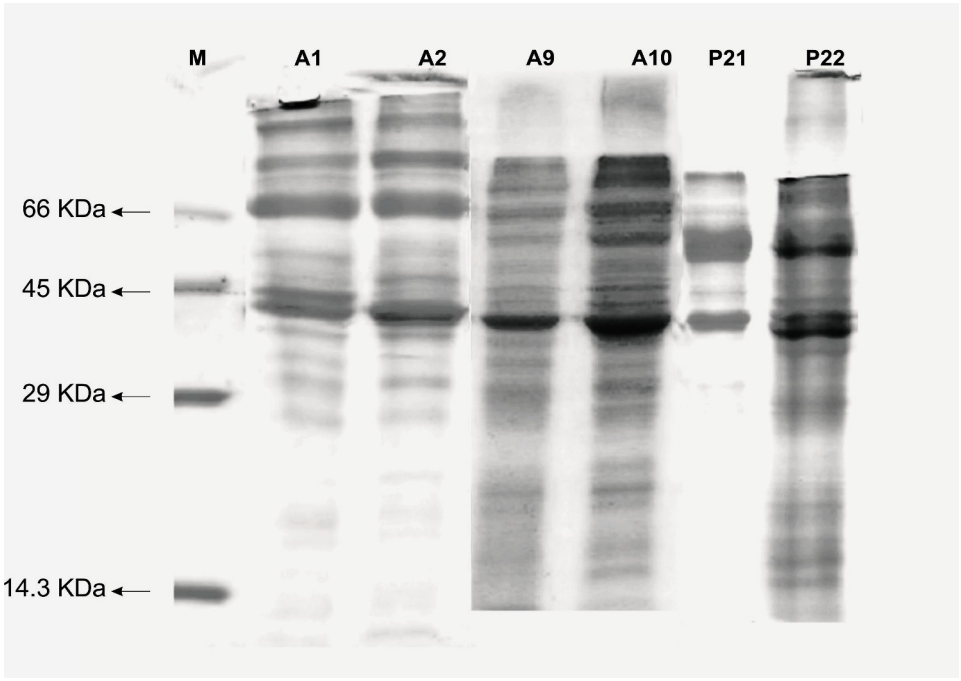
**Table 5**
***RAPD Polymorphism of genotypes of O.abruptus and O.senegalensis***

| Primer | Monomorphic Bands | Pol ymorphic Bands | Total number Of bands | % of polymorphism |
|---|---|---|---|---|
| 970-11 | 3 | 2 | 5 | 40% |
| Opa-02 | 2 | 0 | 2 | 0% |
| Opa-11 | 8 | 0 | 8 | 0% |
| Opa-09 | 4 | 0 | 4 | 0% |
| Opal-05 | 7 | 2 | 9 | 22.2% |
| Opb-10 | 5 | 1 | 6 | 16.6% |
| Opb-18 | 3 | 1 | 4 | 25% |
| Opc-06 | 10 | 2 | 12 | 16.6% |
| Opc-07 | 4 | 2 | 6 | 33.3% |
| Opd-02 | 5 | 2 | 7 | 28.57% |
| | 51 | 12 | 63 | |

**Table-6**
***RAPD Polymorphism of genotypes of C. oxypterus & C. trachypterus***

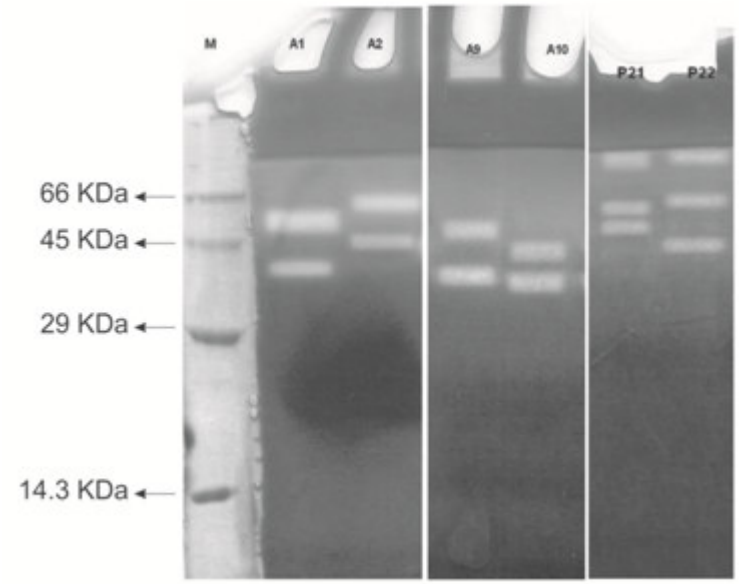
| Primer | Monomorphic Bands | P olymorphic Bands | Total number Of bands | % of polymorphism |
|---|---|---|---|---|
| 970-11 | 0 | 3 | 3 | 100% |
| Opa-02 | 0 | 6 | 6 | 100% |
| Opa-11 | 0 | 4 | 4 | 100% |
| Opaa-09 | 0 | 4 | 4 | 100% |
| Opal-05 | 0 | 8 | 8 | 100% |
| Opb-10 | 0 | 6 | 6 | 100% |
| Opb-18 | Nil | Nil | Nil | Nil |
| Opc-06 | 2 | 12 | 14 | 85.7% |
| Opc-07 | 0 | 3 | 3 | 100% |
| Opd-02 | 0 | 10 | 10 | 100% |
| | 2 | 25 | 55 | |

**Figure 1**
***SDS PAGE profile of proteins***




***A1- A. exaltata, A2 – A. gigantica***
***A9- O. abruptus, A10–O. senegalensis***
***P21- C. oxypterus, P22 – C. trachypterus***

**Figure 2**
***Electrophorosis profile of alpha amylases***



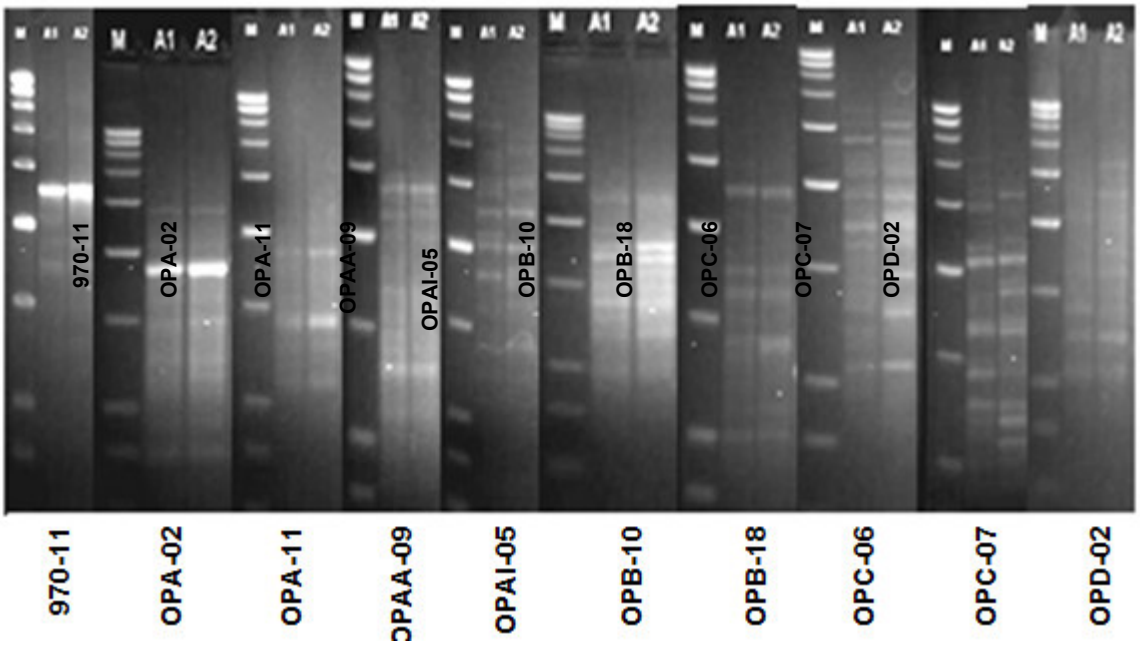

***A1- A. exaltata, A2 – A. gigantica***
***A9- O. abruptus, A10–O. senegalensis***
***P21- C. oxypterus, P22 – C. trachypterus***

**Figure 3a-c**

***Serially arranged RAPD Gel profiles amplified with ten primers 970-11, Opa-02, OPA-11, OPAA-09, OPA1-05, OPB-10, OPB-18, OPC-06, OPC-07, OPD-02 mention below each plate of congeneric grasshoppers.***

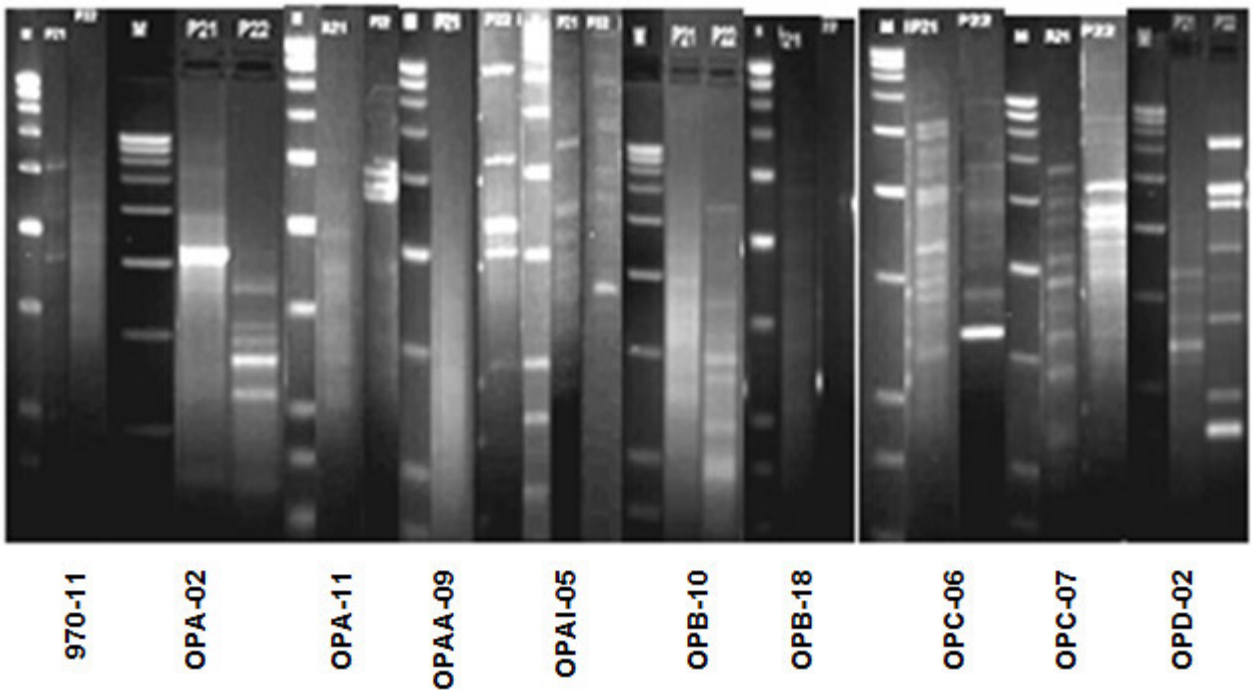
**(3a)**

***A1- A. exaltata, A2 – A. gigantica***

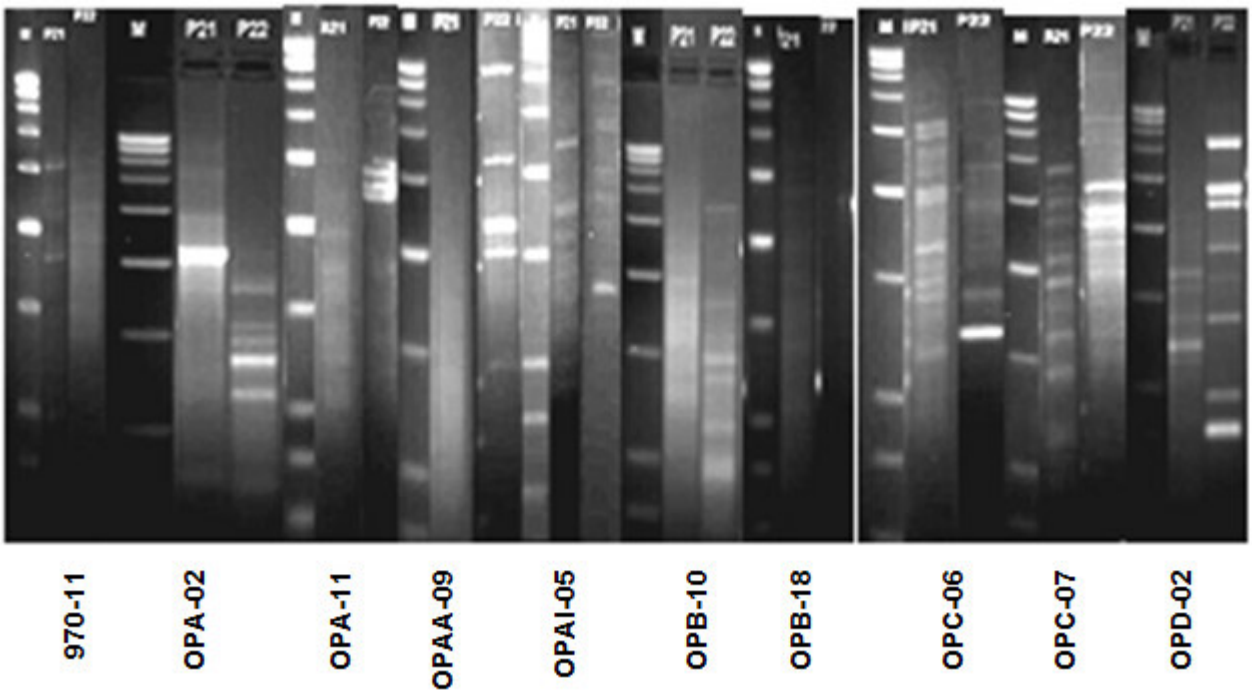



**(3b)**

***A9- O. abruptus, A10–O. senegalensi***



**(3C)**
***P21- C. oxypterus, P22 – C. trachypterus***




## CONCLUSION

All the six congeneric species of grasshoppers have greater genetic diversity among the congeneric group which is due to both polymorphism and duplication of genotype for protein, Alpha amylases and alleles of genomic DNA .In all probability the combined effect of genomic polymorphism and duplication might have played a role in their differentiation in to new species. These species might have originated through different ancestry as in case of *C.oxypterous* and *C.trachypterous* or from a common ancestor as in other cases. The way of attainment of evolutionary independence through reproductive isolation among these congeneric forms remain as an unanswered issue.

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