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Significance of haemolymph protein patterns in biosystematic studies of some grouse locusts (Tetrigidae: Orthoptera)

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Abstract. The grouse locusts (Tetrigidae) represent a group of small sized Caeliferan Orthoptera. These are considered to be primitive orthopterans related to Tridactylids on one hand to Acridids on the other. This family is represented in Indian subcontinent by about 90 species belonging to 35 genera. In the present study haemolymph protein profiles from 7 species from this family have been studied as an additional parameter to understand interrelationship amongst them. It is noted that the species *Paratettix dorsifer* and *Euparatettix personatus* belonging to the same subfamily Tetriginae show remarkable similarity in the haemolymph protein profile, thus justifying their classical taxonomical grouping. In case of subfamily Scelimeninae, however, of the 4 species studied 3 species viz *Eucriotettix flavopictus*, *Criotettix latifrons* and *Thoradonta pruthii* show a marked similarity, however *Eucriotettix harpago* shows a pattern closer to that observed in the subfamily Cladonotinae. This implies that though by classical taxonomic criteria the 4 species are close to each other this may not be a very natural grouping.

From this observation it becomes clear that help from additional parameters like protein profile studies, immunochemical studies, cytogenetic analysis, etc will prove to be very valuable for the real understanding of phylogenetic interrelations and evolution of this family in particular and the status of grouse locusts vis-a-vis the orthopteran insects in general.

Keywords. Haemolymph proteins; electrophoretic analysis; grouse locusts: Orthoptera.

1. Introduction

In the study of systematics of any group of organisms the classical and favourite approach for understanding its evolutionary and phylogenetic relationships, the most customary approach has been to resort to the morphological/anatomical methods. These represent the structural parameters. This 'museum' taxonomic approach is further variously supplemented by zoogeographical, geologically durational and even etho-ecological observations in interpreting the relationships. Undoubtedly, although such a practice is useful, sound and commonly adopted even today, it is not without certain limitations. It is for this reason, of late, that the various supplementary non-structural methods are being used. For example, the genetical, tissue-cultural and above all biochemical and immunochemical ones are being increasingly used as additional parameters to justify the phylogenetic relationships. Therefore, biosystematics can now be called a truely interdisciplinary approach with basic and applied potential.

Like in many other animal groups, insects can also be used in biosystematics. However, insects in general and Orthopterans in particular have been studied from such angles here and there (Blackith and Blackith 1968; Leone 1947; White 1951) only. However, as far as biochemistry of insect plasma/haemolymph proteins is concerned a first ever exhaustive review by Wyatt and Pan has appeared as late as in

1978. Herein it is seen that most of the work relates to the functional aspects of proteins.

At this Centre work is being done for quite sometime on grouse locusts (Tetrigidae: Orthoptera) covering etho-ecological, cytological (Godbole and Paranjape 1977; Paranjape *et al* 1987) and biochemical aspects with special reference to taxonomy and phylogeny of this interesting group of primitive orthopterans. The present paper attempts, by analysing haemolymph protein profile patterns of grouse locusts in particular, to evaluate the classical biosystematic status of this group.

2. Materials and methods

2.1 *Materials*

The insects used for the haemolymph protein studies were 7 species of grouse locusts (Fam. Tetrigidae), 1 species of grasshopper, viz *Chrotogonus* sps. (Fam. Pyrgomorphidae) and 1 species of cockroach, viz *Periplaneta americana* (Fam. Blattidae). Of the 7 species of grouse locusts *Potua sabulosa* belongs to the subfamily Cladonotinae; *Euscelimena harpago*, *Criotettix latifrons*, *Eucriotettix flavopictus* and *Thoradonta pruthii* belong to the subfamily Scelimeninae, while *Paratettix dorsifer* and *Euparatettix personatus* are included in the subfamily Tetriginae of the family Tetrigidae.

Excepting *P. sabulosa*, *E. flavopictus* and *T. pruthii* that were collected at Mahabaleshwar all other insect specimens were collected locally. Prior to their use for haemolymph protein separation the insects were maintained in the insect cages in the laboratory by simulating their natural habitat.

2.2 *Separation of haemolymph for electrophoresis*

0·1 ml of phosphate buffered saline (PBS) (with phenylmethylsulphonyl fluoride) was taken in an Eppendorf tube (1·5 ml). Another Eppendorf tube (0·5 ml) carried a small opening at the basal tip and was introduced into the 1·5 ml Eppendorf tube containing PBS. The hind leg of insect was cut at the base of the coxa and the insect was immediately put into the smaller tube, with its head pointing downwards. The larger tube was then capped. The tube was then centrifuged at 12,000 rpm for 10 min using Remi Micro Centrifuge RM 12C.

The inner tube was carefully removed. The haemolymph collected in the outer tube was maintained at 4 C till further use. This sample was later used for loading on the gel for electrophoresis. By this procedure sufficient haemolymph without cellular elements and other contaminants was available from a single insect.

2.3 *Electrophoretic separation of haemolymph proteins*

Polyacrylamide gel electrophoresis was employed to separate haemolymph proteins. The method used was according to the procedure described by Plummer (1977) and Work and Work (1972). A 7·5% gel system was employed with Tris-Glycine buffer pH 8·9. Gels were cast in glass tubes (10 cm long, 0·5 cm inner diameter). After

polymerization, preelectrophoresis was carried out for 30 min. The haemolymph samples (40 μl each) were loaded on the gels along with bromophenol blue (BPB) as marker dye. Electrophoresis was carried out, with a current 2 mA per tube, till marker dye reached the end of the gel. The gels were stained by Coomassie Blue for 1 h and then destained overnight.

2.4 *Calculation of percent relative electrophoretic mobility of protein bands*

Each protein band was assigned a value to denote its relative electrophoretic mobility as compared to the marker dye. For each tube mobility of the dye was equated to 100. Thus the per cent relative electrophoretic mobility (PREM) for protein bands was derived using the formula:

$$\text{Percent relative electrophoretic mobility} = \frac{\text{Distance (mm) travelled by a band from top of gel}}{\text{Distance (mm) travelled of BPB from top of gel}} \times 100$$

This information on the total number of bands on their PREM was used to compare the protein profile in different species.

3. Results

The observations made on the electrophoretic profiles of haemolymph protein from 7 species of grouse locusts, *Chrotogonus* sps. and *P. americana* are diagrammatically represented in figure 1. Total number of haemolymph protein bands observed in a species and the relative per cent electrophoretic mobility values calculated for each band are given in table 1. It is observed that the total number of protein bands vary between 6 and 13. Every species studied shows a set of protein bands varying in number from 3–6, which move considerably slowly (less than 10% REM). It is also seen that slow migrating bands are quite sharp and narrow as compared to some of the faster moving bands. This implies that the slow migrating proteins may be relatively less in concentration than the faster moving proteins in the haemolymph.

4. Discussion

The classical taxonomy on the basis of the structural and palaeontological information suggests certain affinities and phylogenetic relationships amongst the orthopteroid insects. An attempt has also been made to add to these the usefulness of etho-ecological characteristics with particular reference to the tetrigids (Paranjape *et al* 1987). As far as the Orthopteroid insects are concerned the classical approach can be briefly summarized as follows: (a) The cockroaches (subord. Blattodea = Blattaria) arose from Protoblattoidea (Carboniferous to Jurassic). The latter are either considered allied to or even part of the Protorthoptera that gave rise to Orthoptera (upper Carboniferous) represented by long and short horned grasshoppers, locusts, grouse locusts, crickets and so on. (b) Although cockroaches were formerly included in Orthoptera, these together with the mantids and on the basis of distinctive characters are now included in a separate order, viz Dictyoptera. In other words these are now considered as a natural group, not only from Orthoptera but

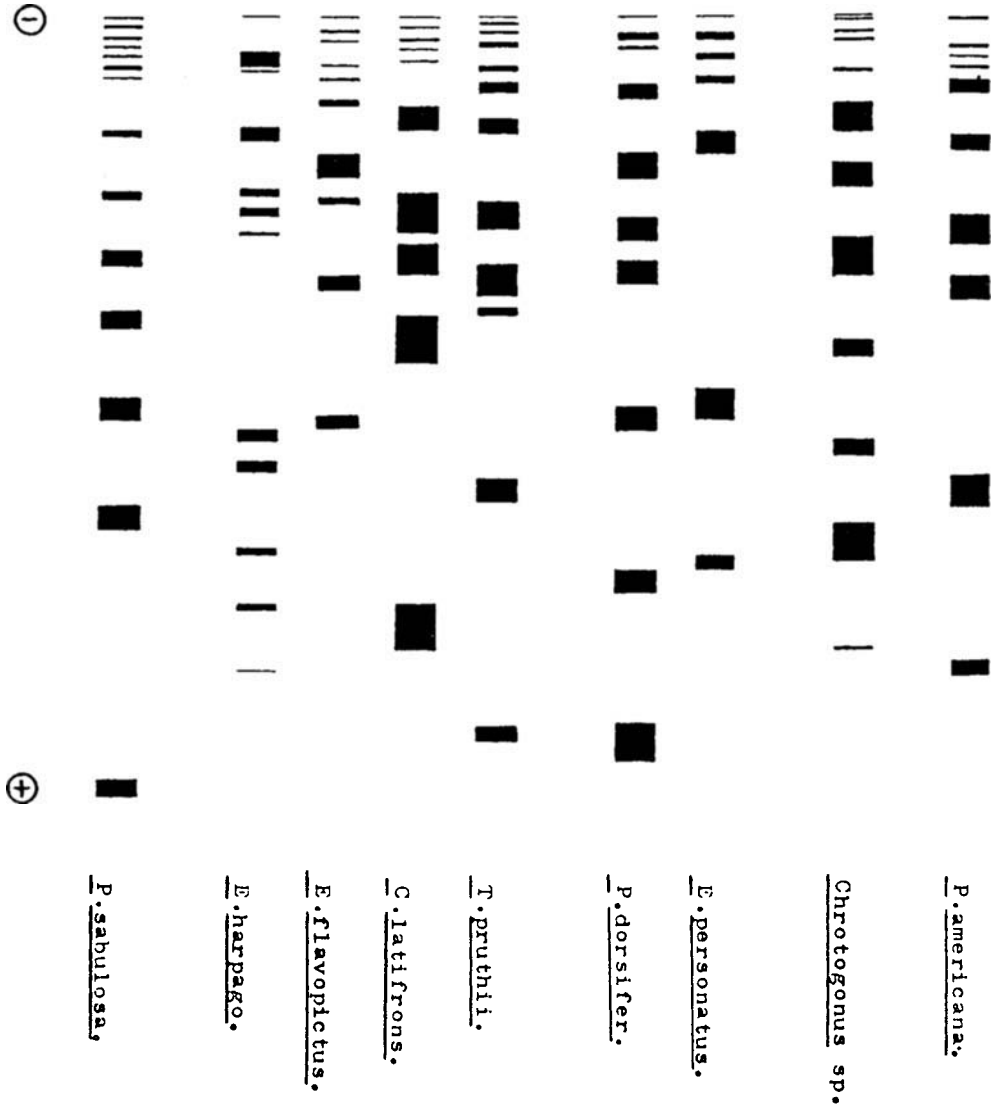

Figure 1. Diagrammatic representation of electrophoretic separation patterns of haemolymph proteins.

also from the Saltatorial Orthoptera and are considered closer to another Orthopteroid order, viz Isoptera. However, even today, cockroaches and grasshoppers can be considered related to one another as Orthopteran insects in an extremely broad sense of the term and their respective orders are definitely included in Orthopteroid insect orders. (c) Amongst Orthoptera and especially so in the Saltatorial Orthoptera the grouse locusts belonging to Tetrigidae are considered primitive and related to Tridactylidae (Pygmy mole and sand crickets) on one hand and Acrididae (short-horned grasshoppers and locusts) on the other (Hubbell 1978). (d) Of the 3 subfamilies from the family Tetrigidae studied by us the Cladonotinae seems to be the primitive,

Table 1. PREM of haemolymph protein bands.

Species \ Band No.	1	2	3	4	5	6	7	8	9	10	11	12	13
P. sabulosa	1·35	2·70	4·05	5·13	6·75	8·10	14·85	22·95	31·05	37·80	49·95	63·45	95·85
E. harpago	5·56	6·95	15·29	22·24	25·02	27·80	52·82	56·99	68·11	75·06	83·40		
E. flavopictus	1·56	3·12	6·24	7·80	10·92	18·72	23·40	34·32	51·48				
C. latifrons	1·41	2·82	4·23	5·64	12·69	25·38	31·02	45·12	77·55				
T. pruthii	1·16	1·74	3·48	5·80	9·28	13·92	25·52	33·64	37·12	60·32	91·64		
P. dorsifer	2·70	4·05	9·45	18·90	27·00	32·40	51·30	71·55	91·80				
E. personatus	2·66	5·32	7·98	15·96	49·21	69·18							
Chrotogonus sps.	0·67	1·99	2·66	6·65	13·30	19·95	30·59	42·56	54·53	66·50	79·80		
P. americana	3·69	4·92	6·15	8·61	15·99	27·06	34·44	60·27	82·41				

with the Scelimeninae having an intermediate position and the Tetriginae related more closely to Acrididae.

The current studies on the haemolymph protein patterns in Orthopteroid insects such as cockroach, grasshopper and grouse locusts generally support the above mentioned phylogenetic relationships. (i) It is observed that the haemolymph protein profile in a cockroach and in a grasshopper shows considerable similarity. (ii) The 7 species of grouse locusts, all representing the family Tetrigidae, show more or less similar profile where the PREM varies between 0·67 and 95·85. (iii) The only member of the subfamily Cladonotinae, viz *P. sabulosa* shows maximum number of bands and also the fastest moving band. (iv) Among the 4 species from the subfamily Scelimeninae, 3 species viz *E. flavopictus*, *C. latifrons* and *T. pruthii* show remarkably similar protein profile, while the fourth species *E. harpago* shows considerable deviation from the remaining 3. (v) Two species included in the subfamily Tetriginae, viz *P. dorsifer* and *E. personatus* show a remarkably similar protein profile.

These observations can be utilized to analyse the classical taxonomical organization of these species at the subfamily level. Placement of *P. dorsifer* and *E. personatus* in the same subfamily is supported clearly by very similar protein profiles. As far as the 4 members of the subfamily Scelimeninae are concerned, it is observed that haemolymph protein profile of *E. harpago* differs from the remaining 3 species. In addition, this protein profile seems closer to that observed in the member of the subfamily Cladonotinae. From this it may be concluded that inclusion of *E. harpago* in the subfamily Scelimeninae may not be a very natural grouping, but it may be placed separately in a group closer to Cladonotinae. Thus, it is clear that haemolymph protein profiles will prove to be very valuable additional parameter for the real understanding of phylogenetic interrelationships and evolution of this group.

From the point of view of cytogenetics, the Orthopteran group has been studied extensively. In the present case, the chromosome complements of the males of the species show distinct groups, one with $2n = 13$ chromosomes and the other with $2n = 17$ chromosomes, as far as the grouse locusts are concerned. The species with 13 as male diploid chromosome numbers are *P. sabulosa*, *E. harpago*, *T. pruthii*, *P. dorsifer* and *E. personatus* and those with 17 are *E. flavopictus* and *C. latifrons*. This information alone by itself is only of a limited value from the viewpoint of taxonomic studies. Additional studies such as chromosome banding, genomic DNA characterization, etc are required to apply them in studies related to establishment of

taxonomic interrelationships. Thus in the present situation, no correlation is established between protein profiles and karyotype.

As an extension to the biosystematic work using haemolymph protein profiles, it is proposed to undertake immunochemical studies on this group with a view to probe further into the affinities and the phylogenetic relationships at subgeneric and species levels. Studies on specific proteins/enzymes for electrophoretic variations are also undertaken as these would provide additional information from the point of view of establishment of interrelationships.

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