RUSSIAN ACADEMY OF SCIENCES
SIBERIAN BRANCH

BIODIVERSITY AND DYNAMICS OF ECOSYSTEMS IN NORTH EURASIA

VOLUME 1

Part 3: Molecular-genetic bases of biodiversity (animals and plants)

Part 4: Human genoms diversity in North Eurasia: evolutional, populational and ecological aspects

Novosibirsk, Russia
August 21-26, 2000

IC&G, Novosibirsk, 2000

AN ANALYSIS OF PHYLOGENY OF SHORT-HORN GRASSHOPPERS DIFFERING IN CHROMOSOME NUMBER AS DEDUCED FROM COMPARISON OF A FRAGMENT OF 16S mtrRNA GENE SEQUENCES

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Keywords: *grasshoppers, phylogeny, systematic, karyotype, gene sequence, mtrRNA 16S.*

Abstract

A 450-bp fragment of 16S mitochondrial ribosomal RNA genes of 18 species of short-horn grasshoppers of the *Acridinae* subfamily was sequenced. Using the reference data , a dendrogram of similarity was built. Its analysis demonstrated that the placements of these species in the generally accepted system does not consistently reflect their phylogeny. Comparisons of the placements of the species on the dendrogram with their cytogenetic features support the assumption that centric fusion of the chromosomes might have occurred repeatedly and independently of each other in the evolving subfamily.

Introduction

There are reasons to believe that the current system of organisms does not faithfully reflect the true phylogeny. This concerns grasshoppers of the *Acridinae* subfamily. Relationships among many tribes remain unclear, the monophyly of certain tribes and even genera, is debatable.

The employment of cytogenetic data was not very helpful in resolving the issues. Thus, in representatives of this subfamily, the diploid number was found to vary from 23 to 15, with the chromosome arm number being 23. A karyotype composed of 23 acrocentric chromosomes in males (24 in females) is considered to be basic, because this is the karyotype observed in the great majority of species of the *Acrididae* family, including representatives of the *Catantopinae* subfamily, thought to be the most ancient. The question is raised whether centric fusion of the chromosomes in the *Acridinae* subfamily occurred many times and independently in different phyletic lineages (Bugrov et al., 1991) or numerous chromosome divisions followed a single fusion (Hewitt, 1979).

The present study was undertaken to elucidate the phylogeny of species of the *Acridinae* subfamily with different chromosome number on the basis of comparisons in a fragment of 16S mtrRNA.

Material and Methods

Insects of 18 species of grasshoppers of various tribes of the *Acridinae* subfamily and one species *Ognevia longipennis* of the *Catantopinae* subfamily were collected in 1989–1999. Isolated testes were fixed in 3 ethanol: 1 glacial acetic acid, then stored in 70% ethanol in refrigerator.

A fragment of the mitochondrial 16S ribosomal gene was isolated by polymerase chain reaction (PCR). The mtDNA primers used for amplification were 252 – CCGGTCTGAACTCAGATCACGT and 253 – CGCCTGTTTATCAAAAACAT. PCR amplification products were sequenced as described by Sanger et al. (1999).

Data concerning three other *Acridinae* species (*Stauroderus scalaris*, *Chorthippus biguttulus* and *Gomphocerus rufus*) and the cricket species (*Gryllus fultoni*) from the NCBI database were added to the final analysis.

Sequences were aligned using the CLUSTAL X computer program. Similar dendrograms were built using PHILIP program package: DNAPARS, the method of maximum parcimony; DNAML, the method of maximum likelihood, and FITCH, the method of matrix distances. The robustness of the dendrogams was assessed by the SEQBOOT program after generating 1000 bootstrap replicates and building a consensus dendrogram using the CONSENSE program.

Results and Discussion

The fragment length in different species varied in the range of 439–443 bp. This was due to deletion-insertion, closely associated with the most variable region of mtrRNA. The studied sequence was found to be AT rich, the percentage of AT-pairs varied from 68,9 to 71,2% nucleotides. The same nucleotides were identified at 250 positions in all the examined species, including cricket.

Pairwise comparison of the sequences demonstrated differences in a range of 0,2–9,8%. The dendrogram of similarities for the fragment of the mtrRNA sequences was built using the DNAPARS program is shown in fig. A notable point is the position of species of the *Chorthippus* genus at significantly different branches of the dendrogram. This can be an evidence of polyphyly of the genus. It should be noted that attempts are made to divide the *Chorthippus* genus into several independent genera. A similar pattern is observed also for the representatives of the *Gomphocerina* subtribe. We suppose that this can also reflect the polyphyletic composition of this particular grasshopper group.

Comparisons of the positions of the *Acridinae* species on the dendrogram with their cytogenetic features do not provide an unequivocal answer to the question of chromosome number evolution in grasshoppers. However, it appears more likely that centric fusion occurred repeatedly and independently from each other in the evolving *Acridinae* subfamily.

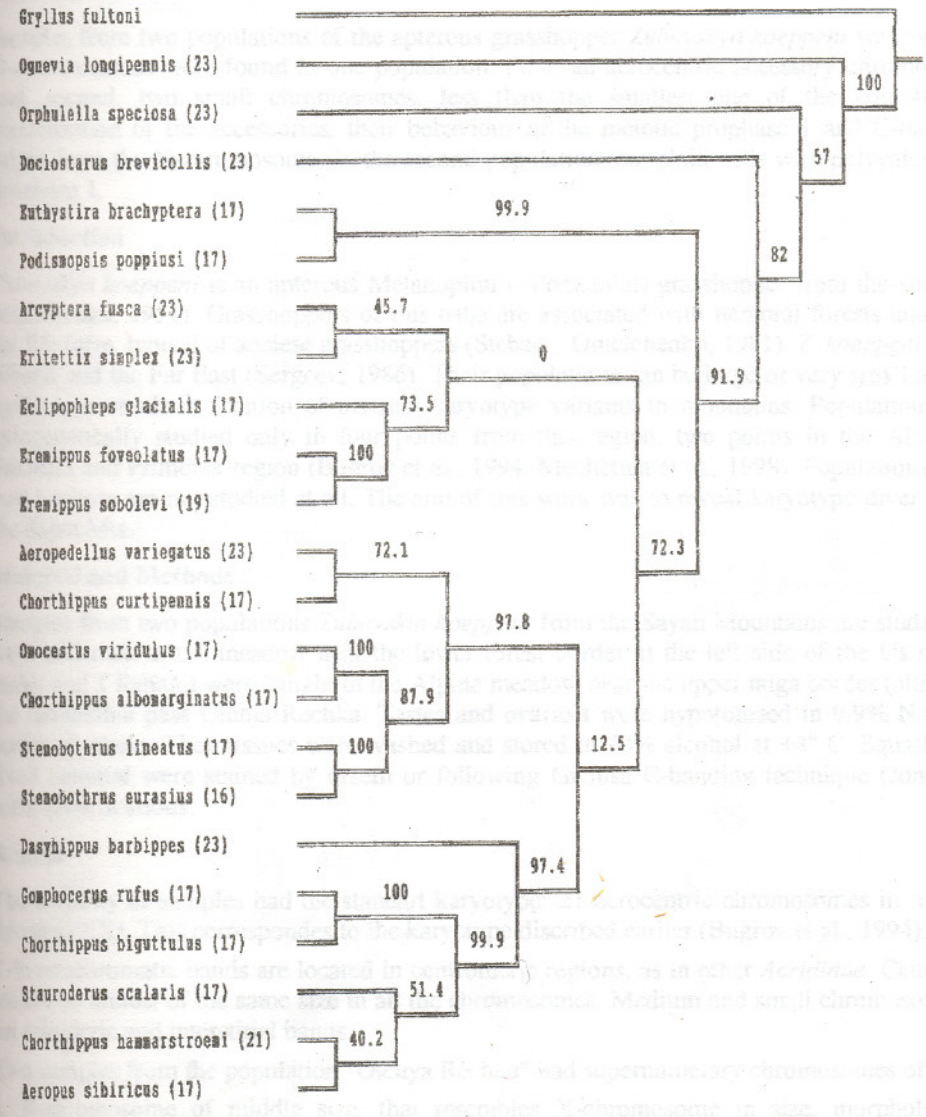

Fig. A dendrogram for similarities of 16S mtrRNA fragment sequences of 22 grasshoppers species and a species of cricket. Bootstrap values are at the base of the branches. Chromosome number in male diploid set are in parentheses.

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