GENETIC STUDIES ON MEDFLY POPULATIONS AND RELATED SPECIES

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Abstract

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Multilocus enzyme electrophoresis (MLEE) and random amplified polymorphic DNA were used to detect genetic markers in *Ceratitis capitata*. The authors employed both types of markers (1) to study the genome organization of the medfly, (2) to determine the level of intraspecific genetic diversity, and (3) to understand the evolution of the geographical populations. Sterility and high mutation rates in interstrain crosses were observed in *C. capitata*, reminiscent of hybrid dysgenesis in *Drosophila*, and may represent the activation of mobile elements, useful for medfly transformation. The biochemical, genetic and molecular characterization of the enzyme alcohol dehydrogenase clarified the peculiarity of this selectable system, compared with that of *Drosophila*, and revealed a surprisingly high sequence variability in medfly populations. The phylogenetic relationships between *C. capitata* and other Tephritidae species of economic importance were analysed by the MLEE approach.

1. INTRODUCTION

Genetic information on insect pest populations is of great importance to applied entomology, in particular to the long term success of sterile insect technique (SIT) programmes. Crosses between populations from different areas may reveal incompatibilities between geographical 'races'. The major fruit pest *Ceratitis capitata* (Mediterranean fruit fly) has not given rise to taxonomic problems, as there is an apparent morphological uniformity within this species. However, *C. capitata* is in reality a complex of several genetically differentiated populations [1].

Genetic analyses of *C. capitata* and related species may also offer a suitable approach to clarify the systematics and phylogeny of the family Tephritidae. Tephritid taxonomy is also of crucial importance to applied entomology [2]. Incorrect identification or the inability to recognize distinct populations can have drastic and costly consequences for pest control management.

Multilocus enzyme electrophoresis (MLEE), random amplified polymorphic DNA (RAPD), and polymorphism detected at single copy nuclear DNA (scnDNA) represent different methodological approaches applied to the study of *C. capitata* genetics. The knowledge of different aspects of variability extends and deepens our understanding of the biology of the medfly and of its populations, and thus improves the prospects of recognizing factors suitable for exploitation in planning new effective control methods.

2. GENOME ORGANIZATION

The genetic segregation of about ninety RAPD polymorphisms generated with six primers has been studied. These RAPD fragments segregate as expected for simple Mendelian inheritance of dominant markers [3]. Their linkage relations have been assessed, and they have been assigned to the five autosomal linkage groups of *C. capitata*, previously marked with morphological and biochemical markers [4]. The combining of morphological, biochemical and molecular markers has enriched the genetic maps of the medfly.

3. HYBRID DYSGENESIS

A syndrome of abnormal genetic effects, which resembles *Drosophila* hybrid dysgenesis, is present in *C. capitata* [5]. This syndrome includes high frequency of partial or complete female gonadal sterility, chromosomal rearrangements (bridges and fragments) at male meiosis and instabilities at the white-eye (*w*) locus. This syndrome was observed in hybrids of *C. capitata* when strains of different origin were mated. The morphology of the undeveloped ovaries recovered in the medfly is apparently very similar to the gonadal dystrophy (GD) which in *D. melanogaster* is associated with the P–M and *hobo* mediated dysgenic syndromes (Fig. 1). The amount of gonadal sterility that can be observed in medfly hybrids depends on the parental strains used, which exhibit specific differences in their inducing abilities. In the considered interstrain combinations there appears to be quantitative variation in the effect of temperature on GD sterility. The highest level of sterility occurs at 25°C. The pattern of abnormal traits observed in medfly hybrids appears to be the phenotypic expression of a rather complex interacting dysgenic system of inducer and

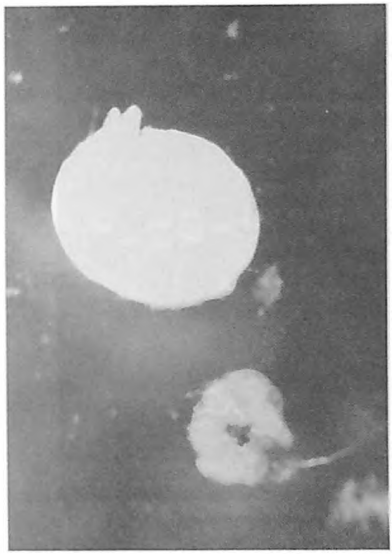


FIG. 1. Illustration showing a normal (top) and a defective ovary of C. capitata, *recovered in a dysgenic cross.*

suppressor effects; probably more than one system is activated in the considered crosses. Genetic instability at the *w* locus has also been observed. Genetic data seem to support the hypothesis that insertional mutations in the *w* locus cause the observed genetic instability [6].

4. GENETIC DIVERSITY AND EVOLUTION

Four African populations of *C. capitata* (Kenya, Libyan Arab Jamahiriya, Morocco, Réunion), five Mediterranean populations (Chios, Crete, Italy, Procida, Sardinia) and two from Guatemala and Hawaii, respectively, were examined by MLEE for genetic variability at 26 enzyme loci. Wright's F_{ST} and Slatkin's estimates, together with tree representations, were used to compare the African populations with the derived ones. Parameters using gene frequencies (F_{ST}, D, Nm) indicate the presence of substantial geographical heterogeneity correlated with the dispersal of the medfly from its source area (Sub-Saharan Africa) to the periphery. Significant

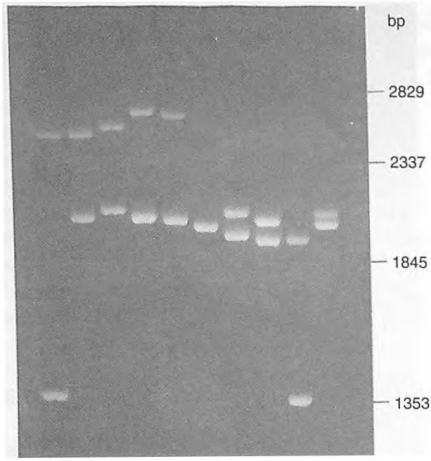


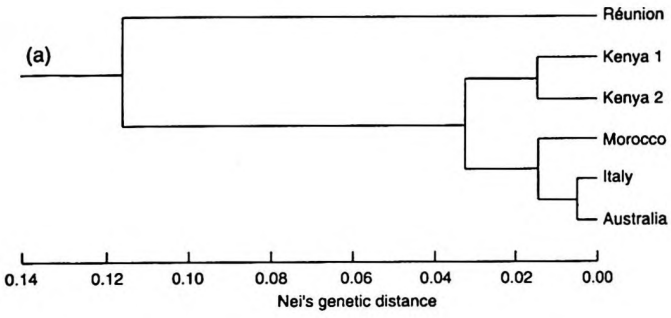
FIG. 2. Adh_1 *first intron size variants isolated from wild Kenyan populations of* C. capitata, *using specific polymerase chain reaction primers.*

estimates of gene flow between the African and derived populations support the hypothesis of recent colonization by *C. capitata* [1]. The analysis suggests that the genetic structure of medfly populations is correlated with the historical events of their colonization. In addition, seasonal and annual variation has been found in a Mediterranean population (Procida island), which chiefly involves the *Mpi* locus [7].

The results obtained by the RAPD markers are significantly correlated with those obtained with MLEE (Fig. 2). They are in agreement with the general trend of decreasing variability from African populations towards peripheral and laboratory ones. However, the RAPD technique reveals larger amounts of genetic variation than conventional MLEE, and can improve discrimination within and between populations. The extremely variable RAPD patterns in medflies from within the ancestral range can represent DNA fingerprints [3, 8].

5. GENETIC, BIOCHEMICAL AND MOLECULAR ANALYSIS OF ADH

The alcohol dehydrogenase (ADH) enzyme system of *C. capitata* has been analysed at the genetic, biochemical and molecular levels. Two ADH proteins exist in the medfly, differing in several features such as pI, tissue localization and



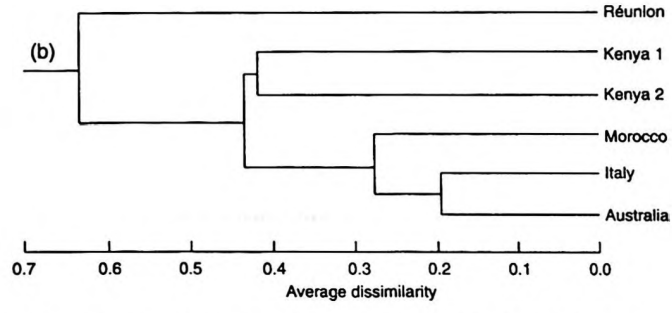


FIG. 3. Dendrograms of geographical populations of C. capitata, *derived from (a) genetic distances evaluated by MLEE and (b) dissimilarity values obtained by RAPD.*

developmental profile [9]. They are encoded by two tightly linked genes (Adh_1 and Adh_2) within 0.49 cmo on the left arm of the second chromosome [10]. These results suggest that the ADH isozymes have arisen by gene duplication. This hypothesis is supported by the immunochemical similarity of the two purified proteins [11].

The cloning of *Adh* genes has been achieved by generation of cDNA probes based on the amino acid sequence of ADH-1 [12]. The molecular characterization of the medfly *Adh* genomic region showed that the first intron of the Adh_1 gene is unexpectedly long, and contains a *mariner*-like element. Variation in the size of this

intron was detected in clones of a genomic library derived from a laboratory strain (Benakeion). Genetic crosses demonstrated codominant Mendelian inheritance for the variants present in this strain.

Further polymorphism at the first intron of Adh_1 has been detected using specific polymerase chain reaction (PCR) primers in a number of wild *C. capitata* populations. Populations from Kenya, within the supposed ancestral range of the species, contain an extremely high level of polymorphism for intron size (Fig. 3). Seventeen or more alleles have been found, each falling into one of three distinct size categories. The number of alleles in each population declines rapidly towards the periphery of the global distribution of *C. capitata*. In the majority of the populations analysed only two alleles, of 2060 and 2600 bp (corresponding to the 1.917 and 2.432 bp introns, respectively), are present. Analysis of the inheritance of the intron polymorphs by means of genetic crosses has confirmed their allelic status. Hybridization of the 1.4 kb intron PCR product to a Southern blot of PCR products from a number of populations indicates that there is a high level of homology between the fragments. Restriction maps of the alleles from the different populations are being constructed. Initial results confirm the presence of a high degree of homology between the fragments. Sequences of the most frequent alleles from the three size categories are nearing completion. It is noteworthy that, in some ancestral populations, intron variants have been found in which the *mariner*-like element is absent. If a functional element should be detected in medfly populations, it will represent the first active transposable element in *C. capitata*.

6. GENETIC AND EVOLUTIONARY RELATIONSHIPS AMONG RELATED SPECIES

There is no generally accepted classification of the Tephritidae, and the systems used are mainly based on morphological traits. Previous MLEE data were inconsistent with the taxonomic classification [13]. Twenty-four orthologous loci (212 alleles) were selected to elucidate the genetic divergence and the phylogenetic relationships among 11 taxa from six genera of the Tephritidae family *(Ceratitis, Anastrepha, Trirhithrum, Capparimyia, Rhagoletis, Bactrocera).* Nei's, Roger's and Cavalli-Sforza's genetic distance values were computed. Two methods of tree construction were employed. The first utilized the unweighted pair group method analysis (UPGMA), the other used the optimality criteria of Fitch and Margoliash [14]. Bootstrap resampling of loci from the original data set [15] was used to test the robustness of the tree topology derived from each method. All trees give the same topology (see Fig. 4). The two *Bactrocera* species, *B. oleae* and *B. dorsalis*, present the greatest range of distances. A common feature of the distance trees is the separation of *Ceratitis rosa* and the *Anastrepha* species *(A. suspensa, A. ludens,*

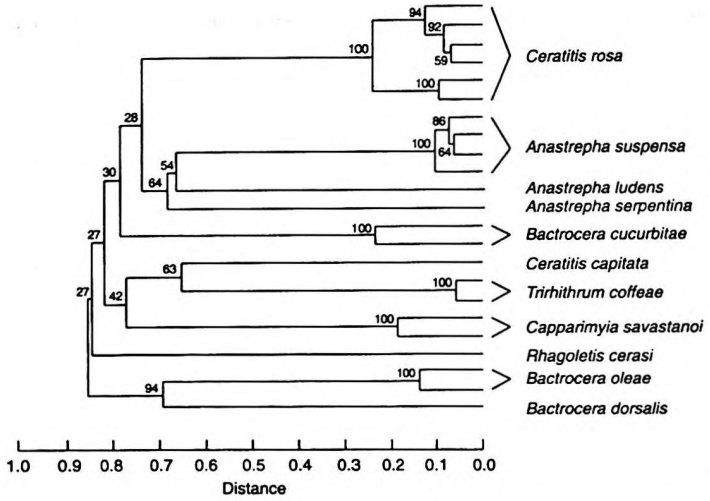


FIG. 4. Consensus tree showing relationships between several Tephritidae species, deduced from the Cavalli-Sforza and Edwards chord distances.

A. serpentina) into a single cluster, and the species *C. capitata*, *Trirhithrum coffeae* and *Capparimyia savastanoi* into another group. The origin of the lineages leading to these two groups of species from two different internodes indicates the possibility of two different ancestors. These findings indicate the need to reconsider the evolutionary relationships between these species.

7. CONCLUSIONS AND PROSPECTS

The biochemical and molecular approaches have provided information adequate to outline the amount and distribution of the genetic variability of the species *C. capitata*. Attention has mainly been centred on the well established multilocus enzyme electrophoretic method for mapping purposes, for assessing the population structures and for determining the phylogenetic relationships between tephritid species. However, the recent use of alternative and/or integrative molecular methods (RAPD and scnDNA) has revealed an additional ample reservoir of cryptic variability in geographical populations of the medfly. Fixed biochemical loci and

DNA fingerprints detected in certain genotypes can represent diagnostic characters for discrimination of medfly populations, and can help elucidate the route of the colonization processes.

The existence of hybrid dysgenic-like phenomena and genetic instability of the white-eye phenotype in *C. capitata* are highly indicative of transposable element activity, and may ultimately provide tools for the development of a germ line transformation system for this species along the lines of the model of *D. melanogaster* [16].

The genetic analysis of the phylogenetic relations of *C. capitata* with several other Tephritidae species can help clarify the controversies of the classification systems based on morphological traits.

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