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Allozyme divergence and phylogenetic relationships among species of tephritid flies

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Multilocus enzyme electrophoresis data from 24 orthologous loci (212 alleles) were used to infer the genetic similarities between 11 Tephritidae pest species from the *Ceratitis, Trirhithrum, Capparimyia, Bactrocera, Anastrepha* and *Rhagoletis* genera. Within some of the considered species, different degrees of genetic variability were demonstrated, which appear to be related to zoogeography and to the biological traits peculiar to each species. Nei (1978) and Cavalli-Sforza & Edwards (1967) genetic distances were used to express the genetic divergence and to infer phylogenetic relationships among the species. The UPGMA clustering algorithm and the optimality criteria of Fitch & Margoliash (1967), with (KITSCH) and without (FITCH) the tree constrained to have contemporary tips, were used. All the methods indicate the same clusters of species. One cluster is composed of *Ceratitis capitata, Trirhithrum coffeae* and *Capparimyia savastanoi,* another is composed of *Rhagoletis cerasi, Bactrocera dorsalis and Bactrocera oleae*. A further loose cluster is comprised of *Ceratitis rosa* and *Anastrepha* spp. The congruence between electrophoretic phylogeny and morphological classification is discussed. Our analysis also elucidated cases, within the *Ceratitis* and *Bactrocera* genera, of interest from the evolutionary point of view, where allozyme dendrograms do not correlate well with morphological taxonomic relationships.

Keywords: cluster analysis, genetic distances, genetic variability, multilocus enzyme electrophoresis, Tephritidae.

Introduction

The family Tephritidae, the true fruit flies, is one of the most economically important dipteran families. Most of the species are pests of soft fruits, including many commercial fruits. Because of their economical importance, detailed research has been carried out on their physiology, ecology, genetics and evolution (for comprehensive reviews see Robinson & Hooper, 1989; White & Elson-Harris, 1992). Fruit flies are represented in all world regions, but the major pest genera, *Ceratitis*, *Bactrocera*, *Rhagoletis* and *Anastrepha*, each have limited natural distributions. However, mankind has played an important part in altering the distribution of some of the more polyphagous species, as well as certain oligophagous species, by extending the range of their plant hosts.

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The question of why only a few species have become major pests has been approached by studies on zoogeography (Maddison & Bartlett, 1989) and on analysis of the life history strategies that each species has evolved (Fletcher, 1989). The degree of phenotypic plasticity that each species has retained has been related to the unpredictability of its habitat, in terms of resource availability in time and space. The majority of these flies belong to the *r*-strategists; however, most species are at the low end of the spectrum. Polyphagous multivoltine tropical and subtropical species such as *Ceratitis capitata*, *Bactrocera dorsalis* and *Anastrepha ludens* have typical *r*-characteristics, whereas oligophagous and stenophagous/monophagous species such as *Bactrocera oleae*, *Anastrepha fraterculus* and *Anastrepha suspensa* have life history characteristics that exhibit a mixture of *r*- and *K*-traits.

Studies on distribution and host relationships have evidenced a prolific speciation resulting in a large

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number of species (>4000) and in a morphological overlap among the higher taxa (White & Elson-Harris, 1992). In addition, a large number of sibling and cryptic species are known in *Rhagoletis* (Bush, 1966, 1969), and the *Anastrepha fraterculus* and *Bactrocera dorsalis* complexes (White & Elson-Harris, 1992). In this context, *Rhagoletis* flies (*R. pomonella* species complex) have been at the centre of a discussion on sympatric speciation (Bush, 1966, 1969; Feder *et al.*, 1988, 1990). Electrophoretic studies of genetic differentiation have provided a powerful tool for the analysis of sympatric speciation and phylogeny in *Rhagoletis* (Berlocher & Bush, 1982; Berlocher *et al.*, 1993), for resolving species complexes in the *A. fraterculus* group (Malavasi & Morgante, 1983; Steck, 1991) and in population genetic analysis in *C. capitata* (Gasperi *et al.*, 1991; Malacrida *et al.*, 1992; Baruffi *et al.*, 1995). All of these studies suggest the great potential of genetic approaches for biogeographical and phylogenetic analysis of Tephritidae flies.

Despite years of taxonomic work, no satisfactory classification and phylogeny exist for these flies (White, 1989). The most common problems are synonymy, homonymy and the establishment of supra- specific groups based on questionable characters. This situation may be the consequence of the large size of the group, the regional nature of most taxonomic work and the fact that it has been hard to find sound taxonomic characters. An immunological (Kitto, 1983) and, more recently, a molecular approach (Han & McPheron, 1994) have been proposed to help the creation of a phylogenetically based classification for the Tephritidae.

In this paper we have attempted to elucidate the relationships among species of the related genera *Ceratitis, Thirhithrum, Capparimyia, Bactrocera, Anastrepha* and *Rhagoletis* using the multilocus enzyme electrophoretic approach (MLEE). The outcome of variability estimates and genetic similarities among the species are discussed in relation to biological characteristics, zoogeography and to the current taxonomic position of each of the considered species.

Materials and methods

Species samples

A total of 23 samples from 11 tephritid species were analysed for electrophoretic variation (Table 1). Each sample was composed of 30–50 flies.

Of the 11 species, five, i.e. *Ceratitis capitata, Ceratitis rosa, Trirhithrum coffeae, Capparimyia savastanoi* and *Bactrocera oleae*, are represented by samples collected from the wild. Samples of the species *C. capitata, C. rosa* and *T. coffeae* are from sympatric populations and were collected together on the same host (coffee berries) in their putative original area (Kenya; White & Elson-Harris, 1992). Wild samples from *C. rosa* were also collected in Réunion Island.

The remaining six species listed in Table 1 were from laboratory colonies.

Electrophoretic procedures

We performed electrophoretic analysis using cellulose acetate gels (Cellogel) adopting the procedures described in Gasperi *et al.* (1991).

The following 24 orthologous enzyme loci (212 alleles) were analysed in each single fly of the 23 samples: *Pgm, Hk_1, Hk_2, Pgi, Pgk, Zw, Pgm, αGpdh, $Acon_1$, $Acon_2$, Idh, Fh, Mdh_1, Mdh_2, Ak_1, Ak_2, Adh_2, Aox, Got_1, Got_2, Gpt, Had, Me, Mpi*. All the considered loci produced consistently interpretable banding patterns in all species studied and the determination of isozyme locus homologies was unambigous.

For each of these biochemical loci, the electrophoretic banding patterns of *C. capitata* were used as a standard because electrophoretic variation in this species is well documented (Gasperi *et al.*, 1991; Malacrida *et al.*, 1992; Baruffi *et al.*, 1995).

Data analysis

For each species sample we calculated standard measurements of polymorphism and heterogeneity: *P* (proportion of polymorphic loci), *A* (average number of alleles per locus) and *H* (average proportion of heterozygous individuals).

The PHYLIP computer package (Felsenstein, 1993) was used for the cluster analysis of the 23 samples. We calculated the genetic distance between each pair of samples using both Nei (1978) and Cavalli-Sforza & Edwards (1967) genetic distances. Two different methods of tree construction were employed. The first utilized the unweighted pair group method using an arithmetic average (UPGMA) clustering algorithm; the other used the optimality criteria of Fitch and Margoliash (1967), first with (KITSCH), and then without (FITCH) the tree constrained to have contemporary tips (Felsenstein, 1984).

We also applied the bootstrap test (Efron, 1982) for assessing the robustness of each node in the tree topology. For this purpose we analysed 100 replicates of bootstrap resampling of the original data

matrix and constructed a consensus tree from the 100 bootstrapped trees obtained.

Results

Parameters of genetic variability

In Table 2 we show the levels of genetic variability estimated in the wild samples of *C. capitata, C. rosa, T. coffeae, C. savastanoi* and *B. oleae* species considering all 24 loci.

Among the three sympatric samples from the native range of *C. capitata, C. rosa* and *T. coffeae* we observed different levels of variability. *Ceratitis capitata* is the most polymorphic ($\overline{H} = 0.138$) whereas *T. coffeae* appears to be the least variable ($\overline{H} = 0.060$). Comparable low levels of variability were found for

Table 1 Origin and date of collection of the samples from the considered Tephritidae species

Species	Origin	Date of collection
Ceratitis capitata		
1 sample*	Kenya (Kabete, Machacos, Ruiru)	1984–92
Ceratitis rosa		
4 samples	Kenya (Kabete, Machacos, Ruiru)	1984–88
2 samples	Réunion Island	1989
Trirhithrum coffeae		
2 samples	Kenya (Kabete, Machacos, Ruiru)	1988
Capparimyia savastanoi		
2 samples	Italy (Pantelleria Island)	1987–88
Bactrocera oleae		
2 samples	Italy (Liguria, Apulia)	1990–93
Bactrocera cucurbitae		
2 samples	Lab. colonies from: Col. Agric., Okinawa (Japan), and USDA, Honolulu (Hawaii)	1991–92
Bactrocera dorsalis		
1 sample	Lab. colony from USDA, Honolulu (Hawaii)	1992
Anastrepha suspensa		
4 samples	Lab. colony from Dept. Agr. Gainesville (Florida)	1990–93
Anastrepha ludens		
1 sample	Lab. colony from Tapachula (Mexico)	1990
Anastrepha serpentina		
1 sample	Lab. colony from Tapachula (Mexico)	1990
Rhagoletis cerasi		
1 sample	Lab. colony from Sissac (Switzerland)	1993

*Gene frequencies in this sample result from the weighted average of 11 samples from the Kenya population. We decided to group them because they appear very similar to each other when considered singly.

Table 2 Parameters of genetic variability in the wild samples of *Ceratitis capitata*, *Ceratitis rosa*, *Trirhithrum coffeae*, *Capparimyia savastanoi* and *Bactrocera oleae*

Species	Origin	$\overline{A} \pm$SD*	$\overline{P} \pm$SD*	$\overline{H} \pm$SD*
C. capitata	Kenya	3.300	0.417	0.138
C. rosa	Kenya	1.625±0.096	0.396±0.024	0.107±0.034
	Réunion	1.450±0.071	0.312±0.029	0.117±0.078
T. coffeae	Kenya	1.300±0.000	0.250±0.000	0.060±0.047
C. savastanoi	Italy	1.300±0.000	0.125±0.059	0.059±0.011
B. oleae	Italy	1.550±0.071	0.291±0.059	0.089±0.002

*SD Standard deviation.

the other two species, *C. savastanoi* ($\overline{H} = 0.059$) and *B. oleae* ($\overline{H} = 0.089$).

General low levels of variability (not reported in Table 2) were found in the considered laboratory strains of *B. cucurbitae* ($\overline{H} = 0.061$), *B. dorsalis* ($\overline{H} = 0.049$), *A. suspensa* ($\overline{H} = 0.053$) and *A. serpentina* ($\overline{H} = 0.089$); in the colonies of *A. ludens* and *R. cerasi* higher levels of variability were detected ($\overline{H} = 0.121$ and $\overline{H} = 0.132$, respectively).

Genetic distances

The matrix of the interspecific Nei genetic distances (Nei, 1978) is shown in Table 3. The lowest genetic distance ($D = 0.78$) is observed between *C. capitata* and *T. coffeae*. The genetic distance observed between the two congeneric species *C. capitata* and *C. rosa* is $D = 1.02$, and those observed between *C. rosa* and the *Anastrepha* species are of the same order of magnitude, i.e. $D = 1.05$ with *A. suspensa*, $D = 1.31$ with *A. ludens* and $D = 0.89$ with *A. serpentina*. Within *Anastrepha* we observed a distance value of 0.81 between the two species (*A. suspensa* and *A. ludens*) from the *fraterculus* group (Norrbom & Kim, 1988). Slightly higher distance values separate these last two species from *A. serpentina* (*serpentina* group), being $D = 0.83$ and $D = 0.93$, respectively. Within the *Bactrocera* genus we observed the highest interspecific distance values: *B. cucurbitae* vs. *B. dorsalis* is 1.53, and vs. *B. oleae* is 2.15.

In Table 4 we summarize the average Nei genetic distance values between the considered genera. The lowest value is the distance between the *Ceratitis* and *Anastrepha* genera ($D = 1.103$) followed by the distance between *Ceratitis* and *Trirhithrum* ($D = 1.242$). The largest distances separate the genus *Bactrocera* from the *Trirhithrum* ($D = 2.679$), *Capparmyia* ($D = 2.534$) and *Anastrepha* ($D = 2.285$) genera.

Cluster analysis

The results of the cluster analysis are shown in Figs 1–3. UPGMA trees computed using Nei genetic distances and Cavalli-Sforza chord measures (Cavalli-Sforza & Edwards, 1967) are shown in Fig. 1(a,b). They represent the consensus trees of 100 bootstrap resamples of the original data set. Both trees show the same topology, confirmed also by relatively high bootstrap values especially at the terminal nodes of the trees. In both trees the first splits separate two of the *Bactrocera* species (*B. dorsalis* and *B. oleae*) and *Rhagoletis cerasi*. The two next splits separate two groups of species: one

Table 3 Matrix of average genetic distances* (±SD) between samples of the considered Tephritidae species

Species	1	2	3	4	5	6	7	8	9	10	11
1 *Ceratitis capitata*	****	1.02±0.02	0.78±0.02	1.14±0.00	2.92±0.08	2.82±0.12	2.60±0.00	1.64±0.03	1.77±0.00	1.54±0.00	1.86±0.00
2 *Ceratitis rosa*	****		1.32±0.04	1.83±0.07	1.92±0.20	1.12±0.05	1.54±0.10	1.05±0.03	1.31±0.01	0.89±0.05	1.75±0.01
3 *Trirhithrum coffeae*		****		1.38±0.06	2.22±0.00	3.46±0.51	2.04±0.07	2.15±0.09	1.46±0.04	1.77±0.06	1.98±0.07
4 *Capparimyia savastanoi*			****		1.88±0.03	2.24±0.00	3.51±1.03	1.79±0.04	2.53±0.01	1.95±0.01	2.15±0.00
5 *Bactrocera oleae*				****		2.15±0.17	0.86±0.03	3.02±0.05	2.87±0.09	3.19±0.10	1.80±0.01
6 *Bactrocera cucurbitae*					****		1.53±0.00	1.66±0.10	1.61±0.08	1.38±0.11	1.73±0.02
7 *Bactrocera dorsalis*						****		2.42±0.02	1.93±0.00	1.44±0.00	1.70±0.00
8 *Anastrepha suspensa*							****		0.81±0.03	0.83±0.03	1.66±0.02
9 *Anastrepha ludens*								****		0.93±0.00	2.02±0.00
10 *Anastrepha serpentina*									****		2.11±0.00
11 *Rhagoletis cerasi*										****	

*Unbiased distances; Nei (1978).

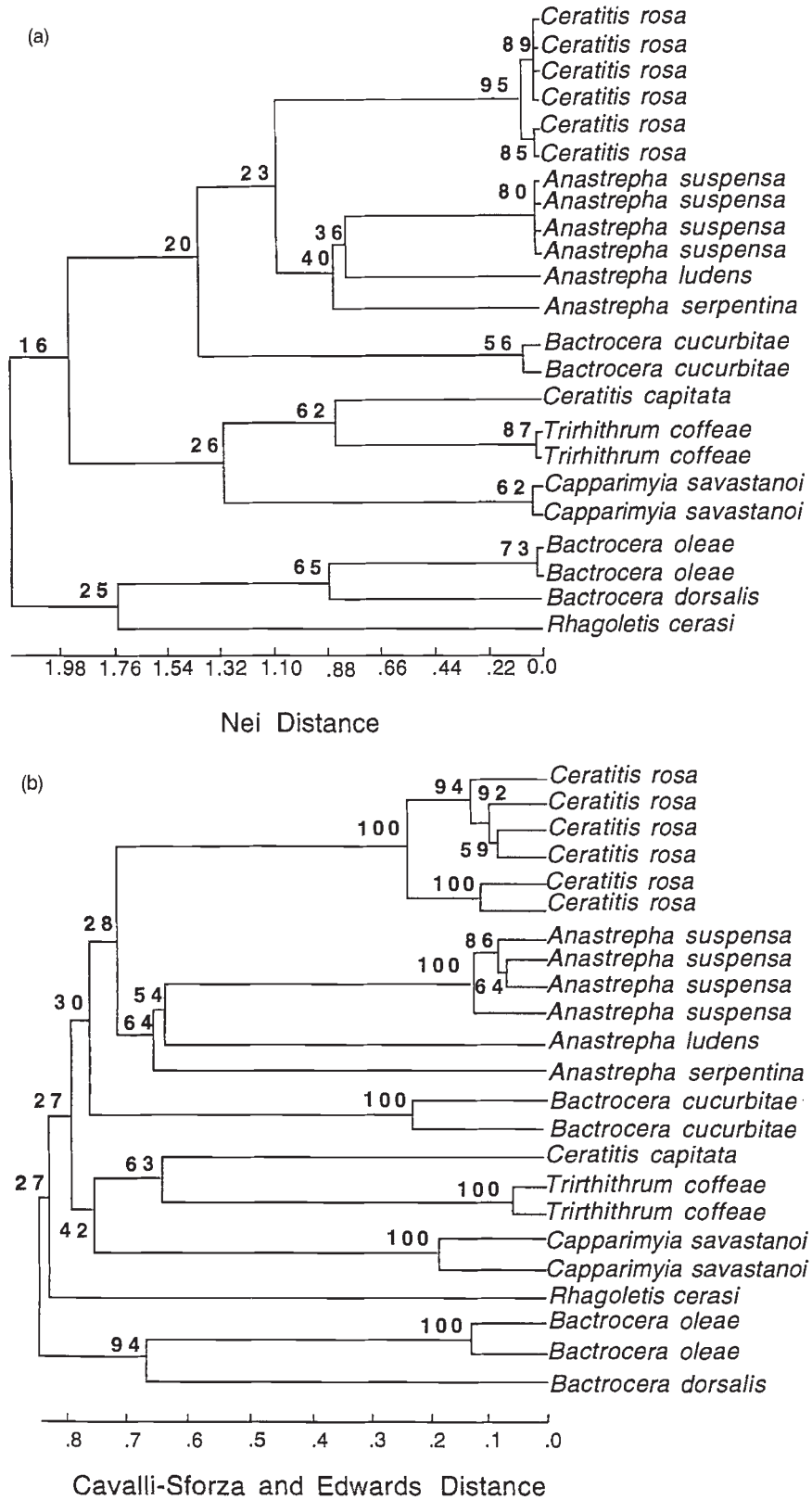

Fig. 1 Tephritid relationships inferred from UPGMA dendrograms obtained from 100 bootstrap resamplings of: (a) Nei unbiased genetic distance (1978) and (b) Cavalli Sforza and Edwards (1967) chord distance. Numbers at the nodes represent the percentage of a group's occurrence in 100 bootstrap replicates.

Table 4 Matrix of average genetic distances* (±sd), between the genera *Ceratitis, Trirhirithrum, Capparimyia, Bactrocera, Anastrepha, Rhagoletis*

	1	2	3	4	5	6
1. *Ceratitis*	*	1.242±0.207	1.735±0.258	1.725±0.580	1.103±0.177	1.772±0.040
2. *Trirhithrum*		*	1.375±0.059	2.679±0.740	1.973±0.292	1.983±0.067
3. *Capparimyia*			*	2.534±1.073	1.942±0.282	2.153±0.005
4. *Bactrocera*				*	2.285±0.679	1.752±0.050
5. *Anastrepha*					*	1.810±0.200
6. *Rhagoletis*						*

*Unbiased genetic distance; Nei (1978).

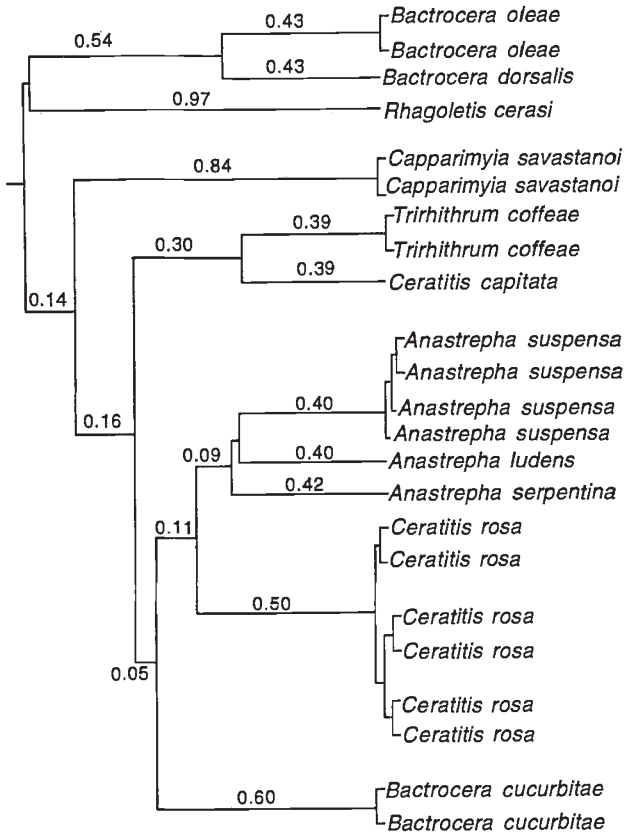

Fig. 2 Tephritid relationships inferred from KITSCH (ultrametric) tree derived from Fitch–Margoliash optimality criteria with the assumption of equal rates of evolution (Felsenstein, 1984). The numbers represent the length of the branches (only those greater than 0.04).

includes *C. rosa* and the three species of *Anastrepha*; the other groups *T. coffeae, C. capitata* and *C. savastanoi*. The hypothetical relationship between species within these two clusters is suggested in general by less than a bootstrap proportion of 50 per cent; however, the affinity between *T. coffeae, C. capitata* and *C. savastanoi* is suggested by a higher value of repeatability, especially in the Cavalli-Sforza & Edwards tree.

The ultrametric tree derived from Fitch–Margoliash optimality criteria (KITSCH) is shown in Fig. 2. This tree confirms the grouping pattern shown in the previous trees which were based on the same assumption of equal rates of evolution among the taxa.

The unrooted tree in Fig. 3, constructed without the constraint of a constant rate of evolution (FITCH), shows similar clustering of species; the clusters *C. savastanoi–C. capitata–T. coffeae* and *R. cerasi–B. dorsalis–B. oleae* are preserved; but we observe that *C. rosa* and the *Anastrepha* species are not accommodated in a single cluster although they separate in subsequent lineages.

Discussion

We used genetic distance to express the total genetic divergence and to infer the phylogenetic relationships among some economically important species of Tephritidae, the majority of which are subject to alternative classifications on morphological bases (Fig. 4).

We used allozyme data from 24 orthologous loci. On the basis that these loci are widely distributed over the genetic maps of some *Rhagoletis* species (Feder, 1989) and *C. capitata* (Malacrida *et al.*, 1990), it is reasonable to assume that these loci may be considered a random, although small, sample of the genomes of the considered Tephritidae species. In addition, these loci code for a variety of enzymes with both singular and multiple physiological substrates, and for both monomeric and multimeric enzymes, which contribute differentially to overall heterozygosity (Zouros, 1976).

In a preliminary attempt to compare the genetic variation among the wild samples of the considered species, we found different levels of intraspecific

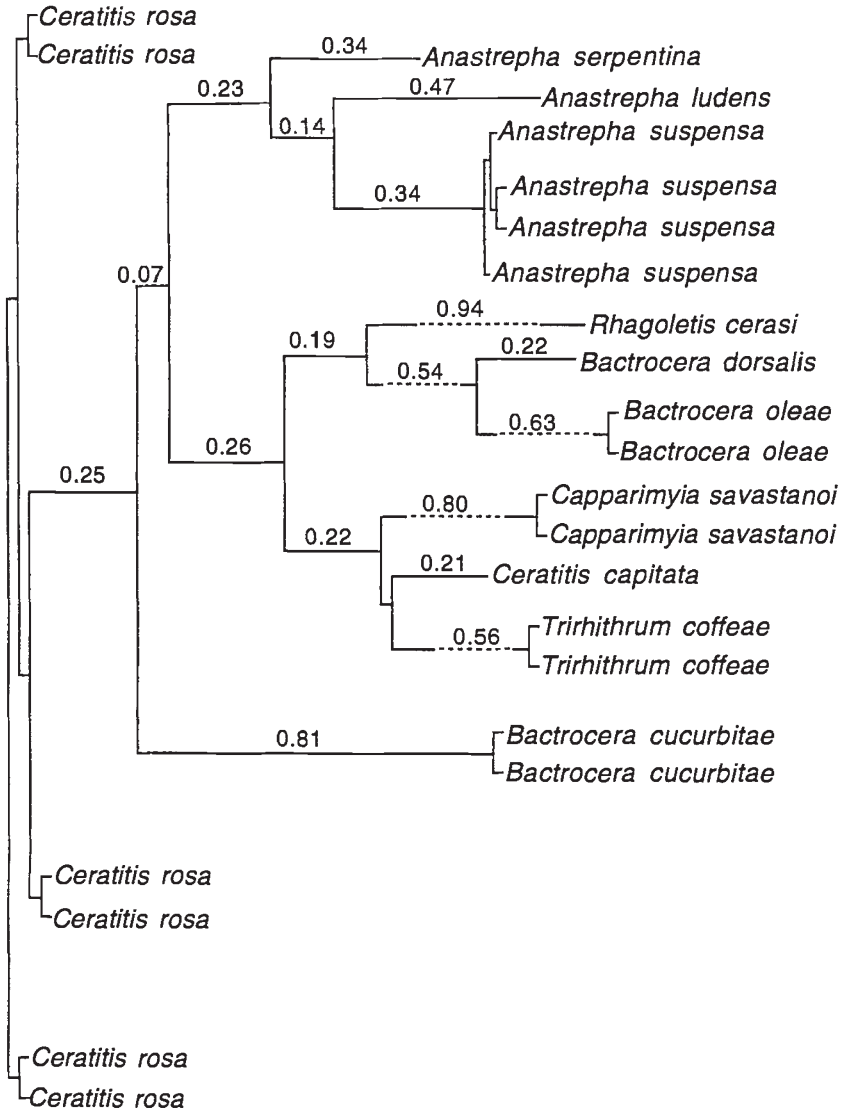

Fig. 3 FITCH unrooted tree (Felsenstein, 1984) derived from Fitch–Margoliash optimality criteria without the assumption of equal rates of evolution for all tip species of Tephritidae considered. The numbers represent the length of the major branches.

variability. In particular, marked differences in genetic variability were found among sympatric samples of the three species *C. capitata*, *C. rosa* and *T. coffeae* collected together in one of their putative original areas (Kenya; White & Elson-Harris, 1992). As these samples are sympatric we can exclude the possibility that the observed intraspecific variability is affected by geographical and/or climatic factors. This level of genetic variability may reflect the specific genetic plasticity and parallels the differential dispersion capacity of these three species. In fact, *C. capitata* which is polyphagous and has a cosmopolitan geographical distribution is the most polymorphic, whereas *T. coffeae* which is monophagous and is considered an endemic species of Western Africa (White & Elson-Harris, 1992) has the lowest level of genetic variability. The intermediate level of genetic variability shown by *Ceratitis rosa* corresponds to the medium level of geographical diffusion of this polyphagous species. During the dispersion processes *C. capitata* loses the greatest part of its variability (Baruffi *et al.*, 1995); its peripheral Mediterranean populations show levels of polymorphism comparable to the ones detected in Kenyan samples of *T. coffeae*. No information is available on the genetic structure or dispersion of geographical populations of *C. rosa* and *T. coffeae*.

The low level of variability detected in the wild samples of the other two species, *C. savastanoi* and *B. oleae*, can be related to their narrow host specialization (Nevo *et al.*, 1984). For *B. oleae* high genetic similarity has been found among distant geographi-

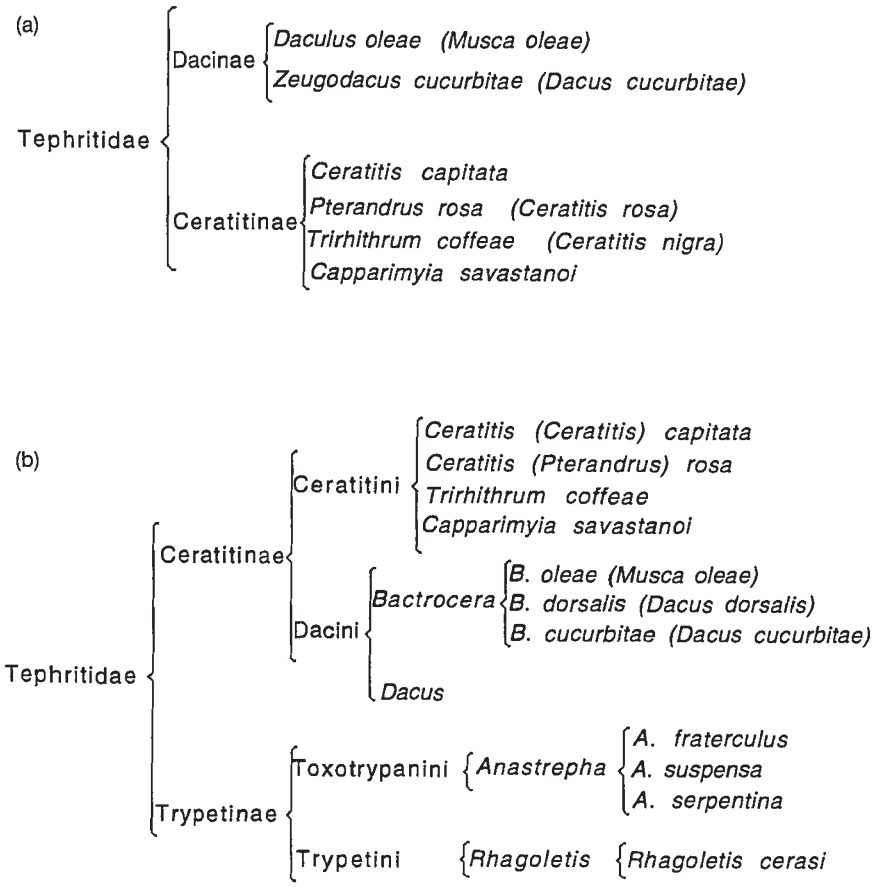

Fig. 4 Morphologically based Tephritidae subfamily relationships proposed by various authors for the species considered in this study. The relationships proposed by Cogan & Munro (1980) and Kugler & Freidberg (1975) are summarized in (a), and those proposed by Hancock (1984, 1987) and White & Elson-Harris (1992) are summarized in (b). The original designations for the species which were subject to alternate classification are given in brackets.

cal populations (Zouros & Loukas, 1989) and this finding has been correlated with the insect's total dependence on the olive fruit.

The specific life history characteristics of these species may also be related to their degree of variabilty. Species such as *C. capitata* and *C. rosa*, which have the attributes of *r*-strategist species (Fletcher, 1989) are also highly polymorphic, whereas species such as *C. savastanoi* and *B. oleae*, which are considered *r-K* strategists, are also less polymorphic.

Congruence between electrophoretic trees

In this study we produced different electrophoretic estimates of the phylogeny of Tephritidae flies, one assuming a molecular clock (UPGMA and KITCH trees) and one making no rate assumptions (FITCH tree). The first conclusion which can be drawn from the tree analysis is that all the topologies obtained are similar, suggesting that we cannot exclude the possibility that rates of enzyme evolution in different clades are similar. All methods indicate the presence of the same clusters of species. One cluster is composed of *T. coffeae, C. capitata* and *C. savastanoi*, and another is composed of *R. cerasi, B. dorsalis* and *B. oleae*. A further loose cluster includes *C. rosa* and the *Anastrepha* species; affinity between them is evident in all the trees; however, only trees based on the assumption of constant evolutionary rate accommodate them under a common hypothetical ancestor.

Congruence between electrophoretic phylogeny and the conventional classification

Figure 4 shows the Tephritidae subfamily relationships based on morphological traits, proposed by various authors in recent years for the species considered in this study. Clearly, there is no generally accepted classification of the Tephritidae.

Nevertheless, there are areas of agreement between the electophoretic trees and some of the proposed classifications. The primary agreement concerns the closely conserved electrophoretic cluster: *C. capitata, T. coffeae* and *C. savastanoi*. That is, the demonstrated genetic affinity parallels

the classical taxonomy in that the three species are grouped under a separate subfamily (Ceratitinae) according to Cogan & Munro (1980) and even under a separate tribe (Ceratitini) according to White & Elson-Harris (1992). *Trirhithrum coffeae* was previously included in the *Ceratitis* genus by the original designation of *Ceratitis nigra* Graham (Cogan & Munro, 1980). The low genetic distance (0.78) between *C. capitata* and *T. coffeae* is in agreement with this previous classification.

The two primary disagreements concern all the electrophoretic trees and involve the species–genus relationships. First, the two congeneric species *C. capitata* and *C. rosa* are separated, secondly, *B. cucurbitae* appears to be genetically unrelated to its congeners *B. dorsalis* and *B. oleae.* In our trees *C. capitata* is closer to *T. coffeae* than to *C. rosa.* This result may indicate how poorly external morphology reflects genetic affinity. Furthermore, *C. rosa*, like *T. coffeae*, has been assigned to various taxonomic relationships. It is regarded now (Hancock 1984, 1987) as a member of the subgenus *Pterandrus* Bezzi of the *Ceratitis* genus, whereas previously Cogan & Munro (1980) regarded *Pterandrus* as a separate genus. From the morphological point of view, *Ceratitis (Ceratitis) capitata* and *Ceratitis (Pterandrus) rosa* are separated on the basis of the male secondary sexual characters, with females being inseparable at the generic level (Hancock, 1984). We can speculate that speciation between these two *Ceratitis* species may have been accelerated by this specific sexual differentiation (Singh, 1988). On the other hand, some caution is necessary to exclude in our study the possibility that by chance we looked at enzyme loci which remain unalterated in some widely separated lines, but were strongly selected in *C. rosa*.

Concerning the relationships deduced from the genetic distances among the three *Anastrepha* species (*A. serpentina*, *A. ludens*, *A. suspensa*), this is an area of agreement within the infrageneric classification of Norrbom & Kim (1988): the two *fraterculus* group species *A. suspensa* and *A. ludens* are more closely related to each other than to *A. serpentina* which belongs to its own subgroup.

The second open question from our electrophoretic results is the unexpected separation within the *Bactrocera* genus. The *Bactrocera* species here considered are members of the following different subgenera: *B. (Daculus) oleae*, *B. (Zeugodacus) cucurbitae* and *B. (Bactrocera) dorsalis* (White & Elson-Harris, 1992). The large genetic distance estimates which separate *B. cucurbitae* from *B. oleae* ($D = 2.15$) and from *B. dorsalis* ($D = 1.53$) are in the range of those expected between different genera (Thorpe, 1982). As reported in White & Elson-Harris (1992) *B. cucurbitae*, like other *Zeugodacus* species, has a pattern of host relationships, which differentiate this species from other *Bactrocera*. This species attacks the flowers rather than the fruit of the Cucurbitaceae, a trait which is more typical of *Dacus* than *Bactrocera.*

Regarding tribe–genus relationships, our electrophoretic data support the close affinities between Ceratitini species and Dacini species proposed by Hancock (1986) and Kitto (1983). White & Elson-Harris (1992) placed these two tribes within a single subfamily of Ceratitinae.

For *Rhagoletis* we have considered only one sample from a single species: *R. cerasi*. In our tree this *Rhagoletis* sample is clustered with *B. dorsalis* and *B. oleae*. The current classification places *Rhagoletis* and *Bactrocera* in different subfamilies. However, genetic affinity between the two *Bactrocera* species (*B. oleae* and *B. dorsalis*) and the primitive *Rhagoletis* species, i.e. *R. cerasi*, parallels a result of Han & McPheron (1994) who recognized a close similarity between two *Bactrocera* species (*B. cucurbitae* and *B. dorsalis*) and one *Rhagoletis* species (*R. striatella*) based on the analysis of nuclear ribosomal DNA.

Conclusion

Several considerations emerge from our results. Individual variation is critical for the study of the systematics of closely related species, unlike higher taxonomic levels (Soto-Adames *et al.* 1994). As expected, electrophoretic data offer a great degree of reliability in ordering genetic similarities between closely related tephritid species. They will be useful in a reanalysis of the morphological data for a reorganization of the formal taxonomic structure of Tephritidae flies, especially at genus–species level. Moreover, our analysis demonstrated cases, within the *Ceratitis* and *Bactrocera* genera, of most interest from the evolutionary point of view, in which allozyme dendrograms do not conform well with the morphological taxonomic relationships. If certain loci can be pinpointed, an important clue to the microevolution of these species may be at hand. Finally, the different degrees of genetic variability demonstrated for the different pest species seem related to zoogeography and to biological traits which are peculiar to each species. This opens the problem of the role of genetic variability in dispersion processes of these species.

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