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Phylogenetic relationships among *Bactrocera* species (Diptera: Tephritidae) inferred from mitochondrial DNA sequences

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Abstract

Several members of the dipteran family Tephritdae are serious pests because females lay eggs in ripening fruit. The genus *Bactrocera* is one of the largest within the family with over 500 described species arranged in 28 subgenera. The phylogenetic relationships among the various species and subgenera, and the monophyly of specific groups have not been examined using a rigorous phylogenetic analysis. Therefore, phylogenetic relationships among 24 *Bactrocera* species belonging to 9 subgenera were inferred from DNA sequence of portions of the mitochondrial 16S rRNA, cytochrome oxidase II, $tRNA_{Lys}$, and $tRNA_{Asp}$ genes. Two morphological characters that traditionally have been used to define the four groups within the subgenus *Bactrocera* were evaluated in a phylogenetic context by mapping the character states onto the parsimony tree. In addition, the evolutionary trend in male-lure response was evaluated in a phylogenetic context. Maximum parsimony analyses suggested the following relationships: (1) the genus *Bactrocera* is monophyletic, (2) the subgenus *B.* (*Zeugodacus*) is paraphyletic, (3) the subgenus *B.* (*Daculus*) is a sister group to subgenus *B.* (*Bactrocera*), and (4) the subgenus *B.* (*Bactrocera*) is monophyletic. The mapping analyses suggested that the morphological characters exhibit a simple evolutionary transition from one character state to another. Male-lure response was identified as being a labile behavior that has been lost on multiple occasions. Cue-lure response was plesiomorphic to methyl-eugenol response, and the latter has evolved independently within the *Bactrocera* and *Zeugodacus* groups of subgenera. The implications of our results for devising a coherent, consolidated classification for *Bactrocera* is discussed.

1. Introduction

Bactrocera Macquart (Tephritidae: Diptera) is among the largest genera within Tephritidae with about 500 described species arranged in 28 subgenera (Drew, 1989; Drew and Hancock, 2000). Members of the genus, formerly included in *Dacus*, constitute one of the most economically important lineages within the family, with several species (e.g., the Oriental fruit fly, *Bactrocera dorsalis*; the Queensland fruit fly, *Bactrocera tryoni*; melon fly, *Bactrocera cucurbitae*) capable of attacking a wide variety of commercially produced fruit (White and Elson-Harris, 1992). *Bactrocera* and *Dacus* are considered sister groups based on the following morphological synapomorphies: radial veins of wings crowded anteriorly and medial cells very broad, female abdominal tergite 6 separate from preceding tergites, and tergite 5 of both sexes with glandular ceromae (Munro, 1984; White, 2000) and Mitochondrial DNA (Smith et al., in press).

Drew (1989) proposed a subgeneric classification of *Bactrocera* (Table 1) based on present/absent permutations of just five morphological characters. Drew (1989) also divided the subgenera of *Bactrocera* into four, presumably monophyletic, groups (Table 1) based on all possible permutations of just two male characters, the shape of male sternite 5 and length of male surstylus lobe. Drew's (1989) subgeneric classification was not based on cladistic principles and as such, may not necessarily reflect the evolutionary history of the genus.

White (2000) conducted the first quantitative cladistic analysis of a subset of *Bactrocera* species based on morphological characters. White's (2000) study included 37

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Table 1
Groups of *Bactrocera* subgenera arranged on the basis of morphology and proposed by Drew and Hancock (2000)

Bactrocera Group	*Queenslandacus* Group	*Zeugodacus* Group	*Melanodacus* Group
Afrodacus	*Queenslandacus*	*Asiadacus*	*Hemisurstylus*
Apodacus		*Austrodacus**	*Hemizeugodacus*
*Bactrocera**		*Diplodacus*	*Melanodacus*
Bulladacus		*Hemigymnodacus**	*Daculus**
Gymnodacus		*Hemiparatridacus*	
*Notodacus**		*Javadacus*	
Semicallantra		*Nesodacus*	
Tetradacus		*Niuginidacus*	
Trypetidacus		*Papuodacus*	
		*Paradacus**	
		Paratridacus	
		Parazeugodacus	
		Sinodacus	
		*Zeugodacus**	

*Indicates *Bactrocera* subgenera included in the present study.

morphological characters from 51 economically important species, representing nine *Bactrocera* subgenera. White's (2000) analysis suggested that *Bactrocera* is paraphyletic. The subgenus *B.* (*Bactrocera*), which contains about 60% of the described species, was paraphyletic in both unweighted and weighted analyses. Furthermore, *Dacus* was not basal to *Bactrocera* as proposed by Drew and Hancock (2000). The phylogenetic proposal of White (2000), although lacking strong branch support (a limitation he points out in his paper), raised doubts about the phylogenetic hypotheses of Drew (1989) and Drew and Hancock (2000) and suggested a need for further investigation utilizing a set of independent characters, such as DNA sequences, and rigorous phylogenetic analyses. Although nucleotide data have been used to examine the phylogenetic relationships of other economically important tephritids (e.g., *Anastrepha* and *Rhagoletis*), currently few extensive molecular phylogenetic studies have been published on *Bactrocera*. Muraji and Nakahara (2001) were the first to use mitochondrial DNA sequence (16S rRNA + 12S rRNA; ~1600 bp total) to examine the evolution of *Bactrocera*. Muraji and Nakahara's (2001) study, which included 18 *Bactrocera* species from four subgenera, indicated that *B.* (*Bactrocera*) was paraphyletic and *B.* (*Zeugodacus*) was monophyletic.

In addition to resolving some of the outstanding taxonomic issues concerning *Bactrocera*, a well-resolved phylogeny based on cladistic principles could provide insight into the evolution of various biological traits. In this regard, an intriguing trait of *Bactrocera* and *Dacus* is the attraction of males to chemicals referred to as "male-lures." The most attractive chemicals are methyl-eugenol (4-allyl-1,2-dimethoxybenzene) and cue-lure 4(*p*-acetoxyphenyl)-2-butanone) (reviewed in White, 2000). Species in the *Bactrocera* group of subgenera are attracted to either methyl-eugenol or cue-lure (never both), while most species in the *Zeugodacus* group of subgenera and *Dacus* are attracted to cue-lure (White and Hancock, 1997). In addition, several species are not attracted to either lure and/or there may be more as yet undiscovered lures. Drew and Hancock (2000) hypothesized that cue-lure response is plesiomorphic relative to methyl-eugenol response. However, it is not known if there was a single or multiple evolutionary transitions from cue-lure to methyl-eugenol response within *Bactrocera*. Furthermore, it is not known if all non-responding species constitute a single monophyletic lineage or represent multiple independent losses of lure-response. A well-supported phylogenetic tree could provide insight into the evolution of male-lure response in *Bactrocera*.

The objective of this study was to infer phylogenetic relationships among selected *Bactrocera* and *Dacus* species using mitochondrial DNA sequences and cladistic analysis. Specifically, the DNA sequence of portions of the mitochondrial 16S rRNA and cytochrome oxidase II + $tRNA_{Lys}$ + $tRNA_{Asp}$ genes were used in separate and combined analyses to infer a phylogeny for 24 *Bactrocera* species. The inferred relationships are compared to those proposed by Drew and Hancock (2000), White (2000), and Muraji and Nakahara (2001). In addition, the evolutionary pathways of selected morphological characters and male-lure response are examined in a phylogenetic context.

2. Materials and methods

2.1. Specimens

A list of analyzed taxa, including country of origin and male-lure response, is presented in Table 2. Specimens were either field-collected using male-lure-baited traps by PTS and SK, or provided by colleagues in the form of genomic DNA, or as dried or ethanol preserved adults or larvae. The Mediterranean fruit fly, *Ceratitis capitata* (Tephritidae: Dacini: Ceratitidina) and *Paragastrozona japonica* (Tephritidae: Dacini: Gastrozonina) were used as outgroups. The taxonomic identification of

Table 2
List of taxa examined with geographic origin, male-lure response (ME, methyl-eugenol; CL, cue-lure; TM, trimed lure; NA, unknown or no lure response) and GenBank accession numbers arranged by species and gene fragment

Species	Origin	Lure	16S rRNA	CO2-K-D
Bactrocera (*Austrodacus*) *cucumis* (French)	Australia	NA	AY037349	AY037392
Bactrocera (*Bactrocera*) *albistrigata* (Meijere)	Malaysia: Petaling-Jaya	CL	AY037382	AY037425
Bactrocera (*Bactrocera*) *aquilonis* (May)	Australia: NT: Leanyer	CL	AY037351	AY037394
Bactrocera (*Bactrocera*) *carambolae* Drew and Hancock	Malaysia: Ipoh and Penang	ME	AY037367	AY037410
Bactrocera (*Bactrocera*) *caryeae* (Kapoor)	India: Karnataka: Mudigere and Mudikere	ME	AY037384	AY037427
Bactrocera (*Bactrocera*) *correcta* (Bezzi)	Thailand: Bangkok; India: Hyderabad	ME	AY037375	AY037418
Bactrocera (*Bactrocera*) *curvipennis* (Froggatt)	New Caladonia	CL	AY037356	AY037399
Bactrocera (*Bactrocera*) *dorsalis* (Hendel)	USA: Hawaii; Tahiti; Taiwan: I-Lan	ME	AY037372	AY037415
Bactrocera (*Bactrocera*) *frauenfeldi* (Schiner)	Soloman Islands: Guadalcanal	CL	AY037358	AY037401
Bactrocera (*Bactrocera*) *kirki* (Froggatt)	Tonga	CL	AY037357	AY037400
Bactrocera (*Bactrocera*) *nigrotibialis* (Perkins)	Malaysia: Selangor: Gombok	CL	AY037365	AY037408
Bactrocera (*Bactrocera*) *papayae* Drew and Hancock	Australia: Cairns; Malaysia: Penang; Philippines	ME	AY037377	AY037420
Bactrocera (*Bactrocera*) *psidii* (Froggatt)	New Caladonia	CL	AY037355	AY037398
Bactrocera (*Bactrocera*) *trilineola* Drew	Vanuatu	CL	AY037353	AY037396
Bactrocera (*Bactrocera*) *tryoni* (Froggatt)	New Caladonia	CL	AY037388	AY037431
Bactrocera (*Bactrocera*) *umbrosa* (Fabricius)	Indonesia; Brunei Darussalem	ME	AY037365	AY037408
Bactrocera (*Daculus*) *oleae* (Rossi)	Italy; USA: California: Ventura Co.	NA	AY037380	AY037423
Bactrocera (*Hemigymnodacus*) *diversa* (Coquillett)	Sri Lanka: Kandy	ME	AY037379	AY037422
Bactrocera (*Notodacus*) *xanthodes* (Broun)	Fiji	ME	AY037352	AY037395
Bactrocera (*Paradacus*) *longicaudata* (Perkins)	Malaysia: Selangor: Gombok	CL	AY037364	AY037407
Bactrocera (*Zeugodacus*) *atrifacies* (Perkins)	Malaysia: Selangor: Gombok	CL	AY037381	AY037424
Bactrocera (*Zeugodacus*) *caudata* (Fabricius)	Brunei Darussalem: Bandar Seri Begawan	CL	AY037363	AY037406
Bactrocera (*Zeugodacus*) *signata* (Hering)	India: Karnataka: Mudigere	CL	AY037362	AY037405
Bactrocera (*Zeugodacus*) *tau* (Walker)	India: Karnataka; Mudikere	CL	AY037391	AY037434
Dacus (*Callantra*) *pedunculatus* (Bezzi)	Philippines: Los Banos: Mt. Makiling	CL	AY037390	AY037433
Dacus (*Dacus*) *demmerezi* (Bezzi)	Reunion: La Bretagne	CL	AF388192	AY037530
Dacus (*Didacus*) *vertebratus* Bezzi	Malawi: Chirombo Bay	CL	U39384*	AY037535
Outgroups				
Ceratitis (*Ceratitis*) *capitata* Wiedemann	Italy; Brazil; USA: Hawaii	TM	AJ242872*	AJ242872*
Paragastrozona japonica (Miyake)	Japan: Hokkaido: Sapporo, Jozankei	NA	U39385*	AY037535

*Indicates previously published sequence.

specimens collected by PTS and SK was carried out using CABIKEY (White and Hancock, 1997), an interactive identification tool. Specimens of several species were obtained by KA from various sources, already identified. We had the identifications of a few species confirmed by Ian White, Natural History Museum, London. DNA or specimens of *C. capitata*, *P. japonica*, *Dacus demmerezi*, and *Dacus vertebratus* were supplied by Bruce McPheron, Pennsylvania State University already identified. Voucher specimens and/or genomic extracts are located in the Insect Genetics Laboratory, Department of Entomology, Kansas State University.

2.2. DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from individual flies following the procedures outlined by Kambhampati and Smith (1995) and Armstrong et al. (1997). For some flies, DNA was extracted using the DNAeasy Tissue Kit (Qiagen) according to manufacturer's instructions. Two mitochondrial DNA fragments representing portions of the 16S rRNA (16S) and cytochrome oxidase II + $tRNA_{Lys}$ + $tRNA_{Asp}$ (CO2KD) genes were PCR-amplified and sequenced using the oligonucleotide primers listed in Table 3.

Polymerase chain reaction (PCR) amplifications were performed in 50 μl volume as previously described (Kambhampati et al., 1992; Smith et al., 1999). The temperature profile for PCR amplification included an initial denaturation step of 95 °C for 2 min followed by 40 cycles of 94 °C for 45 s, 41–46 °C for 45 s and 72 °C for 45 s. A final extension step of 72 °C for 7 min was also added. Amplified products (40–50 μl) were electrophoresed on a 1% agarose gel. The band corresponding to the target PCR product was excised with a sterile razor blade, and purified on a QiaQuick PCR column (Qiagen). Sequencing was performed using the ABI d-Rhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit (ABI–Perkin–Elmer) in 5 μl volume according to the manufacturer's instructions. Sequencing was undertaken by the University of Florida's DNA Sequencing Core Facility an ABI 377 DNA sequencer. Both strands of the DNA fragments were sequenced.

The DNA sequences were read from electropherograms into a computer using ABI's Sequence Navigator software and any ambiguities between complementary strands were corrected. The sequences were initially aligned using CLUSTALX (Thompson et al., 1997) and manually adjusted as needed. The DNA sequences used in this study have been deposited in GenBank under the accession numbers provided in Table 2.

2.3. Phylogenetic analysis

Permutation tail probability (PTP; Archie, 1989; Faith, 1991; Faith and Cranston, 1991) as implemented in PAUP* 4.0b6 (Swofford, 1998) and relative apparent synapomorphy analysis (RASA; Lyons-Weiler et al., 1996) were used to evaluate the extent to which the data were hierarchically structured. RASA, a tree-independent regression based method, was carried out using RASA Ver. 3.0T (Lyons-Weiler, 2001). To explore the conflict between data sets, the incongruence length difference (ILD) test (Cunningham, 1997a, b; Farris et al., 1994, 1995; Mickevich and Farris, 1981) was applied to the combined data matrix (invariant characters removed; 100 randomizations) using the partition homogeneity test option in PAUP*.

Phylogenetic analyses were conducted using maximum parsimony (MP) and neighbor-joining (NJ) methods in PAUP*. Neighbor-joining analysis (Saitou and Nei, 1987) was conducted using the Tajima–Nei distance to account for the nucleotide bias in animal mtDNA (Tajima and Nei, 1984). Unweighted MP analyses were carried out using the multiple equally parsimonious heuristic search option with tree bisection–reconnection and 100 random addition sequence replicates. Support for specific nodes on the MP and NJ trees was estimated by bootstrapping (10,000 replications; "fast" stepwise addition search) (Felsenstein, 1985) and decay analysis (Bremer, 1994). Decay indices were determined using AUTODECAY Ver. 4.0 (Eriksson, 1998) and extracted onto our MP tree using TREEVIEW (Page, 1996). Finally, MacClade 4.0 (Maddison and Maddison, 2000) was used to explore character state changes on our MP tree.

3. Results

3.1. Characterization of the nucleotide data

A total of 841 aligned bases (including gaps) of DNA sequence were obtained from portions of the mito-

Table 3
Oligonucleotide primers used for polymerase chain reaction (PCR) amplifications

Name[a]	Sequence
(16S-F) LR-J-13756	5′-TAGTTTTTTTAGAAATAAATTTAATTTA-3′
(16S-R) LR-N-13308	5′-GCCTTCAATTAAAAGACTAA-3′
(C2KD-F) C2-J-3549	5′-CAAATTCGAATTTTAGTAACAGC-3′
(C2KD-R) TD-N-3884	5′-TTAGTTTGACAWACTAATGTTAT-3′

[a] Nomenclature based on Simon et al. (1994); 3′ nucleotide position based on sequence of *Drosophila yakuba* (Clary and Wolstenholme, 1985).

chondrial 16S rRNA (473 bp) and cytochrome oxidase II + $tRNA_{Lys}$ + $tRNA_{Asp}$ (374 bp) genes for the taxa included in this study. Of the 841 characters, 243 (29%) were variable and 158 (19%) were parsimony informative. A χ^2 test of homogeneity of base frequencies across taxa for the combined data set yielded a P value of 1.0 ($\chi^2 = 17.83$, df = 84). The estimated transition (ti)/transversion (tv) ratio was 1.13. Among transitions, 48% were A ↔ G transitions and 52% were C ↔ T transitions. The estimated relative proportions of the eight types of transversions were: A ↔ T, 76%; A ↔ C, 11%; G ↔ T, 12%; and G ↔ C, 1%.

3.2. Phylogenetic signal and congruence testing

Phylogenetic signal in the molecular data sets was explored using PTP and RASA tests. All PTP tests were statistically significant ($P < 0.01$), indicating that the data exhibited more character congruence overall than would be expected by chance alone. Rooted RASA analyses were conducted on the individual and combined data sets with gaped, constant, and missing/ambiguous nucleotide sites excluded. All *t*RASA values were statistically significant (*t*RASA: 16S = 14.44; CO2KD = 8.39; 16S + CO2KD = 11.80), which suggested the data sets contained a significant degree of hierarchy in the distribution of character states among taxa.

To explore the conflict between data sets, pairwise incongruence was estimated using the ILD test, which indicated that the data sets were homogeneous (Table 4). Therefore, the data sets were combined and analyzed as a single data set.

3.3. Phylogenetic analyses

A summary of character statistics and results of unweighted parsimony analysis (gaps = missing) for individual and combined data sets is given in Table 4. Among the individual data sets, unweighted parsimony analysis identified seven equally parsimonious trees of 473 steps each for the 16S rDNA sequence and three equally parsimonious trees of 374 steps each for the CO2KD sequences (Table 4). The topologies of the 16S rDNA and CO2KD trees differed in the placement of several ingroup taxa, even though the ILD test indicated the lack of significant incongruence between the data sets; in addition, many of the clades were not supported in greater than 50% of the bootstrap replicates (not shown). Analyses of the individual data sets failed to recover *Dacus* basal to *Bactrocera*, and *Bactrocera* was not monophyletic in either tree (not shown).

The strict consensus tree for the combined (16S + CO2KD) data set is shown in Fig. 1. Many of the clades were supported in >50% of the bootstrap replicates and in trees that were 1–10 steps longer than the shortest tree. The consensus tree indicated the following relationships: (1) *Bactrocera* and *Dacus* were monophyletic, (2) *B.* (*Zeugodacus*) was paraphyletic, (3) *B.* (*Daculus*) was a sister group to *B.* (*Bactrocera*), and (4) *B.* (*Bactrocera*) was monophyletic. These same relationships were upheld in a neighbor-joining analysis based on the Tajima–Nei distance (not shown).

3.4. Character evolution

We used the phylogenetic tree shown in Fig. 1 to examine the evolution of specific morphological characters and male lure-response by mapping the associated character states onto the tree. The following morphological characters were taken from the literature and examined: (1) length (short or long) of the posterior lobe of the male surstylus (Fig. 2) and (2) shape (shallow "V" or deep "V") of male sternite 5 (Fig. 3). These characters were selected because they are commonly used to classify the subgenera of *Bactrocera* into four, presumably monophyletic, groups (Drew, 1989). However, their evolutionary transitions, and therefore phylogenetic utility, has not previously been examined using cladistic methods.

Character state mapping of the two morphological characters indicated that both characters exhibit a simple transition from one state to the other. Shallow V-shaped emargination of sternite 5 and a short posterior lobe of the male surstylus were plesiomorphic (Figs. 2 and 3). The mapping of male-lure response suggested that cue-lure response was plesiomorphic relative to methyl-eugenol response (Fig. 4). These results are in general agreement with the hypothesis of Drew and Hancock (2000). However, the ability to respond to either lure has been lost on multiple occasions. Moreover,

Table 4
Summary of character statistics and results of parsimony analysis

Data partition	Characters (including gaps)	PIC	TL	EPT	CI	RI	RCI	ILD	*t*RASA
16S rRNA	473	74	318	7	0.54	0.63	0.34	NA	14.44
CO2KD	368	84	374	3	0.43	0.52	0.27	NA	8.39
16S + CO2KD	841	158	710	40	0.47	0.60	0.29	$P = 0.03$	11.80

PIC, number of parsimony informative characters; TL, most parsimonious tree length; EPT, number of equally parsimonious trees; CI, consistency index; RI, retention index; RCI, rescaled consistency index; ILD, incongruence length difference; *t*RASA: relative apparent synapomorphy analysis (*t* value).

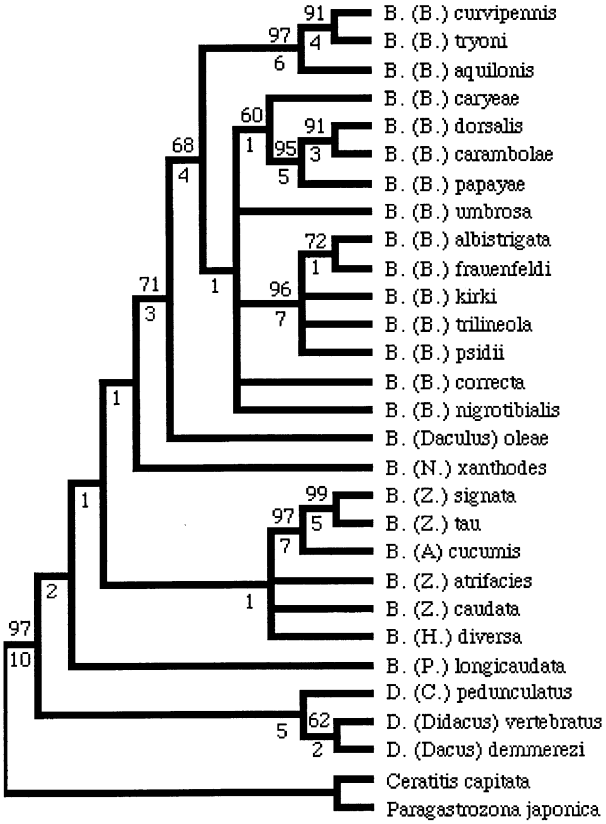

Fig. 1. Strict consensus of 40 equally parsimonious trees for 24 species of *Bactrocera* based on the combined DNA sequence (841-bp including gaps) of the mitochondrial 16S rRNA and cytochrome oxidase II + $tRNA_{Lys}$ + $tRNA_{Asp}$ genes and equal weighting of characters; gaps were excluded. Tree length: 710. Numbers above branches are bootstrap values (%) and those below the branches are decay indices. Bootstrap values below 50% and decay indices of 0 are not shown. See Table 4 for tree statistics.

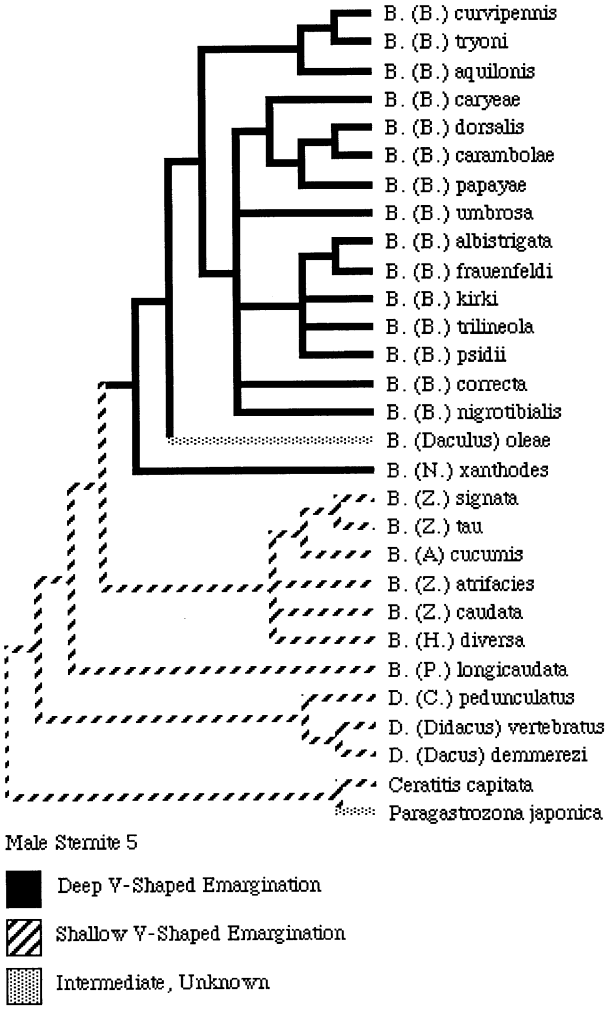

Fig. 2. Evolution of the posterior lobe of the male surstylus in *Bactrocera* based on the tree shown in Fig. 1. See text for details.

the response to methyl-eugenol has evolved independently within *Bactrocera* several times. Thus, our results suggested that lure-response has been labile within this group of flies.

4. Discussion

4.1. Data analysis

In this paper a phylogenetic analysis of relationships among 24 *Bactrocera* taxa, representing three subgeneric "groups" and seven subgenera, based on the DNA sequence of portions of the mitochondrial 16S rRNA and cytochrome oxidase II + $tRNA_{Lys}$ + $tRNA_{Asp}$ genes was presented. The characteristics of the mtDNA sequences are consistent with those of other insect mtDNA sequences. For example, a bias toward adenine and thymine (76% of total) is consistent with the base composition of mtDNA sequences of other insects (Simon et al., 1994), including other tephritids (Han and McPheron, 1997; McPheron and Han, 1997).

Congruence testing indicated that the data sets were not significantly heterogeneous. Thus, we will discuss the consensus tree (Fig. 1) from the combined analysis. The issue of whether to combine data sets in phylogenetic analysis is a subject of much debate (e.g., Bull et al., 1993; De Queiroz, 1993; De Queiroz et al., 1995; Huelsenbeck et al., 1996; Nixon and Carpenter, 1996), and no clear consensus has emerged. However, in most cases combined analyses are more likely to recover a phylogenetic tree close or identical to the "true" tree, if for no other reason than the amount of information available to infer a phylogenetic tree is maximized. In addition, all the genes used here were mitochondrial and thus, presumably, share the same evolutionary history.

4.2. Phylogenetic relationships

Drew and Hancock (2000) proposed a non-quantitative "tree-like diagram" for the four groups of *Bactrocera* subgenera and a scenario for the origin,

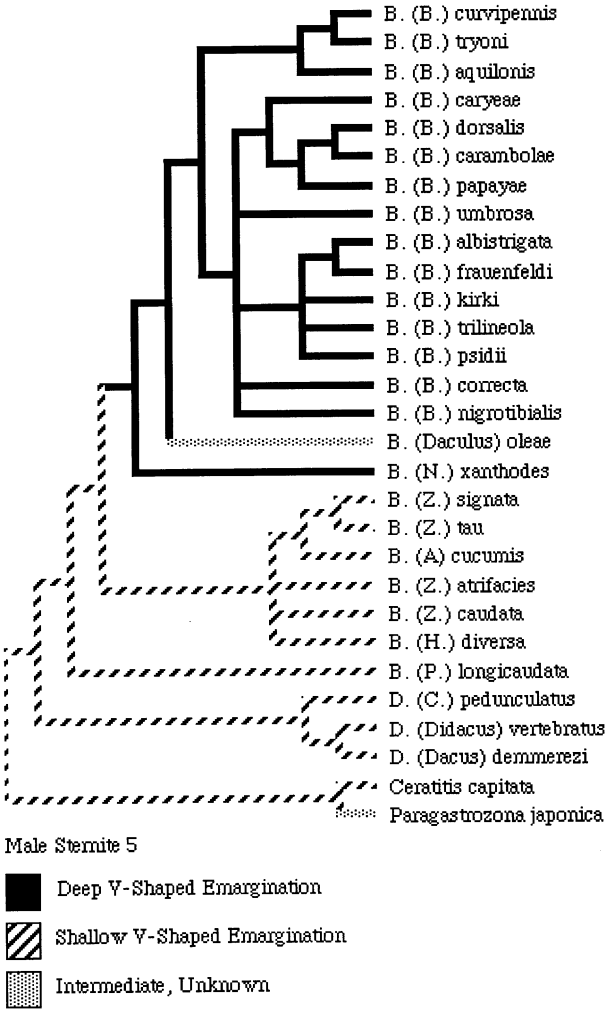

Fig. 3. Evolution of male sternite 5 in *Bactrocera* based on the tree shown in Fig. 1. See text for details.

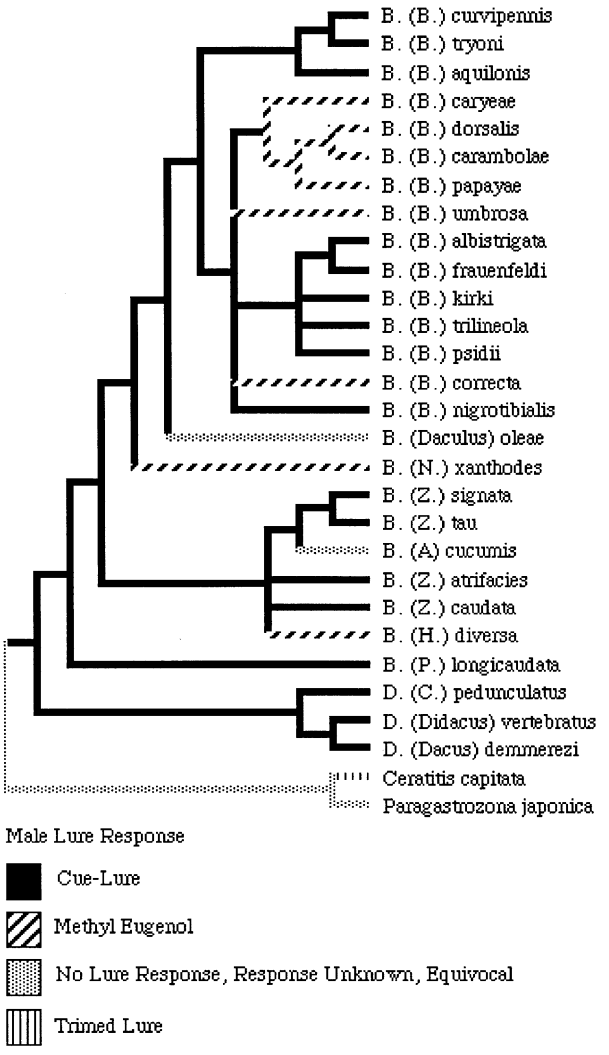

Fig. 4. Evolution of male-lure response in *Bactrocera* based on the tree shown in Fig. 1. See text for details.

evolution, and diversification of numerous subgenera. The present study represents one of the first extensive molecular-based phylogenetic treatment of *Bactrocera* and allows us to comment on the hypothesized groupings of Drew and Hancock (2000). In addition, White's (2000) phylogenetic analysis based on morphological characters provides a basis to compare our molecular-derived trees to those based exclusively on morphology.

Certain relationships occur in most or all of our analyses regardless of how the data were treated: *B.* (*Zeugodacus*) was paraphyletic, *B.* (*Daculus*) was a sister group to *B.* (*Bactrocera*), and *B.* (*Bactrocera*) was monophyletic. Drew and Hancock (2000) suggested that the *Zeugodacus* group of subgenera is monophyletic and is most closely related to the *Melanodacus* group of subgenera, represented here by *B.* (*Daculus*). Although our results (Fig. 1) suggested that members of the *Zeugodacus* group were indeed more closely related to each other than they were to other taxa in this study, the subgenus *B.* (*Zeugodacus*) was not recovered as a monophyletic lineage. These findings also differ from those of Muraji and Nakahara (2001) who recovered *B.* (*Zeugodacus*) as monophyletic; however, due to limited taxon sampling they were unable to ascertain whether the *Zeugodacus* group of subgenera are monophyletic.

Drew and Hancock (2000) also suggested that the *Bactrocera* group of subgenera, represented here by *B.* (*Bactrocera*) and *B.* (*Notodacus*) is monophyletic; however, our results did not support this hypothesis as *B.* (*Daculus*) *oleae* (*Melanodacus* group) consistently fell within the *Bactrocera* group. This latter result was also supported by the results of White (2000).

The four members of the *B.* (*B.*) *dorsalis* complex (as defined by Drew and Hancock, 1994) included in this study were monophyletic, a result not surprising considering that they have been presumed to be closely related (Drew and Hancock, 1994). However, since the complex presumably includes more than 60 geographically diverse species (Drew and Hancock, 1994), more

taxa must be included to test their monophyly. Moreover, the genes used in this study showed little variation among the *B.* (*B.*) *dorsalis* complex species, signalling a need for more characters to address the issue of monophyly.

In light of our results it is apparent that Drew and Hancock's (2000) grouping of subgenera and subgeneric designations are not phylogenetically sound because they do not necessarily represent monophyletic lineages, an assumption implicit in Drew and Hancock's (2000) hypotheses. Therefore, a rational consolidation of the numerous subgenera is warranted. Ian White (pers. comm.) suggested it is likely that *B.* (*Austrodacus*) is a member of *B.* (*Zeugodacus*), the only major difference between them being the loss of a pair of scutellar setae in the latter taxon. The first step toward consolidation could include eliminating the subgeneric designations proposed by Drew and Hancock (2000) and treating the *Bactrocera* and *Zeugodacus* "groups of subgenera" simply as subgenera of *Bactrocera*. Thus, *Bactrocera* would contain three or four subgenera depending on the phylogenetic position of the monotypic subgenus *B.* (*Queenslandacus*) (not included in our study) relative to the other three groups. A similar proposal has been suggested by White (2000). Regardless of how many subdivisions the genus *Bactrocera* is presumed to contain, each such division must be based on the identification of monophyletic lineages in a rigorous phylogenetic analysis, rather than a sole reliance on presumed morphological synapomorphies.

4.3. Character evolution

We examined the evolution of specific morphological characters and male lure response by using the phylogenetic tree based on maximum parsimony analysis of the combined data set. All possible permutations of just two morphological characters were used by Drew (1989) and Drew and Hancock (2000) to define the four groups of *Bactrocera* subgenera: the 5th abdominal sternite and the posterior lobe of the surstylus in male flies. A deep V-shaped emargination of sternite 5 + short surstylus lobe defines the *Bactrocera* group, whereas a shallow V-shaped emargination of sternite 5 + long surstylus lobe defines the *Zeugodacus* group. About 99% of all *Bactrocera* species belong to one of these two groups. However, eight species do not conform to the above combination of characters and were placed in separate subgeneric groups by Drew (1989). The monotypic *Queenslandacus* group is defined by a deep V-shaped emargination of sternite 5 coupled with a long surstylus lobe. The remaining seven species were placed within the *Melanodacus* group and possess a shallow V-shaped emargination of sternite 5 and a short surstylus lobe. White (2000) discussed the biological significance of these two characters and suggested that the differences in structure of the male terminalia among *Bactrocera* spp. imply that the details of copulation probably differ among the groups.

With regard to the shape of the surstylus lobe, our analysis suggested that a short posterior lobe of the male surstylus is plesiomorphic relative to a long posterior lobe among the ingroup taxa (Fig. 2). However, the taxa that possessed a long posterior lobe were not monophyletic. This result may be due to limited taxon sampling. With regard to male sternite 5, a shallow V-shaped emargination is plesiomorphic and a deep V-shaped emargination is apomorphic (Fig. 3). The character state for *B. oleae*, however, appears to be intermediate, although Drew and Hancock (2000) considered this species to be closely allied with the *Melanodacus* group which exhibit a shallow V-shaped emargination of sternite 5. However, Drew and Hancock's (2000) assertion is not compatible with our phylogenetic tree, as *B. oleae* is placed between two taxa that exhibit a deep V-shaped sternite 5 (Fig. 3). Thus, our analyses support the assertion by Drew and Hancock (2000) that these two characters are phylogenetically useful in defining monophyletic lineages within the genus *Bactrocera*. Unfortunately, we were unable to obtain specimens of the sole representative of the *Queenslandacus* group, *Bactrocera* (*Queenslandacus*) *exigua*, for inclusion in this study. From a taxonomic perspective it is of critical interest to see where this species is positioned relative to the *Zeugodacus* and *Bactrocera* groups. Inclusion of *B.* (*Q.*) *exigua* in a phylogenetic analysis would help elucidate which character (sternite 5 vs. surstylus lobe) is phylogenetically more important, and which character is homoplasious. Inclusion of *B. oleae* in our study did shed some light on this issue with the *Melanodacus* group. Our analyses suggested that the male surstylus lobe is the more important of the two characters in tracking evolutionary history. However, additional species from the *Melanodacus* group are needed to verify this hypothesis.

An interesting aspect of *Bactrocera* and *Dacus* biology is that the males are strongly attracted to methyl-eugenol and cue-lure. These male-lures have proven to be useful for population monitoring and taxonomic surveys. Drew and Hancock (2000) and White (2000) discussed the biological and taxonomic significance of male-lure response within *Bactrocera* and *Dacus*. In some cases, lure response is the only diagnostic feature that discriminates between some closely related pairs of species (White and Hancock, 1997). Here, we were interested in examining the evolution of male lure-response from a phylogenetic perspective. Our analyses both recovered evolutionary trends in lure response within *Bactrocera* (Fig. 4) and strongly suggested that within the subgenus *Bactrocera*, lure response is labile. Specifically, response to cue-lure was plesiomorphic relative to methyl-eugenol response. Our data also in-

dicated that lure-response has been lost on multiple occasions, and that response to methyl-eugenol has evolved independently several times. Our results could potentially be used to predict lure-response for a taxon whose lure response is unknown simply by identifying where it is grouped in relation to other species. For example, if a species whose male lure is unknown falls within a clade of exclusively cue-lure responding species, it can be assumed that the species in question either responds to cue-lure, or exhibits no lure-response at all.

4.4. Summary and future research needs

Our results indicated that the combination of mitochondrial genes examined here were useful for inferring a phylogenetic tree for the *Bactrocera* taxa included in our study. Although there has been considerable work done on the biology and taxonomy of *Bactrocera*, much of it has been descriptive in nature, and few of the data have been analyzed using cladistic methods. Our study represents a first attempt at resolving some of the outstanding taxonomic and evolutionary issues concerning *Bactrocera* using DNA sequence data. Clearly, more taxa must be analyzed, and more data, particularly from genes that exhibit sufficient informative variation to resolve some of the internal nodes, are needed. However, our phylogenetic tree represents a sorely needed starting point by which a coherent classification could be constructed; one not based on numerous subdivisions but, rather, on the identification of monophyletic lineages.

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