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Abstract

Oedipodine grasshoppers occur throughout the major continents, making them the most widely distributed of the 30 subfamilies that comprise the Acrididae. Most species have been allocated to one of 15 tribes; some remain unassigned. The subfamily, according to Vickery, had an ancient origin, just after the breakup of Pangaea but before the separation of Laurasia from Africa. Thereafter, Oedipodinae continued to evolve in separate continental centers; some Nearctic species apparently descended more recently from Palearctic ancestors when land bridges still connected the two continents.

Our objectives are to independently assess these biogeographic accounts, to examine the validity of several tribal constructs, and to shed light on problematic taxa such as *Stethophyma* and *Machaerocera* which have had, over the years, an ambivalent affiliation with Oedipodinae. To realize these goals, we sequenced and phylogenetically analyzed portions of four mitochondrial genes (coding for cytochrome oxidase subunits I and II, cytochrome b, and NADH dehydrogenase subunit V), totaling up to 2254 bp, in specimens collected in the Americas, Eurasia, Africa and Australia. Methodology entailed applying weighted and unweighted maximum parsimony, maximum likelihood and Bayesian techniques. A member of the Pyrgomorphidae served as the outgroup. The ages of evolutionary divisions were estimated using the program "r8s" ; the date of 100 Mya, previously estimated as the time of divergence between the subfamilies Oedipodinae and Gomphocerinae, was used to calibrate our chronogram.

In general, taxa appear to assort themselves according to continental land mass, rather than by tribe. Aiolopini, Bryodemini, Oedipodini and Sphingonotini proved to be nonmonophyletic, whereas there was no evidence to reject monophyly in Acrotylini, Chortophagini, Locustini and Psinidini. Phylogenetically, both *Machaerocera* and *Stethophyma* were well-positioned within the Oedipodinae, with *Machaerocera* closely aligned with *Chortophaga* and *Encoptolophus*, and *Stethophyma* tightly linked to *Aiolopus*. *Duroniella*, presently regarded as an oedipodinid, emerged strongly connected to the Gomphocerinae.

The current biogeographical distribution of Oedipodinae is the result of widespread intercontinental dispersion. In particular, with the assistance of DIVA analysis, we argue that Asiamerica was the center of initial oedipodinid radiation about 94 Mya. Through a series of early dispersals, the remaining clusters of taxa were established. Somewhat surprisingly, this includes the branch leading to the Australian genera *Austroicetes* and *Chortoicetes*. In contrast, the multiple dispersals to the African continent occurred more recently. It would appear that North American oedipodinids had both an ancient and a more recent ancestry. The single South American species analyzed evolved very recently from North American ancestors.

Key words

Orthoptera, Oedipodinae, Gomphocerinae, phylogeny, biogeography, mitochondrial DNA, molecular clock

Introduction

Molecular phylogenetic studies of intercontinental taxa above the species level provide opportunities for investigating the evolutionary impact of geological and climatological processes in the distant past. For the past decade, the number of such studies has steadily increased for several insect orders, for example: Coleoptera (*e.g.*, Pearson & Vogler 2001, Davis *et al.* 2002), Diptera (*e.g.*, Barrio & Ayala 1997, Martin *et al.* 2002), Hemiptera (*e.g.*, Buckley *et al.* 2002, von Dohlen *et al.* 2006), Hymenoptera (*e.g.*, Leys *et al.* 2002, Kawakita *et al.* 2004), Lepidoptera (*e.g.*, Zakharov *et al.* 2004, Hundsdoefer *et al.* 2005), to name just a few. Challenges to traditional views on place and time of origin and directionality of migration (*e.g.*, Pearson & Vogler 2001, Costa *et al.* 2003, Hundsdoefer *et al.* 2005, von Dohlen *et al.* 2006) have often been the result.

Within the Orthoptera, however, there have been few comparable investigations. In one recent example, a mitochondrial DNA (mtDNA) phylogenetic analysis (Lovejoy *et al.* 2005) of Old and New World *Schistocerca* species demonstrated that the genus originated in Africa and not the Western Hemisphere, as had been proposed in an earlier morphological investigation (Song 2004). Rather, a single east-west, trans-Atlantic dispersal event took place, eventually leading to the establishment of the numerous species presently distributed throughout the Americas. Rowell and Flook (2004), also employing mtDNA, speculated on the place of origin of the neotropical subfamily Proctolabinae, identifying proto-Central and South American land areas as alternative locations. Recent findings (Chapco *et al.* 2001, Amédégnato *et al.* 2003) challenged the prevailing view (Vickery 1989) that the subfamily Melanoplinae originated in Laurasia and that during the Pliopleistocene Great Interchange, incursions from the north led to the establishment of taxa in South America. Instead, molecular phylogenetic analyses of mitochondrial genes showed the reverse, that the subfamily originated in South America, probably in the Early Cenozoic, and subsequently, via island-hopping, progressed to establish the Holarctic fauna. A similar analysis (Contreras & Chapco 2006) of Holarctic Gomphocerinae supported Vickery's (1989) contention that there were at least three dispersal events from Eurasia to North America. More recently, with the inclusion of taxa from the southern hemisphere, preliminary analyses (unpub.) have not contradicted that conclusion; however, they further suggest the possibility that the subfamily originated still earlier in Gondwanaland.

Another subfamily, whose distribution surpasses even those of Gomphocerinae and Melanoplinae, is Oedipodinae (= Locustinae), or the band-winged grasshoppers. Its over 900 species and 185 genera occur throughout the major continents, making the subfamily the most cosmopolitan among the 30 subfamilies of the Acrididae

(Vickery & Kevan 1985, Otte 1995). Among their numbers can be counted several infamous pests, such as the migratory locust (*Locusta migratoria*), the Australian plague locust (*Chortoicetes terminifera*), and the clear-winged grasshopper (*Camnula pellucida*).

Taxonomically, oedipodine grasshoppers over the years, have been grouped as a tribe, as a subfamily and at times, as a family [see Guliaeva *et al.* (2005) for a summary]. The subfamily designation is now generally accepted (Otte 1984), but not by all (see Rentz 1996). In the most recent version of the Orthoptera Species File (OSF2) (Eades *et al.* 2007), Oedipodinae is subdivided into 15 tribes, of which a few such as Locustini and Sphingonotini occur on two or more continents. Most however, are restricted to only one land mass. Morphological similarities among continentally separated taxa have led to speculations about the subfamily's historical origins, a topic of interest to orthopterists for about half a century, starting with Rehn's (1958) seminal paper on North American species. Rehn made brief reference to connections with Eurasian taxa, but on the whole focused on identifying probable centers of origin in the New World. Vickery (1987, 1989) proposed that initially the subfamily had evolved over 100 Mya, before the complete sundering of Pangaea; subsequently, diversification continued in separate Nearctic, Palearctic and Ethiopian centers. Vickery (1989) also viewed some Nearctic elements as descendants of more recent invaders from the Old World, entering North America via one of several land bridges that had connected the two land masses. The subfamily is poorly represented in the Neotropics (about seven genera), which would suggest that the incursion from the north was fairly recent (Rehn 1958, Carbonell 1977). The subfamily is well represented on the African continent (Otte 1984), but apart from Vickery's brief statement cited above, very little (see Ritchie 1981, 1982) has been proposed on the origin of that continent's oedipodinid fauna.

Unfortunately, the few published phylogenies — both morphologically based (*e.g.*, Otte 1984) and molecularly based (*e.g.*, Chapco *et al.* 1997, Rowell & Flook 2004, Guliaeva *et al.* 2005, Lu & Huang 2006) — are inadequate for testing these biogeographic hypotheses. Trees appear either somewhat arbitrary in their construct (*e.g.*, Otte 1984), or they include too few oedipodinids (Rowell & Flook 2004, Guliaeva *et al.* 2005), or they focus on only one continent (Chapco *et al.* 1997, Lu & Huang 2006). The present study is a phylogenetic analysis of mtDNA sequences from a selected group of oedipodine grasshoppers sampled from both Old and New-World continents. Our objectives are 1) to shed light on the subfamily's origins and thereby test the aforementioned biogeographic hypotheses, and 2) to add to our ongoing understanding of taxonomic relationships and organization within the subfamily Oedipodinae. Of possible interest to orthopterists, the Australian oedipodinids, *Austroicetes* and *Chortoicetes* — neither of which has been assigned to tribe — are included. We also provide further insight into the phylogenetic affinities of *Machaerocera* and *Stethophyma*, two genera that have had a somewhat uncertain relationship with Oedipodinae (Otte 1984).

Materials and Methods

Species, along with sources, are listed in Table 1. Included are 22 species from Eurasia/Africa, 12 from North America, one from South America and two from Australia. Collectively, these represent 12 of the tribes listed in the OSF2. Two tribes, Bryodemini and Sphingonotini, contain both New and Old-World genera. In order to assist in estimating times of divergence (see below), five members of the closely related subfamily, Gomphocerinae, were included. *Pyrgomorpha conica* was employed as the outgroup. An earlier in-

vestigation (Flook & Rowell 1997) had established that within the Acridoidea, the Pyrgomorphidae are basal to the Acrididae.

DNA was extracted from specimens using either the DTAB/CTAB method outlined in Philips and Simon (1995) or using a QIAGEN DNeasy tissue kit (Mississauga, Canada). Portions of the mitochondrial genes encoding NADH dehydrogenase subunit V (ND5), cytochrome oxidase subunit I (CO1) and II (C02), and cytochrome b (cytb) were amplified and sequenced. Primer sequences, PCR gene amplification conditions, as well as DNA sequencing methods, are described elsewhere (Litzenberger & Chapco 2001a, 2001b; Contreras & Chapco 2006). [Two additional primers used for amplifying cytb sequences are:

> mtd26 5'-TATGTACTACCATGAGGACAAATATC-3' and
> mtd28 5'- ATTACACCTCCTAATTTATTAGGAAT-3'].

Sequences were easily aligned by visual inspection, imported into MacClade (Maddison & Maddison 2004) and analyzed using the software packages PAUP* (version 4.0b8 — Swofford 2003) and MrBayes (MB) (Version 3.0b4 — Huelsenbeck & Ronquist 2001). Both standard maximum parsimony (MP) and weighted maximum parsimony (wMP), following Farris' (1969) iterative reweighting scheme, were used. In addition to using parsimony and Bayesian methods, maximum likelihood (ML) was applied, also available in PAUP*. In order to reduce the run-time for ML, parameter estimates provided by the program Modeltest (Version 3.6 — Posada & Crandall 1998) were used as input values. Levels of support for parsimony-derived relationships were estimated through 1000 bootstrap replicates. Bayesian analyses provided measures of nodal support in the form of posterior probabilities (PP). For all analyses, the four sequences were treated as a combined unit, a procedure that, as in our previous studies (Chapco *et al.* 2001; Litzenberger & Chapco 2001a, 2001b), always yielded trees with greater resolution and support when compared to those based on single genes.

In order to place biogeographic events within a geological context, it was important to estimate the times of divergence for various nodes. We initially applied the maximum likelihood ratio test (Page & Holmes 1998) to determine whether sequences evolved in a clock-like manner. Because sequences did not in fact conform to a model of rate constancy, we estimated divergence times by employing a semiparametric penalized-likelihood (PL) method, which can accommodate rates that vary over lineages (Sanderson 2002). To this end, the program r8s, version 1.70 (Sanderson 2004) was used. As recommended by Sanderson, the TN (Truncated Newton) algorithm was applied in conjunction with PL. A cross-validation analysis was first performed to determine the most likely smoothing parameter (a measure of the relative contributions of parametric and nonparametric models that underlie PL), necessary for estimating optimal divergence times. Zero-length branches were collapsed. A more extensive description of the method and theory is given by Sanderson (2002). The program yields estimates of absolute times of divergence if at least one known divergence date is provided as input. Usually these times are based on the fossil record, which in the case of Acrididae is rather poor (Vickery 1989) [To date, the earliest fossil on record that is unequivocally an oedipodinid dates to the Miocene (Stidham & Stidham 2000), too recent for the specimen to be ancestral]. Instead, we relied on the work of Gaunt and Miles (2002) who, calibrating their molecular clock using dated ancient cockroach fossils, estimated the time of split between subfamilies Oedipodinae and Gomphocerinae at about 100 Mya. Accordingly we have used this value to calibrate our chronogram.

Table 1. Species analyzed, locations and GenBank Accession numbers of mtDNA sequences.

Subfamily/Tribe*	Species	Source	Accession Nos CO1, CO2, cytb, nd5
Oedipodinae			
/Acrotylini	*Acrotylus blondeli*	Torodi, Niger[ab]	EF151860, --, EF151895, EF151922
	Acrotylus insubricus	Malatya, Turkey	EF151836, EF151816, EF151870, EF151904
	Acrotylus patruelis	Salobrena, Spain	EF151859, --, EF151894, EF151921
/Aiolopini	*Ailopus simulatrix*	Niamey, Niger[abc]	EF151862, --, EF151897, --
	Aiolopus strepens	Sierra Nevada, Spain	EF151841, EF151821, EF151875, EF151907
	Aiolopus thalassinus	Nerja, Spain	EF151842, EF151822, EF151876, --
	Duroniella fracta	Malatya, Turkey[ab]	DQ230738, DQ230807, DQ230827, DQ230774
	Heteropternis couloniana	Mt Nimba, Guinea[abc]	EF151858, EF151830, EF151892, EF151920
/Arphiini	*Arphia conspersa*	Jameson, SK[d]	EF151839, EF151819, EF151873, --
/Bryodemini	*Angaracris barabensis*	Gansu Prov., China[a]	EF151856,--, EF151890, --
	Bryodema luctuosum	Gansu Prov., China[a]	EF151854, EF151829, EF151888, EF151917
	Circotettix carlinianus	Condie, SK[d]	EF151845, --, EF151879, EF151909
/Chortophagini	*Chorthophaga viridifasciata*	Jameson, SK[d]	EF151864, --, EF151899, EF151924
	Encoptolophus costalis	Regina, SK[d]	EF151850, EF151827, EF151884, EF151913
/Locustini	*Gastrimargus africanus*	Niamey, Niger[abc]	--, EF151831, EF151893, --
	Locusta migratoria	Genbank[abc]	X80245
	Oedaleus decorus	Massif Central, France[abc]	EF151834, EF151814, EF151868, EF151903
	Oedaleus asiaticus	Gansu Prov., China	EF151865, --, EF151900, --
/Macherocerini	*Machaerocera mexicana*	Oaxaca, Mexico[d]	EF151861, --, EF151896, EF151923
/Oedipodini	*Celes variabilis*	Massif Central, France[a]	EF151855, --, EF151889, EF151918
	Oedipoda caerulescens	Massif Central, France[ab]	EF151835, EF151815, EF151869, --
	Oedipoda miniata	Erzingan, Turkey	EF151840, EF151820, EF151874, EF151906
/Psinidiini	*Metator pardalinus*	Last Mountain, SK[d]	EF151849, EF151826, EF151883, EF151912
	Trachyrhachys kiowa	Condie, SK[d]	EF151846, --, EF151880, EF151910
/Sphingonotini	*Dissosteira carolina*	Bimidji, WISC[d]	EF151851, EF151828, EF151885, EF151914
	Spharagemon collare	Jameson, SK[d]	EF151852, --, EF151886, EF151915
	Spharagemon campestris	Condie, SK	EF151838, EF151818, EF151872, --
	Sphingonotus caerulans	Sierra Nevada, Spain[abc]	EF151844, EF151824, EF151878, EF151908
	Sphingonotus yenchihensis	Gansu Prov., China	EF151853, --, EF151887, EF151916
	Sphingonotus nebulosus	Malatya, Turkey	EF151866, EF151832, EF151901, --
	Trimerotropis pistrinaria	Findlater, SK[de]	EF151848, EF151825, EF151882, EF151911
	Trimerotropis pallidipennis	Uspallata, Argentina	EF151863, --, EF151898, --
/Unassigned	*Austroicetes sp.*	Canberra, Australia[c]	EF185880, --, EF185881, --
	Chortoicetes terminifera	Merriwah, Australia[c]	EF185877, EF185876, EF185878, EF185879
	Wernerella pachecoi	El Llano, Canary Is.[b]	EF151857, --, EF151891, EF151919
Acridinae	*Stethophyma gracile*	Last Mountain, SK[ad]	DQ230737, DQ230806, DQ230826, DQ230773
	Stethophyma grossum	Massif Central, France	DQ230735, DQ230804.-- , DQ230771
Gomphocerinae	*Aeropedellus clavatus*	Dilke, SK	DQ230708, DQ230777, EF565468, DQ230741
	Chorthippus curtipennis	Pinawa, MB	DQ230709, DQ230779, DQ230809, DQ230743
	Dociostaurus maroccanus	Montpellier, France	DQ230714, DQ230784, DQ230814, DQ230748
	Paracyptera brevipennis	Montpellier, France	DQ230713, DQ230783, DQ230813, DQ230747
	Stenobothrus zubowskyi	Malatya, Turkey	DQ230716, DQ230786, DQ230816, DQ230750
Outgroup	*Pyrgomorpha conica*	Malatya, Turkey	EU031777, EU031776, EU031778, EU031779

* - according to the OSF2; MB = Manitoba, SK = Saskatchewan, WISC = Wisconsin; Distribution of genera: a – Eurasia, b – Africa, c – Australia, d – North America, e – South America

Ancestral geographic areas were reconstructed with the assistance of the program "DIVA" (Version 1.1) (Ronquist 1996, 1997). Analysis was simplified by collapsing the dataset to genera. Each genus was coded as 0 or 1 according to its presence in Africa, Australia, Eurasia, North America or South America (even though the particular species analyzed was restricted to one continent). For "maxareas", a parameter that limits the range of ancestral distributions, two sets of values were employed: the default (which favors vicariance) and 2 (which favors dispersal). While the results from DIVA proved helpful, some artifacts appeared. For instance land masses assigned to certain nodes proved unlikely as ancestral areas, given the tectonic events during the times suggested by our temporal analysis; consequently output was interpreted and modified, taking those features into account.

Results

The overall A+T content in Oedipodinae is about 69.6%, virtually the same as that obtained for subfamilies Melanoplinae (Litzenberger & Chapco 2001a) and Gomphocerinae (Contreras & Chapco 2006). Base compositions did not differ significantly among the 37 taxa, averaging 31.6% (A), 16.2% (C), 14.2% (G) and 38.0% (T). Across the four genes, spanning up to 2254 bp, 1254 sites were variable, of which 873 were phylogenetically informative.

Based on parsimony analysis, maximum resolution was achieved when sites were weighed according to their rescaled consistency indices (Fig. 1A). Bayesian methods (Fig. 1B) recovered the same associations and branching orders (Fig. 1B), with a couple of minor differences (see below). The ML tree (presented as a chronogram in Fig. 2) is essentially the same as the Bayesian topology, with the exception that the *Aiolopus-Stethophyma* clade is positioned as in Fig. 1A. Bootstrapping using ML was abandoned owing to excessive run-times. Modeltest identified the general time reversal model (GTR) with variable rates (G) and invariable sites (I) as the one best fitting the data; Modeltest parameter estimates were very similar to Bayesian values.

In broad terms, all approaches agree in identifying groups of Old and New World genera, labeled in the figures A to F (note that, between methods, configurations of taxa within some groups vary slightly – see below). On the whole, the Bayesian tree is somewhat less resolved: *Oedipoda* occupies an unresolved position within groups A to C and cluster D occupies an unresolved position within A to E. In the parsimony tree, *Oedipoda* is basal to groups B and C, and in the ML tree it is basal to A. Another difference between the outcomes of the methods concerns group C. It is monophyletic in the Bayesian and ML trees, but paraphyletic (to B) in the parsimony tree. Within group C, the association of *Celes* and *Sphingonotus nebulosus* with other taxa differs among methods. All approaches show that clades D and E (the Australian group) are external to groups A to C. Clade F, consisting of *Encoptolophus* to *Machaerocera*, is outside all remaining oedipodinids, excepting *Duroniella*. The latter is external to the five gomphocerine genera clustered as a monophyletic group.

The following tribes emerged as monophyletic assemblages (the numbers of genera sampled from the complete list in OSF2 are indicated in brackets): Locustini (4/12), Acrotylini (1/2) and Chortophagini (2/4). Remaining tribes: Aiolopini, Bryodemini, Oedipodini, Psinidini and Sphingonotini proved to be nonmonophyletic. Trees in which each tribe was constrained to be monophyletic were constructed and all, except Psinidini, had significantly lower likelihood values compared to that of the ML tree [comparisons

were based on Kishino-Hasegawa and Shimodair-Hasegawa tests, available in PAUP*]. Monophyly on the part of Psinidini, here consisting of *Metator* and *Trachyrhachys*, could not be statistically rejected. Members of some tribes are widely separated phylogenetically. For example, *Heteropternis*, listed among the Aiolopini, belongs to group A, whereas another member, *Aiolopus*, connects with *Stethophyma* within group D.

Discussion

Monophyly or lack thereof

Previously considered part of Acridinae (Bei-Bienko & Mischenko 1964), *Duroniella* is now listed in the OSF2 among the Oedipodinae. However, according to our analysis, *Duroniella* is very closely associated with the Gomphocerinae (99% bootstrap and PP). Whether this position remains invariant will depend on the outcome of studies underway which include members of Acridinae. In any case, without this genus, Oedipodinae can be regarded as monophyletic. Tribal groupings are another matter.

Given a lack of support for monophyly of most tribes, it would appear that the very traits used to define this category are the result of convergence, probably brought about by natural selection factors imposed by similar habitats and conditions. Morphological similarities between continentally-separated, but phylogenetically unrelated, taxa have been noted in other acridids as well and have been similarly attributed to ecological convergence (Amédégnato 1993, Rowell 2005).

It is entirely possible that, with further sampling, Locustini, Acrotylini, Chortophagini, and perhaps Psinidini, may also prove to be nonmonophyletic. Indeed, this is probably true of Locustini, Guliaeva *et al.* (2005) having discovered that one member, *Psophus*, was far removed from *Locusta* and *Oedaleus*. In an earlier work Otte (1984) included the Old World genus *Acrotylus* in Psinidia, but our data provide no support for that association.

Sphingonotini and Bryodemini

Sphingonotini is the largest oedipodine tribe with 22 genera listed in the OSF2, and, according to Rehn (1958) and Otte (1984), has an affinity with Bryodemini. Although the present study does (strongly) support a connection between the two tribes, neither is monophyletic, a conclusion also reached by Guliaeva *et al.* (2005). However, there is a clean separation between North American and Eurasian members (some genera also occur in Africa and Australia; see below) of Sphingonotini and Bryodemini. On the Eurasian side, these elements together with *Wernerella* and *Celes*, form a monophyletic or a paraphyletic group according to the method used. Internal within the North American group is *Circotettix*, which, according to Otte (1984) and the OSF2, is the single North American representative of the otherwise Eurasian Bryodemini. Our results question that particular allocation, and appear to confirm earlier cytological findings (White 1973, Weissman & Rentz 1980, Weissman 1984) which showed that *Circotettix*'s chromosomes greatly resemble those of *Trimerotropis*, and in particular, of those species belonging to "Section B" (in White's scheme). This resemblance is reflected in our phylogeny in which *Circotettix* is directly linked to *T. pallidipennis*, a Section B member. The morphological similarity between *Circotettix* and some members of Bryodemini is probably the result of convergent evolution.

A discord between morphology and molecules, of course, is

not universal, as illustrated by *Wernerella*'s close relationship to *Sphingonotus*. In this case, both genera are very similar morphologically (Bland & Gangwere 1998). *Wernerella* has yet to be assigned to tribe.

A case could therefore be made for redefining the two tribes, along continental lines: a New World group consisting of *Spharagemon*, *Trimerotropis*, *Dissosteira* and *Circotettix* and (if the Bayesian tree proves to be correct) a largely Old World group, consisting of *Sphingonotus*, *Wernerella*, *Bryodema*, *Angaracris* and perhaps *Celes* (Guliaeva *et al.* also connected this genus to *Sphingonotus*, but with moderate bootstrap support). [It should be noted that three species of *Sphingonotus* also occur on some Caribbean islands (Otte 1984), but at least one of them seems to have been recently introduced by humans. The one species of *Sphingonotus* that occurs in Australia (Rentz *et al.* 2004), like most congenerics with Eurasian relatives (see below), is regarded as a recent invader (Key 1959).] Not withstanding these exceptions, the above suggestion of dividing these taxa into Old and New World groups is worth further consideration.

Aiolopus and *Stethophyma*

Rowell and Flook (2004), employing mitochondrial rDNA sequences, positioned the genus *Mecostethus*, a member of the same tribe as *Stethophyma* (Storozhenko & Otte 1994), externally to eight species of Oedipodinae; within the latter, the most basal was *Aiolopus*. However, given that no members of Gomphocerinae or Acridinae (at various times *Stethophyma* had been assigned to one or other subfamily) were included in that study, the subfamily affiliation of *Stethophyma*/*Mecostethus* remained unclear. Curiously, in a recent ordination analysis (Petit *et al.* 2006) of several tegminal characters in Acrididae, *Aiolopus* proved to be far removed from *Stethophyma* but much closer to *Mecostethus*. In contrast to both analyses, our results link *Stethophyma* directly with *Aiolopus*, well within the Oedipodinae. For now, it would appear that the question of *Stethophyma*'s subfamily affiliation has been resolved.

Phylogenetic relationships uncovered among the three species of *Aiolopus* aid in interpreting some earlier findings. In crossing experiments (Fuzeau-Braesch & Chapco 1977), the three species mate quite readily, deposit egg pods, but produce no viable offspring. Among the six possible two-way crosses, those involving *A. strepens* yielded the fewest number of egg pods (none in one reciprocal cross). The extent of reproductive isolation is thus reflected phylogenetically, with *A. strepens* occupying a position external and therefore, more genetically distant to the other two species. Our topology also makes sense biogeographically. *A. strepens* has the most restricted geographical distribution, occurring along the Mediterranean coast; in contrast, *A. simulatrix* and *A. thalassinus* occur throughout Eurasia, Africa and, in the case of *thalassinus*, Australia (Bei-Bienko & Mishchenko 1964, Rentz *et al.* 2004). The basal position of *A. strepens* in our phylogeny would indicate that the common ancestor (of at least these three species) occupied the Mediterranean region and subsequently spread out and diversified.

Austroicetes and *Chortoicetes*

According to Rentz *et al.* (2004), these genera are often confused with one another. They also state that *Austroicetes* superficially resembles *Aiolopus* and *Heteropternis*. Based on their morphological similarities and biogeographic distributions, Key (1954) constructed a provisional phylogeny that first linked eight species of *Austroicetes* to *Chortoicetes* and then that group to *Aiolopus*. Despite these apparent affinities, neither Australian genus has yet been assigned to a tribe. The two genera are most certainly closely related (Fig. 1A, B), but no direct relationship with any of the current tribes emerged in our study. As a pair, they are basal to groups A, B, C and perhaps D (parsimony tree).

Machaerocera and Chortophagini

This clade constitutes the most ancient offshoot at the base of the oedipodinid tree. We are however, unaware of other studies that would suggest this outcome, at least for the Chortophagini. *Macherocera* does appear to share ecological and morphological features with both Oedipodinae and Acridinae and as a result, Otte (1984) regards the genus as a link between the two subfamilies. Nevertheless, the OSF2 lists *Machaerocera* among the Oedipodinae, as the sole member of the tribe Macherocerini. [*Machaerocera* is known to possess an unusual set of multiple chromosome associations during meiosis (Helwig 1942) compared to most Acrididae; this feature, however, seems to have very little phylogenetic value.]

Early history and biogeography of Oedipodinae

The chronogram presented in Fig. 2 is topologically the maximum likelihood tree with lengths between internal and external nodes replaced by estimated times of divergence. The earliest time of oedipodine radiation is the late Cretaceous, about 94 Mya, a date considerably older than Flook and Rowell's (2004) estimate, but in remarkable agreement with Vickery's (1989) date. This initial split gave rise to the basal North American clade and the remaining taxa. Thereafter, major lineages branched off in relatively rapid succession, giving rise to the Australian pair *Austroicetes* and *Chortoicetes* (88 Mya), the cluster consisting of *Aiolopus* and *Stethophyma* (86 Mya), followed by the lineage leading to cluster A, composed of *Locusta* to *Oedipoda* (76 Mya). About 65 Mya, a split between the (largely) Eurasian group C and the (largely) North American cluster B occurred. Thus, the North American oedipodinids, as suspected by Vickery (1989), had both ancient and recent origins.

What then can we say about the place of origin of the subfamily? As a first approximation, DIVA proved useful in identifying ancestral areas and probable dispersal events, but as we shall see, some adjustments were required after geological events were taken into account. DIVA analysis identified three widespread distributional areas as possible roots of Oedipodinae: 1) Eurasia-North America – Australia, 2) North America – Australia, and 3) Eurasia – North America. The first area was recovered when the default "maxareas" option was used, but clearly it can be dismissed, given that by 100 Mya Pangaea had already split into two supercontinents. The last two area outcomes resulted when the "maxareas" was set equal to 2. As with 1), the second possibility is unlikely for the same reason, thus leaving Eurasia – North America (Laurasia) as a logical choice for the place of initial radiation of the subfamily.

DIVA then places Eurasia at each backbone node, except for the ancestor leading to the Australian genera. For that node, DIVA identifies both Australia and Eurasia as sites occupied by that ancestor. Since these two land masses were not conjoined at the time, the implication is that Australia was reached by dispersal. While this is a possibility, it is more likely that movement to the southern continent took place more recently (see below). We therefore decided to assign Eurasia to all the backbone nodes, with North America-Eurasia (Laurasia) at the root. Also, as another adjustment, we decided to regard dispersal as playing the primary role in the establishment of
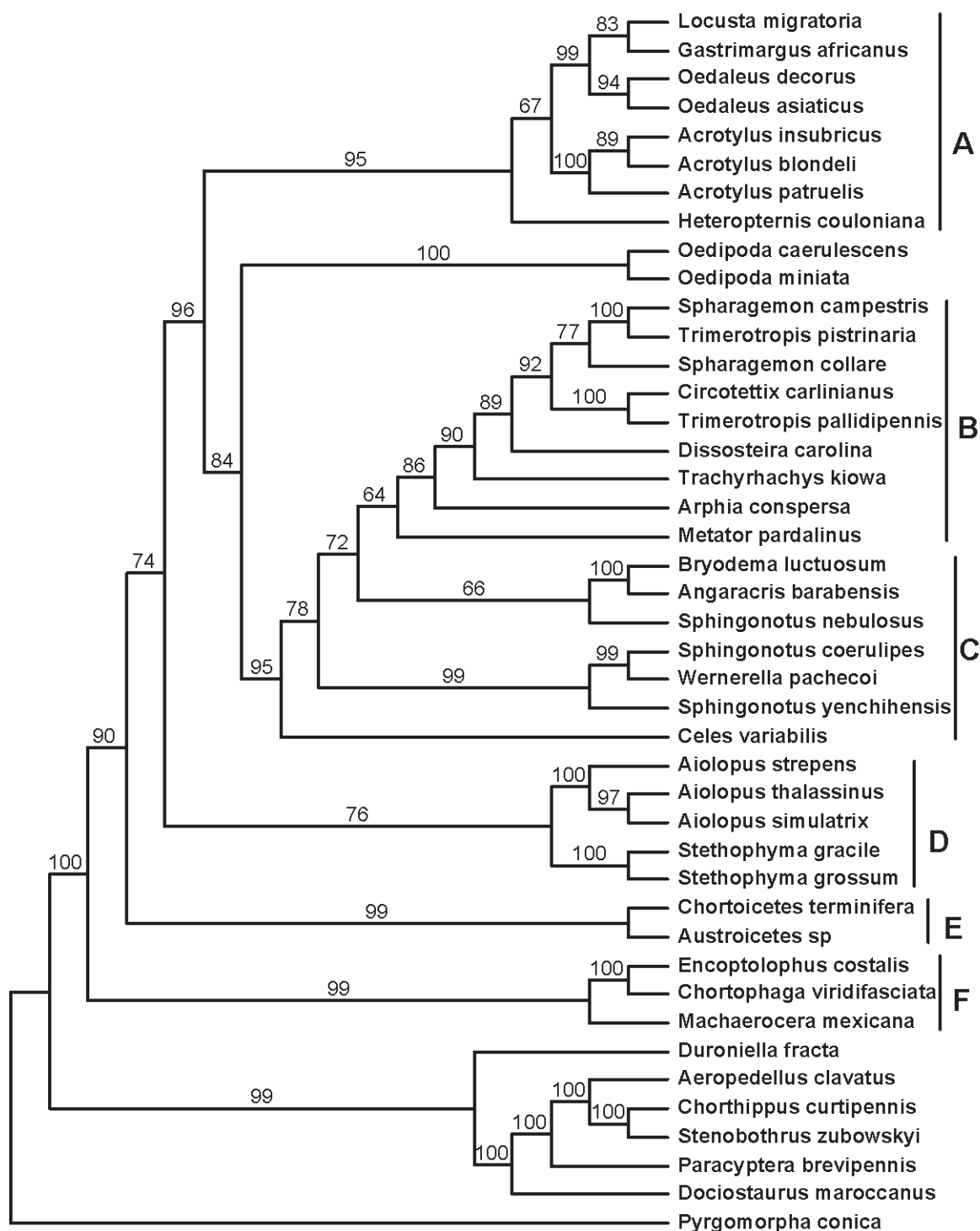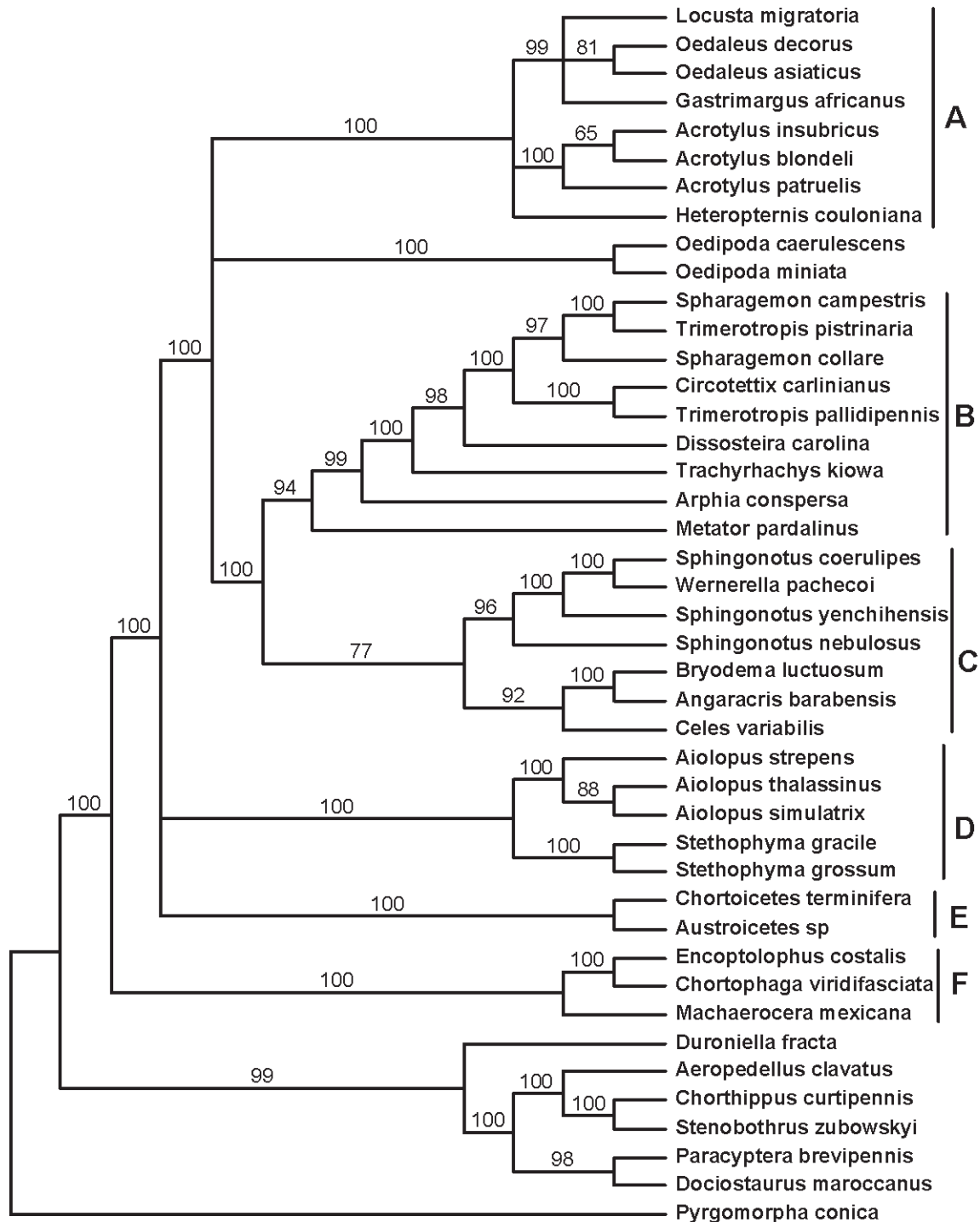
Fig. 1a. Relationships recovered using weighted parsimony. Letters A to F refer to groups of taxa identified in text. Numbers indicate bootstrap levels of support using 1000 replicates.

Fig. 1b. Bayesian tree based on model: GTR + G + I. Eight Monte Carlo Markov chains, one cold and seven heated, were run simultaneously for 1×10^6 generations. Trees were saved every 200 generations, yielding 5000 trees; the last 2500 were used to estimate the topology, parameter values and posterior probabilities (indicated in figure). Parameter estimates are: $R_{AC} = 0.0555$, $R_{AG} = 0.2543$, $R_{AT} = 0.0914$, $R_{CG} = 0.0477$, $R_{CT} = 0.5169$, $R_{GT} = 0.0341$, $\pi_A = 0.3170$, $\pi_C = 0.1409$, $\pi_G = 0.1129$, $\pi_T = 0.4290$, $\alpha = 0.7767$, $p_{inv} = 0.3110$. Letters A to F refer to groups of taxa identified in text.

species distributions because the (two) vicariant events identified by DIVA analysis occurred at a time when the participating continents (*e.g.,* Eurasia and North America) were clearly still together or at least, passage between them was possible. A thoughtful discussion of DIVA's shortcomings when dispersal events predominate can be found in Cook and Crisp (2005). Focusing on dispersal as a major factor is not an unreasonable inference to make considering the flying proclivities of many insect species (*e.g.,* Prüser & Mossakowski 1996, Fuller *et al.* 2005). Indeed, long-distance dispersal is not unheard of in grasshoppers, as illustrated for example by *Chortoicetes* (700 km, Uvarov 1977) and more recently, by the remarkable transatlantic invasion by *Schistocerca* in 1988 (5000 km, Rosenberg & Burt 1999).

As noted, DIVA analysis suggests a vicariant event took place at the base, leading to a split between North America and Eurasia, but contact between these land masses, together constituting Laurasia, was not broken until much later (Askevold 1991). Moreover, approximately 90 Mya, Laurasia consisted of two land masses, Euramerica (eastern North America and Europe) and Asiamerica (western North America and Asia) separated by two epicontinental seas (San Martin *et al.* 2001). Given that the present distribution of the Mexican genus *Machaerocera* overlaps the southern region of the western North American component of Asiamerica, one might provisionally suggest that of the two Laurasian land masses, Asiamerica was the site of oedipodinid radiation. [By about 41 Mya, the common ancestor of *Chortophaga* and *Encoptolophus* had appeared, by which time dispersal into eastern North America was made possible by the disappearance of the Mid-Continental Seaway (San Martin *et al.* 2001)]. Additional support for Asiamerica as the center of initial oedipodinid radiation is that this possibility presents a reasonable explanation for how an early passage to Australia may have occurred (see below).

The lineage giving rise to the Australian clade split off about 88 Mya. Presumably between that time and 44 Mya, when the common ancestor of *Austroicetes* and *Chortoicetes* appeared, their progenitors had somehow dispersed from Laurasia. [While there are several other oedipodines in Australia, a large proportion of them, such as *Gastrimargus* and *Oedaleus*, have congenerics in the Old World (Rentz *et al.* 2004) and are probably the result of recent invasions via New Guinea (Key 1959, Ritchie 1981).] One route may have been via Asia, Southeast Asia and then on to Australia. If passage took place during the earlier part of the time span, long distance dispersal would have been necessary in the latter step, because it was not until 25 to 30 Mya that Australia and Southeast Asia were connected by a series of islands, making island-hopping possible (Jønsson & Fjeldså 2006). It could be argued that, given the vagaries associated with dating molecular clocks and geological events, the upper limit of 30 Mya is not that far removed from 44 Mya. The Australian continent could therefore have been reached in that manner. During 88 to 44 Mya, a range that encompasses a time when climate was favorable to insects in Australia (Raven & Axelrod 1972), alternative pathways might also have been feasible. Two routes, apparently used by other insects, are: Laurasia – South America – Australia via Antarctica (Hundsdoefer *et al.* 2005) and Laurasia – Africa – India – Australia (Fuller *et al.* 2005). The problem with the first scenario is that, as already stated, there are not very many South American oedipodines, and in any case the latter were most likely derived from North American ancestors only recently (Carbonell 1977, Confalonier *et al.* 1998, this study: see *Trimerotropis pallidipennis* below). It is possible, but unlikely, that at the time there were ancestral oedipodinids in South America, these

having since become extinct. Conditions in the Neotropics were certainly favorable during the Early Cenozoic to other subfamilies of Acrididae (Carbonell 1977), and it is therefore difficult to envisage how or why early Oedipodinae in particular might have perished. For the second scenario, that involving Africa and India, at least two transoceanic dispersal events would be required, depending on the timing and positioning of India relative to Africa and Australia as it moved northward toward the Asian continent. For now, our working hypothesis, pending analysis of additional Old-World genera (in particular those endemic to the African continent), is that Oedipodinae originated in Laurasia (probably Asiamerica), and sometime between 88 and 44 Mya, via southeast Asia, reached Australia.
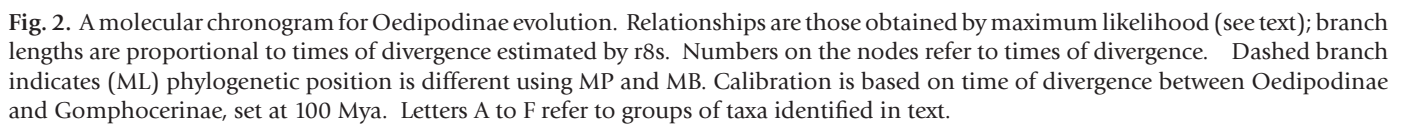
From about 75 to 58 Mya, a series of radiations led to groups D, A, C and B. That Eurasian/African taxa are basal and paraphyletic to the North American clade B, supports the conclusion that this second New World ensemble evolved from Old World ancestors. According to the chronogram, this occurred in the early Tertiary, about 65 Mya. During this period, climatic conditions for insect activity were favorable and both Atlantic and Bering Land Bridges offered possible pathways of incursion (Tiffney 1985, Askevold 1991).

Two sets of species with African connections were analyzed here. The first consists of five species (Table 1) collected in Africa: *Acrotylus blondeli, Aiolopus simulatrix, Heteropternis coulonianus, Gastrimargus africanus,* and *Wernerella pachecoi.* Except for the latter, all these species, or their genera, occur elsewhere in the Old World, and, in some cases, Australia (Dirsh 1965). The second set consists of *Locusta, Oedaleus, Oedipoda,* and *Sphingonotus,* collected in Europe or Asia. All these genera also occur in Africa and except for *Oedipoda,* in Australia.

DIVA analysis indicates that several dispersals from Eurasia to Africa and Australia probably took place recently. We cannot provide dates of entry into Africa for the second set, but we can for the first group of species. Our chronogram (Fig. 2) indicates that *Acrotylus blondeli* and *Wernerella pachecoi* diverged from Eurasian ancestors some time in the late Oligocene, a period when the Arabian Bridge linked Asia and Africa (Jolivet & Faccenna 2000) and when a substantial number of intermigrations took place (Hallam 1994). It is somewhat more difficult to account for the earlier divergence of *Gastrimargus* and *Heteropternis* from Eurasian ancestors, respectively about 50 and 67 Mya, in light of the vast (open-water) distance from Africa during those times (Smith *et al.* 2004). Moreover, if ancestors had come from Asia, as suggested above, then two gaps would have had to be traversed: the Turgai Sea (which did not disappear until 30 Mya (Sanmartin *et al.* 2001) and the still present Tethys Sea, although the latter separating southern Europe and northern Africa would have been rather narrow (Smith *et al.* 2004). Still another avenue from Asia to Africa may have been via India during the latter's northern migration (Briggs 1987). Clearly a wider geographical sampling of Oedipodinae needs to be assayed to help decide which passageway was more likely.

Trimerotropis pallidipennis is the only South American species examined (other museum specimens from that continent were refractory to DNA extraction methods). It branched off from a common ancestor shared with *Circotettix* about 14 Mya, well within the period in which passage between the two Americas would have been possible (Vickery 1989), but somewhat before the estimate of 3 Mya produced by Confalonieri *et al.* (1998) in their analysis of mitochondrial RFLP patterns.

Given the basal position of Old World taxa within clade D, it is

Fig. 2. A molecular chronogram for Oedipodinae evolution. Relationships are those obtained by maximum likelihood (see text); branch lengths are proportional to times of divergence estimated by r8s. Numbers on the nodes refer to times of divergence. Dashed branch indicates (ML) phylogenetic position is different using MP and MB. Calibration is based on time of divergence between Oedipodinae and Gomphocerinae, set at 100 Mya. Letters A to F refer to groups of taxa identified in text.

logical to conclude that the North American species, *Stethophyma gracile*, evolved from Eurasian ancestors, as hypothesized by Vickery (1989). According to our r8s calculations, this species split from its Eurasian counterpart, *S. grossum*, about 40 Mya. This value is approximately in the lower range of values determined previously (Contreras & Chapco 2006). The time of dispersal into North America still remains considerably earlier than the time-frame envisaged by Vickery (1989), namely before the last glaciation.

Acknowledgments

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