

Molecular Phylogeny of the *Ceratosolen* Species Pollinating *Ficus* of the Subgenus *Sycomorus sensu stricto*: Biogeographical History and Origins of the Species-Specificity Breakdown Cases¹

Carole Kerdelhue,² Isabelle Le Clainche, and Jean-Yves Rasplus

CNRS équipe INRA, Laboratoire Populations, Génétique et Evolution, Batiment 13, F-91198 Gif-sur-Yvette Cedex, France

Received March 31, 1998

The 14 species of *Ficus* of the subgenus *Sycomorus* (Moraceae) are invariably pollinated by *Ceratosolen* species (Hym. Chalcidoidea), which in turn reproduce in the fig florets. They are distributed mostly in continental Africa, Madagascar, and the Mascarene and Comoro Islands, but 1 species extends its geographical range all over the Oriental region. Fig-pollinator relationships are usually strictly species specific, but exceptions to the 'one-to-one' rule occur within the group we studied. In order to understand both the biogeographical history of the *Ceratosolen* species associated with *Ficus* of the subgenus *Sycomorus* and the origins of the specificity breakdown cases, we have used cytochrome *b* sequences to reconstruct a phylogeny of the fig wasps. The results show that the pollinators from the Malagasy region and those from continental Africa form two distinct clades, which probably diverged after the crossing of the Mozambique Channel by an ancestral population. The Oriental wasp species show strong affinities with the African species. The two species-specificity exceptions are due to different evolutionary events. The occurrence of the two West African pollinators associated with *F. sur* can be explained by successive speciation events of the mutualistic partner without plant radiation. In contrast, we hypothesize that *C. galili* shifted by horizontal transfer from an unknown, presumably extinct, *Ficus* species to *F. sycomorus* after this native Malagasy fig species colonized Africa. © 1999 Academic Press

INTRODUCTION

Mutualisms have long been underemphasized in ecological studies, but there has been growing evidence

¹ Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. AJ235226 to AJ235252.

² To whom correspondence should be addressed at present address: INRA, Laboratoire Modélisation et Biologie Evolutive, 488 rue Croix de Lavit, F-34090 Montpellier, France. Fax: 33 (0) 4 67 54 94 58. E-mail: kerdelhue@zavez02.ensam.inra.fr.

that they represent a very widespread phenomenon (Begon *et al.*, 1986). Yet, few of these systems have been studied from an evolutionary viewpoint (see for instance (Brown *et al.*, 1994; Pellmyr *et al.*, 1996). Understanding the origins and the maintenance of mutualisms in a phylogenetic context is, however, an outstanding topic of evolutionary biology. In this respect, the fig-pollinator model is of great interest, because of the amazing species diversity of the system and the high specificity of the relationship [see Wiebes (1965, 1979) and Rasplus (1996) for reviews of the specificity breakdown cases]. Moreover, the mutualism between fig trees (*Ficus*, Moraceae) and their pollinating wasps (Hym.: Chalcidoidea: Agaonidae: Agaoninae) is obligate, and it is usually considered as an example of plant-insect coevolution (Ramirez, 1974; Wiebes, 1979; Kjellberg *et al.*, 1987; Anstett *et al.*, 1997). This hypothesis, however, has yet to be supported by conclusive evidence (Lachaise, 1994). The plant strictly depends on its partner for seed setting and pollen dispersal, while the insect relies on the fig ovaries for egg laying and larval development (Galil and Eisikowitch, 1968; Galil, 1977; Janzen, 1979). Unlike information on their taxonomy and ecology, very little is known about fig pollinator evolutionary history, and only a few phylogenetic hypotheses (based on either morphological or molecular characters) have hitherto been proposed to understand the evolution of the fig tree-fig pollinator mutualism (Yokohama, 1994, 1995; Herre *et al.*, 1996; Machado *et al.*, 1996). Most of these studies, however, aimed to resolve the history of pollinator genera in order to test the hypothesis of *Ficus*-Agaoninae coevolution at a higher taxonomic level (i.e., pollinator genera vs fig subgenera and sections). Previous phylogenies of Neotropical pollinators have been reconstructed at the species level (Herre *et al.*, 1996; Machado *et al.*, 1996), but no such studies have been conducted concerning Old World pollinator genera.

Within the genus *Ficus*, the subgenus *Sycomorus* (*sensu stricto*: see Berg and Wiebes, 1992) is a small subunit of 14 monoecious species that shows strong

taxonomic affinities with Asiatic groups of dioecious *Ficus* (subgenus *Sycomorus sensu lato*: Berg, 1989). Its distribution is centered on the Afrotropical region, that is, continental Africa and the Malagasy region (=Madagascar, Comoro Islands, and Mascarene Islands). One of the species, however, *Ficus racemosa* L., has an atypical Oriental distribution (from India to North Australia; see Fig. 1). The pollinators of 12 out of the 14 fig species are described and belong to the genus *Ceratosolen*, subgenus *Ceratosolen* (Wiebes, 1964, 1968, 1981, 1989; Wiebes and Compton, 1990; Berg and Wiebes, 1992; Rasplus *et al.*, unpublished). They are active pollinators with mesothoracic pollen pockets; the behavior and biology of some of them have previously been studied in detail (Galil and Eisikowitch, 1968, 1969a,b, 1971; Galil, 1973; Galil and Eisikowitch, 1974; Abdurahiman and Joseph, 1976, 1981; Kerdelhué *et al.*, 1997). Interestingly, two cases of fig–pollinator specificity breakdown exist within the subgenus *Sycomorus*. One case implicates *Ficus sur*, which is pollinated throughout its geographical range by three different *Ceratosolen* species (Kerdelhué *et al.*, 1997); the other exception to the one-to-one rule is more complicated and involves both *F. sycomorus* and *F. mucosa*. Each of these two fig species is associated with both *Ceratosolen arabicus* and *C. galili*, which occur in sympatry across their whole geographical range. Moreover, it has been proven that the former species is solely responsible for the pollination of *F. sycomorus*. *C. galili*, even though having fully developed pollen pockets, exhibits no pollination behavior and is therefore known to be a ‘cuckoo

wasp’ (Galil and Eisikowitch, 1968, 1969a, 1974; Compton *et al.*, 1991). The same is probably true for *F. mucosa*, as Wiebes, after dissections of wasps found in figs of *F. mucosa*, was unable to find any pollen grains in *C. galili*’s pollen pockets, whereas he found some in *C. arabicus*. *C. galili* is the only nonpollinating aganous species known.

We conducted a phylogenetic study of the *Ceratosolen* species associated with *Ficus* of the subgenus *Sycomorus* to generate evolutionary hypotheses in order to address some major questions about the history of mutualism between fig trees and fig wasps. In particular, this approach can contribute to understanding the affinities between species from Africa and the Malagasy region and clarify the origins of the *Ceratosolen* species endemic to the Mascarene and Comoro Islands. The placement of the Oriental species associated with *F. racemosa* is also of interest. Moreover, reconstructing the group phylogeny will shed light on the exceptions to the one-to-one rule and will enable us to determine whether *Ceratosolen* species occurring in the same host share a recent common ancestor or if one of the pollinators has shifted from a separate host *Ficus*, or in other words, whether the exception is due to a speciation event in the pollinator lineage without host diversification or if the existence of more than one pollinator is due to horizontal transfer. Understanding the phylogenetic affinities of *C. galili* should also help to build hypotheses explaining the lack of pollination behavior in this particular species.

In fig wasps, ecological pressures are expected to be

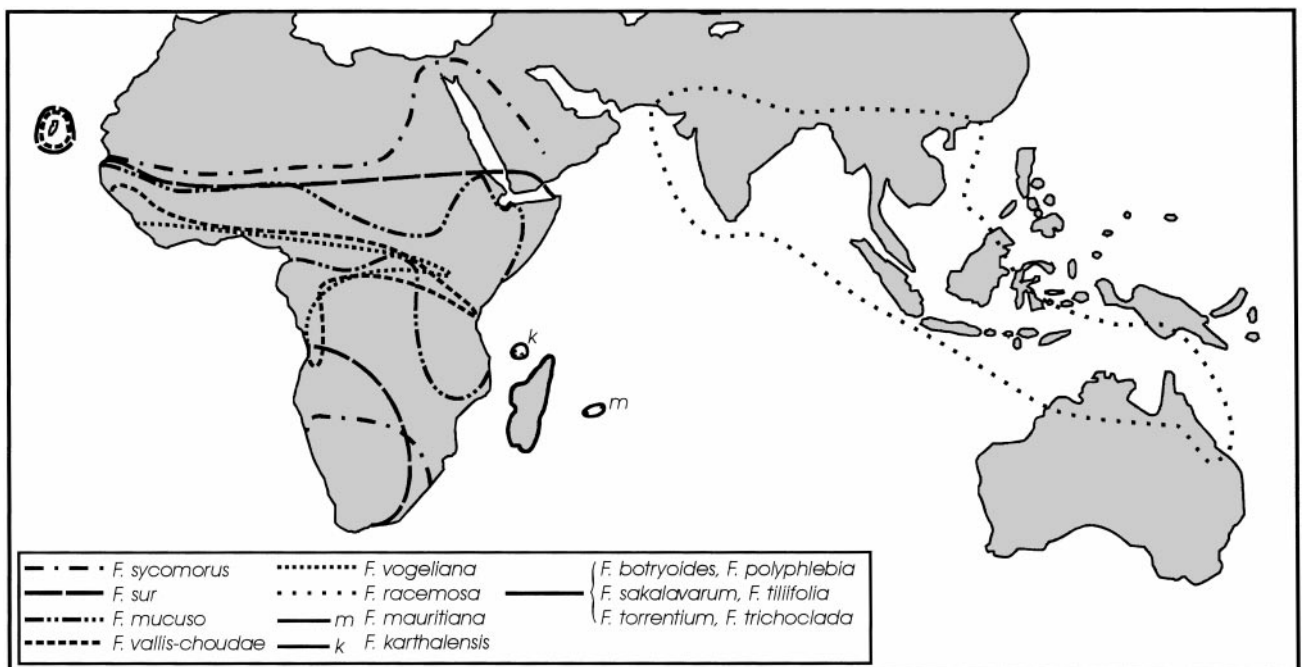


FIG. 1. Geographical distribution of the 14 *Ficus* species of the subgenus *Sycomorus sensu stricto*.

strong. Most female morphological characters are possibly shaped by its host and can be adaptive with regard to some of the features of the figs, such as ostiole length, bract forms, style length, wall width, or host density. Consequently, pollinator morphology is likely to be prone to adaptive convergence and thus to homoplasy. For this reason, we decided to use molecular markers to reconstruct *Ceratosolen* phylogeny. We tested the phylogenetic utility of the mitochondrial cytochrome *b* gene, which has been broadly used in vertebrates (Kocher *et al.*, 1989; Irwin *et al.*, 1991; Janczewski *et al.*, 1995; Ledje and Arnason, 1996; Yoder *et al.*, 1996; Griffiths, 1997), but has not been fully developed in insect phylogenetic studies (Crozier and Crozier, 1993, 1994; Jermini and Crozier, 1994; Crozier *et al.*, 1995; Harry *et al.*, 1998).

MATERIAL AND METHODS

Material and Collection Sites

Among the 14 fig species of the subgenus *Sycomorus*, 4 are restricted to continental Africa (namely *Ficus sur* Forsskål, *F. vallis-choudae* Delile, *F. vogeliana* Miquel, and *F. mucuso* Ficalho); 1 can be found in Africa, in Madagascar, and in the Arabic peninsula (*F. sycomorus* L.); 6 are endemic to Madagascar (*F. tiliifolia* Baker, *F. botryoides* Baker, *F. polyphlebia* Baker, *F. sakalavarum* Baker, *F. trichoclada* Baker, and *F. torrentium* Perrier); 1 is found only in the Comoro Islands (*F. karthalensis* Berg); 1 is restricted to the Mascarene Islands (*F. mauritiana* Lamark); and 1 is distributed in most of the Oriental region (*F. racemosa* L.). The geographical distribution of the 14 *Ficus* is shown in Fig. 1. The host–pollinator relationships, as well as the collecting sites and the collector names, are summarized in Table 1. Several species have been sampled from more than one locality; in particular, *C. arabicus* and *C. galili*, which have the largest geographical ranges, were collected six and five times, respectively. All species but the undescribed pollinators of *F. karthalensis* and *F. torrentium* have been collected and preserved in absolute ethanol. Insect rearing and collections have been described elsewhere (Kerdelhué and Rasplus, 1996; Kerdelhué *et al.*, 1997).

We have strong morphological evidence supporting the monophyly of the genus *Ceratosolen* within the Blastophagini. In particular, convincing apomorphic characters which are absent from all other genera are described in Wiebes (1994) and Boucek (1988). The genus is separated into three subgenera, namely *Ceratosolen* (to which the species studied here belong), *Rothropus*, and *Streptitus*. These latter two subgenera are restricted to the Indo-Australian region and pollinate various dioecious *Ficus* sections. Following Wiebes (1994), we agree that the subgenus *Ceratosolen* is monophyletic with respect to the other two subgenera. The morphological apomorphies shared by species of *Rothropus* on the one hand and by species of *Streptitus*

TABLE 1

Ceratosolen Species Sampled, with Host *Ficus*, Collection Site and Collector Name

<i>Ceratosolen</i>	Host- <i>Ficus</i>	Collection site	Collector
<i>arabicus</i> Mayr	<i>sycomorus</i>	LT, TZ, SA, MA	CK & JYR, S. van Noort
	<i>mucuso</i>	LT, TZ	CK & JYR
<i>galili</i> Wiebes	<i>sycomorus</i>	LT, SA, MA	CK & JYR, S. van Noort
	<i>mucuso</i>	LT, TZ	CK & JYR
<i>silvestrianus</i> Grandi	<i>sur</i>	LT	CK & JYR
<i>flabellatus</i> Grandi	<i>sur</i>	LT	CK & JYR
<i>capensis</i> Grandi	<i>sur</i>	TZ, SA	CK & JYR, S. van Noort
<i>megacephalus</i> Grandi	<i>vallis-choudae</i>	LT	CK & JYR
<i>acutatus</i> Grandi	<i>vogeliana</i>	TAI	CK
<i>namorokensis</i> (Risbec)	<i>sakalavarum</i>	MA	CK & JYR
<i>stupefactus</i> Wiebes	<i>tiliifolia</i>	MA	CK & JYR
<i>blommersi</i> Wiebes	<i>botryoides</i>	MA	CK & JYR
<i>longimucro</i> Wiebes	<i>polyphlebia</i>	MA	CK & JYR
<i>desideratus nomina provis</i>	<i>trichoclada</i>	MA	CK & JYR
<i>coecus</i> (Coquerel)	<i>mauritiana</i>	LR	CK & JYR
<i>fusciceps</i> (Mayr)	<i>racemosa</i>	AUS, IN	G. Weiblen, D. McKey

Note. LT: Lamto, Côte d'Ivoire; TAI: Tai forest, Côte d'Ivoire; TZ: Tanzania; MA: Madagascar; SA: South Africa; LR: La Réunion; AUS: Australia; IN: India; CK: Carole Kerdelhué; JYR: Jean-Yves Rasplus.

on the other hand are considered derived compared to the apomorphies of the subgenus *Ceratosolen*, which can be considered basal. Apart from the pollinators of the monoecious subgenus *Sycomorus* sampled in the present study, the subgenus *Ceratosolen* also comprises 19 described Indo-Australian species associated with dioecious *Ficus* of various sections (Wiebes, 1994). Except for one species (*C. fuscipes*), these latter have not been sampled; the phylogenetic placement of the Oriental species of subgenus *Ceratosolen* is thus not tested here.

Ficus sakalavarum is considered by Berg as a synonym of *F. sycomorus*. However, given that both 'forms' may stand next to each other in the same habitat, while being pollinated by distinct *Ceratosolen* species, we here treat *F. sakalavarum* as a valid species. Moreover, we sampled the pollinator of *F. trichoclada*, which was unknown so far. This species is currently under study and will soon be described by the authors. In the present work, it is referred to as *Ceratosolen desideratus nomina provis*.

Fig pollinators have long been classified in the family Agaonidae (subfamily Agaoninae) together with most fig-inhabiting chalcids (subfamilies Sycophaginae, Sycoryctinae, Epichrysomallinae, Otitesellinae, and Sycocinae; see Boucek, 1988). The Agaonidae were then assumed to form a monophyletic group. This hypothesis has, however, recently been rejected (Rasplus *et al.*, 1998), and the family Agaonidae is now restricted to the fig pollinators. The nonpollinating fig wasps were proven to belong to different chalcid families (in particular to the distant family Pteromalidae), and the pollinators are now thought to be of ancient origin compared to other fig-developing chalcids. These latter groups can thus no longer be considered as outgroups. Concerning the other chalcid families, very little is known about their evolution, and very few hypotheses exist about their phylogenetic affinities. This lack of knowledge is largely due to the difficulty of differentiating the characters shared by common ancestry from those due to adaptive convergence (Gibson, 1993). However, one of the strongest hypothesis based on morphological evidence is that Agaonidae and Torymidae are phylogenetically related (Grissell, 1995). Moreover, within the Torymidae, Megastigminae are seed feeders as the supposed ancestor of Agaonidae, whereas most Chalcidoidea are parasitoids (Boucek, 1988; Grissell, 1995) and this subfamily is considered as the stem group of all other Torymidae. A species of Megastigminae thus seemed to be a good choice to root our phylogenetic trees. As a consequence, *Megastigmus spermotrophus* Wachtl (Torymidae: Megastigminae) was chosen as outgroup in the present study.

DNA Protocols

Total DNA was prepared from adult females using standard phenol-chloroform extraction (Sambrook *et al.*, 1989). We amplified ca. 800 bp of the mitochondrial cytochrome *b* (cyt. *b*) gene using PCR. The primers used were CP1 (5'GATGATGAAATTGGATC3'; Harry *et al.*, 1998) and CB2 (5'ATTACACCTCCTAATTTATTAGG-AAT3'; Jermiin and Crozier, 1994). Using the Promega Taq package, 30 cycles of amplification were performed as follows in 50- μ l reaction volumes: denaturation step at 92°C for 1 min, annealing at 48°C for 1 min and 30 s, and extension at 72°C for 1 min. PCR products were then purified with QIAquick PCR purification kit (QIAGEN) and directly sequenced on an ABI 373 automated sequencer using TaqFS and dye-labeled terminators (Perkin-Elmer). CP1 and CB1 (5'TATGTACTACCAT-GAGGACAAATATC3'; Jermiin and Crozier, 1994) were both used as sequencing primers. All sequences were carefully checked at least twice, and confidence can be given to the obtained data.

Data Analyses

Sequence alignment was performed using ClustalW software (Thompson *et al.*, 1994). When nucleotide sequences were analyzed, we obtained low branch

supports and low resolution of consensus trees, due to a large amount of noise in this kind of data (data not shown). A technique to counter such difficulties when a substantial sequence divergence is apparent is to translate the sequences into amino acids (Swofford *et al.*, 1996). This approach has already been used successfully to analyze cytochrome *b* data (see for instance Normark *et al.*, 1991; Harry *et al.*, 1998), and was judged satisfactory. Only the amino acid sequence analyzes are presented here.

Maximum parsimony analyses (MP) were performed with PAUP 3.1.1 (Swofford, 1993). The most parsimonious trees were obtained by heuristic search with 50 random stepwise taxon addition replicates, using the branch-swapping tree bisection-reconnection (TBR) option. A step matrix was constructed using McClade 3.05 (Maddison and Maddison, 1992) and a protein parsimony analysis was completed with PAUP 3.1.1 using the options stated above. In such an approach, changes between pairs of amino acids are weighted by whether they are adjacent or whether a change to a third, intermediate amino acid is required to bridge the gap between them. In the protein parsimony analysis, a bootstrap procedure (500 iterations with the same options of heuristic search) was used to establish the score of each node (Felsenstein, 1985) by retaining groups compatible with the 50% majority rule consensus. Concerning the tree obtained from the amino acid sequences, decay indices were also determined for each node using AutoDecay 2.9.10 (Eriksson, 1997). Biogeographical characters were mapped onto the phylogeny using McClade 3.07. Constrained trees were sought using PAUP* (written by David L. Swofford).

In addition, analyses were conducted using the distance-matrix method with the Neighbor-Joining (NJ) algorithm (Saitou and Nei, 1987) on *p*-distance, with MEGA (Kumar *et al.*, 1993); a bootstrap procedure of 500 iterations was completed. We also performed a maximum likelihood analysis (quartet puzzling tree search; hereafter ML) with Puzzle 3 (Strimmer and Von Haeseler, 1997) in order to compare the topologies obtained from three different reconstruction methods. The substitution model used was that of Adachi and Hasegawa (1996), adapted to mitochondrial genes (option 'mtREV24'). Other parameters were directly estimated from the data. Reliability of nodes was tested by 1000 reiterations.

RESULTS

Sequences

For all pollinators sampled but *C. acutatus* (associated with *Ficus vogeliana*), we obtained cyt. *b* sequences of 699 bp, corresponding to 233 amino acids (aa). Among these, 92 aa sites are variable and 65 are phylogenetically informative. The A-T content reaches 76.3%, which is similar to A-T contents of drosophilid cytochrome *b* gene (Clary and Wolstenhome, 1985; Garesse, 1988). Table 2 gives the *p*-distance for cyt. *b*

sequencing data, calculated on amino acids. Between-species divergences are high, reaching a maximum of 23% within the ingroup and 29% among ingroup and outgroup. To obtain evidence that such distances do not represent the plateau of maximum saturation at all variable sites, we sequenced a similar portion of the cytochrome *b* gene for genera of Sycoryctinae (namely *Apocrypta*) and Sycophaginae (*Apocryptophagus*, *Sycophaga*, and *Idarnes*) subfamilies. The amino acid distances then calculated between these genera and the *Ceratosolen* ranged from 25 to 42% (Kerdelhue, unpublished data), which can be an indication that the data analyzed here are not saturated. It further shows that the choice of a *Megastigmus* species as outgroup is satisfactory.

The genetic distances calculated in our data set are distributed in four groups (Fig. 2): intraspecific distances ranged from 0 to 0.04; interspecific distances between closely related species ranged from 0.04 to 0.13; interspecific distances between separate species groups ranged from 0.15 to 0.23; genetic distances between any *Ceratosolen* and the outgroup ranged between 0.24 and 0.29. However, it is noteworthy that some intraspecific distances are exceptionally high (especially *C. galili* and *C. fuscipes*), whereas the two most closely related species (*C. silvestrianus* and *C. flabellatus*), which occur in sympatry on the same host, show a genetic distance of 0.04.

Phylogenetic Reconstructions

The phylogenetic reconstructions were performed on subsamples of *Ceratosolen* species containing a maximum of two populations per species. The topologies remain the same regardless of the populations chosen for *C. arabicus* and *C. galili*. The trees obtained from MP and NJ analyses are given in Figs. 3 and 4, respectively, with bootstrap values for both and Decay

indices for MP. The mapping of the species biogeographical distribution obtained by McClade is shown in Fig. 3. The topology of the phylogenetic reconstruction obtained by ML was very similar to that obtained by MP. For the analysis conducted on amino acid sequences, there were 14 most parsimonious trees, 252 steps long, with a consistency index and a retention index of 0.718 and 0.722, respectively. The protein parsimony analysis led to 2 most parsimonious trees, 271 steps long.

Regardless of whether NJ, MP, or ML algorithms were used, the grouping of all African species but *C. arabicus* with the Asiatic taxon *C. fuscipes* as sister species is strongly supported (scores 91, 65, and 99 in NJ, MP, and ML, respectively). Within this group, it is noteworthy that *C. silvestrianus* and *C. flabellatus*, both associated with the same *Ficus* host (*F. sur*), are closely related (distance 0.04; node support 95, 85, and 80 in NJ, MP, and ML, respectively). On the other hand, the exact placement of *C. capensis* (which also pollinates *F. sur*; but in eastern and southern Africa) within the African clade is unclear. It is unresolved in NJ analysis (bootstrap value <50), whereas it is considered as the sister species of *C. silvestrianus* and *C. flabellatus* in the ML algorithm (with a support value of 60) and as a sister group of all other African species in the MP tree. However, when we constrained *C. capensis* into a monophyletic group with *C. silvestrianus* and *C. flabellatus*, we found 2 most parsimonious trees 252 steps long that were included in the 14 minimal unconstrained trees. We thus cannot derive conclusions about the phylogenetic position of *C. capensis* relative to the other African species. Three Malagasy species, namely *C. blommersi*, *C. longimucro*, and *C. desideratus*, form a strongly supported clade whatever the reconstruction method used (scores being 100 for NJ, MP, and ML); the remaining Malagasy species

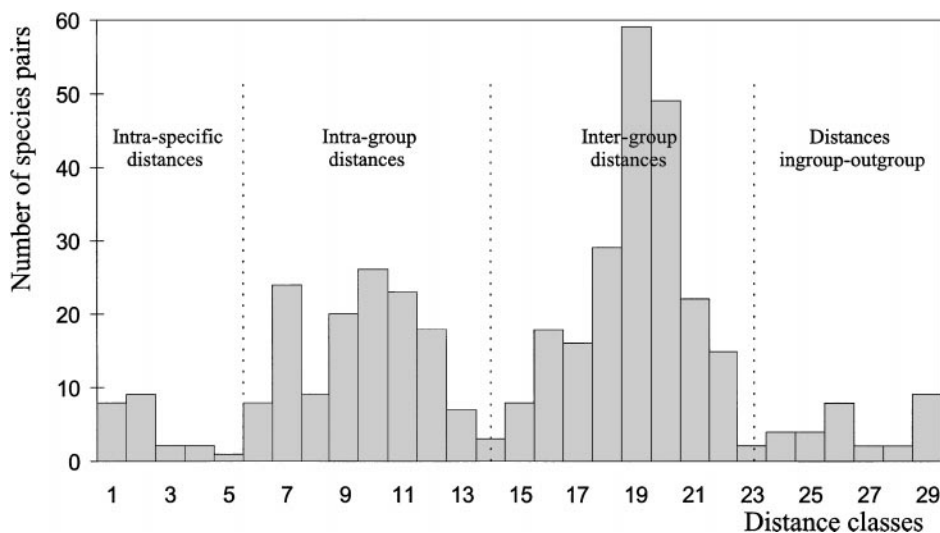


FIG. 2. Histograms of *p*-distance frequencies based on amino acid sequences of cytochrome *b* gene. Each class represents a step of 0.01 (class 1, distances from 0 to 0.01; class 2, from 0.01 to 0.02, . . . class 29, from 0.28 to 0.29).

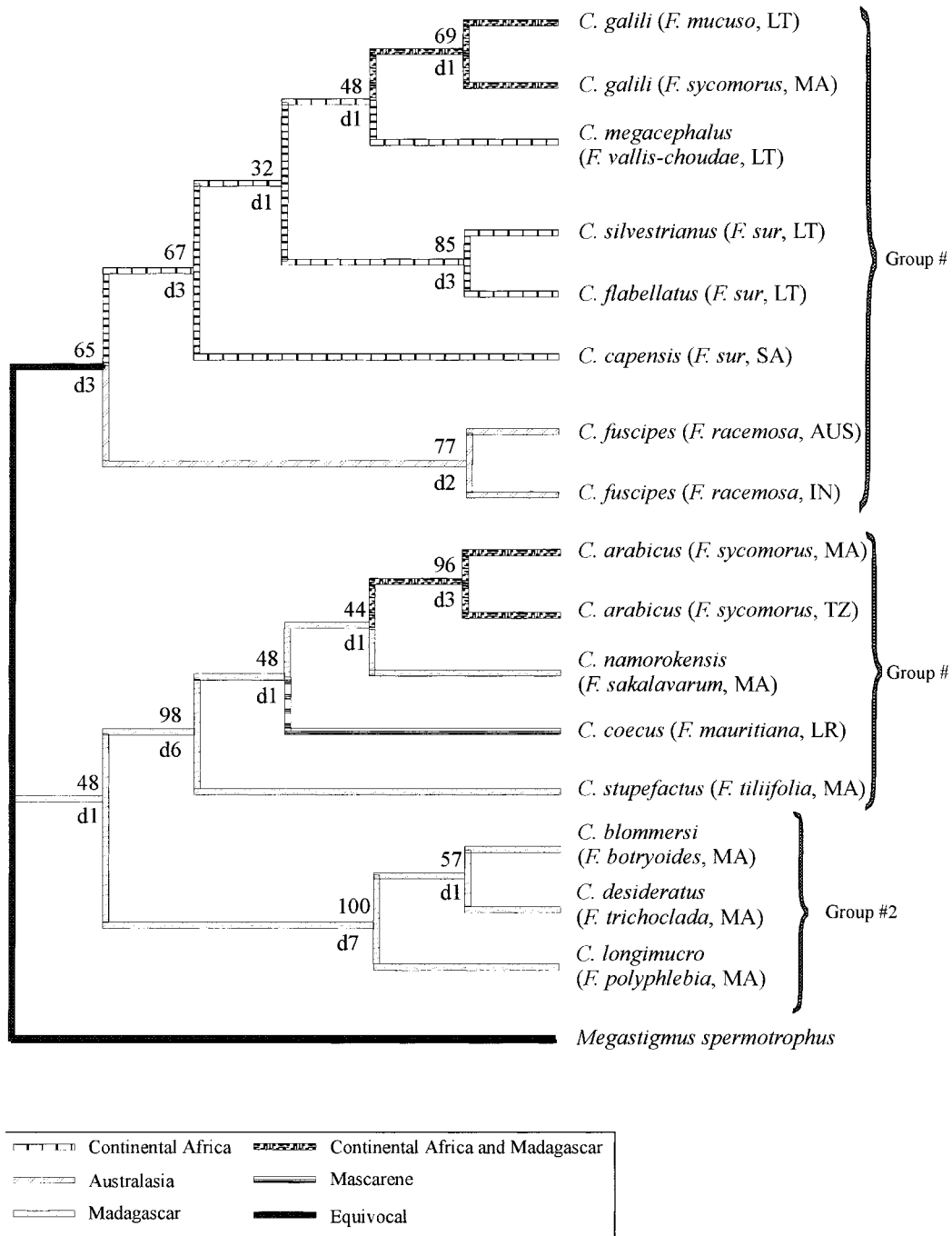


FIG. 3. Phylogenetic tree reconstructed from the amino acid sequences of cyt. *b* gene using maximum parsimony (MP) analysis. Bootstrap values (calculated in a protein parsimony analysis, see text) and decay index value (obtained on the amino acid sequence analysis) are given at each node, above and beneath lines, respectively.

(*C. stupefactus* and *C. namorokensis*) are significantly grouped with *C. arabicus* and *C. coecus* (endemic in the Mascarene). NJ and ML analyses support the clustering of these two species groups (scores 66 and 89, respectively) into a general clade of species from the Malagasy region. However, this node is supported by a lower bootstrap value (48) in the MP tree. Whatever the analysis performed, *C. arabicus* and *C. galili* (both

associated with *F. sycomorus* and *F. mucoso*) unequivocally belong to phylogenetically distinct clades.

DISCUSSION

The Cyt. *b* sequences indicated a robust phylogeny of the *Ceratosolen* associated with *Ficus* of the subgenus *Sycomorus*. The results allow the building of strong

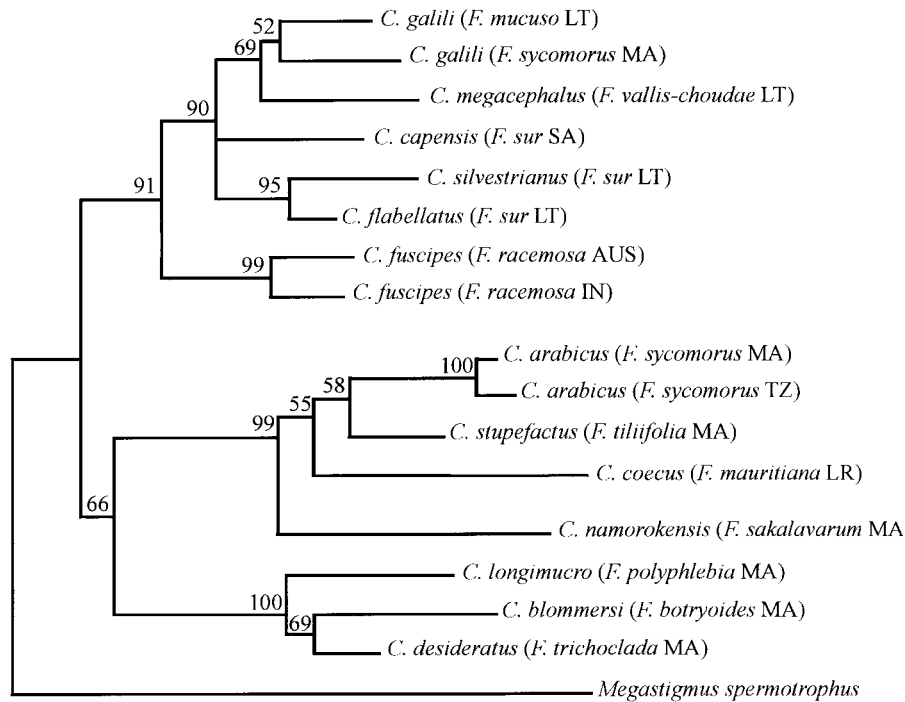


FIG. 4. Phylogenetic tree reconstructed from the amino acid sequences of cyt. *b* gene using neighbor-joining (NJ) analysis. Bootstrap values are given at each node.

evolutionary hypotheses concerning *Ceratosolen* history. In particular, we shall discuss the biogeographical events that occurred within the genus and will analyze the exceptions to the one-to-one rule in the light of the obtained phylogeny.

Biogeographical History

The phylogenetic tree of the *Ceratosolen* associated with the *Ficus* of the subgenus *Sycomorus sensu stricto* is divided into three distinct clades which closely reflect the geographical range of the constituent species. One species group (1) contains all taxa occurring in continental Africa (except for *C. arabicus*) and the Oriental species *C. fuscipes*. The other two clusters (2 and 3) are composed of Malagasy (including *C. arabicus*) and insular *Ceratosolen* and show phylogenetic affinities. Our results suggest two possible evolutionary scenarios. The first is that an ancestral speciation event happened between the African mainland and Madagascar (leading to the splitting into group 1 and groups 2 + 3), closely followed by a second speciation event within Madagascar. A second possibility is that the two Malagasy lineages arose from two independent colonization events. Consequently, the *Ceratosolen* evolutionary history deals with two vicariant main clusters, corresponding to African and Malagasy regions, the latter consisting of two species groups. On the other hand, African and Asiatic *Ceratosolen* appear to be sister groups. However, sampling of the other Oriental *Ceratosolen* species would be necessary to understand the history of the genus between the two biogeographi-

cal areas. Nevertheless, our results favor the hypothesis of a connection between Africa and Asia over that of a colonization corridor between Madagascar and Asia ('stepping stone hypothesis'; see Lachaise *et al.*, 1996; Schatz, 1996).

Given the age of the physical splitting between Africa and Madagascar (160 to 120 million years ago), we can assume that these two blocks were already disconnected when the ancestor of the *Ceratosolen* associated with the *Sycomorus* subgenus arose. This further implies that colonization of Madagascar from Africa occurred through the crossing of the Mozambique Channel by an ancestral population. A similar hypothesis has recently been proposed to explain the colonization of Madagascar by lemurs (Mittermeier *et al.*, 1994; Yoder *et al.*, 1996) and ants (Fisher, 1997). On the other hand, we believe that the migrations between Africa and Asia occurred via a continental connection (Arabic Peninsula) rather than through the contacts that may have existed between Africa and India during the long derivation of the latter (Briggs, 1989).

Concerning the Malagasy species, differences exist in the geographical range, as well as in the behavior of the taxa constituting the clades 2 and 3. One group contains dark-colored species restricted to particular habitats in Madagascar (northern and eastern humid evergreen forest belt for *C. blommersi* and *C. longimicro*, dry southern central highlands for *C. desideratus*). In contrast, the species of the other group are yellowish (which is atypical for *Ceratosolen* and usually associ-

ated with a nocturnal way of life; pers. obs.) and show a wider geographical range: *C. stupefactus* can be found in most parts of Madagascar and has colonized the Comoro Islands; *C. namorokensis* is distributed in north and south Madagascar; *C. coecus* is endemic in the Mascarene islands and has probably arisen from a local speciation event after colonization of the Archipelago by an ancestral Malagasy population; and *C. arabicus* has spread in Madagascar and in most of continental Africa. The phylogenetic cluster 2 thus consists of a group of diurnal species arising from recent local speciation events, whereas cluster 3 is composed of colonizing nocturnal species.

Exceptions to the One-to-One Rule Revisited

Two hypotheses can be proposed to explain the occurrence of more than one pollinator species on a single host fig (Michaloud *et al.*, 1996). (i) In the first evolutionary scenario, the existence of two agaonine species on a single *Ficus* is due to a recent speciation event in the pollinator (due for instance to habitat specialization) not followed by any such event in the host fig (see Fig. 5). (ii) The second scenario is based on the coexistence in a given habitat of two related fig species, each one being pollinated by its own specific wasp. If one of the pollinators shifts to the other fig species, it may be locally maintained on that new host even if its native host becomes extinct. The presence of two pollinating wasps on a single host is then due to horizontal transfer (see Fig. 6). In view of our phylogenetic reconstructions, we will now try to clarify the origin of fig–pollinator specificity breakdowns within the *Ficus* of the subgenus *Sycomorus sensu stricto*.

Ficus sur and its three pollinators. In West Africa, *F. sur* is pollinated by either *Ceratosolen silvestrianus* or *C. flabellatus*, and both pollinators can eventually be

found sympatrically, even in the same fig (Kerdelhué *et al.*, 1997). *C. flabellatus* has also been reported from Uganda and Kenya (Berg and Wiebes, 1992). In eastern and southern Africa, *F. sur* is associated with *C. capensis* (Fig. 7). Interestingly, the genetic distance separating *C. flabellatus* and *C. silvestrianus* is the shortest measured (4%). This suggests that these two species recently diverged from a common ancestor, without speciation in the *Ficus* lineage. In other words, the occurrence of two pollinators in *F. sur* in West Africa can be explained by hypothesis (i) (Fig. 5). *C. silvestrianus*, which has untransformed antennal segments, is specialized for open habitats in which *F. sur* is present at high densities. On the other hand, *C. flabellatus*, which has pectinated antennal segments that increase the number of antennal sensilla (Fig. 8), is favored in forests in which host densities are lowest (Kerdelhué *et al.*, 1997). They can, nonetheless, be found in sympatry in mosaic ‘forest–savanna’ areas such as Lamto. The speciation experienced by the ancestral pollinator of *F. sur* in West Africa could thus be related to habitat specialization and differences in host densities in forests and savannas. We can hypothesize that *C. flabellatus* has secondarily colonized East African forests, but we would need samples collected in East Africa to better understand the history of that species.

On the other hand, our phylogenetic results do not allow us to choose between scenarios (i) and (ii) in the case of *C. capensis*. This species is not significantly closer to *C. silvestrianus* and *C. flabellatus* than to any other continental African *Ceratosolen*. This could be explained by a horizontal host shift (either of *C. capensis* or of the ancestor of the two other species associated with *F. sur*) followed by a geographic separation of the pollinators east and west of the African Rift (hypothesis

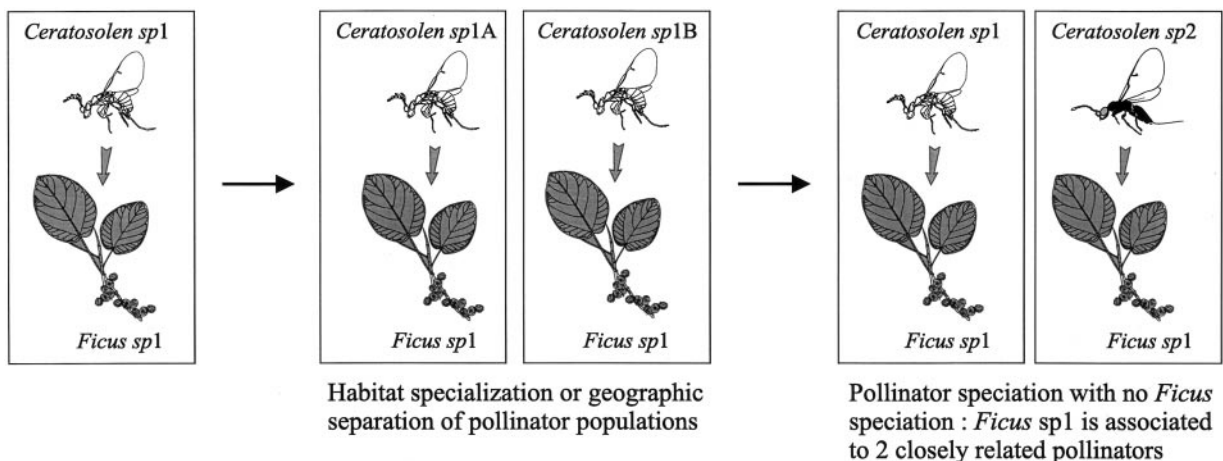


FIG. 5. Schematization of hypothesis (i). The pollinator associated with a fig species can become differentiated into subpopulations if habitat specialization occurs (e.g., forest vs savanna populations) or if geographical gaps or barriers exist over the host distribution (e.g., the African Rift). If there is sufficient reproductive isolation, the populations can further diverge into different, but closely related, species even if the host plant does not speciate. This scenario can explain the existence of both *C. flabellatus* and *C. silvestrianus* on *F. sur* in West Africa (see text for details).

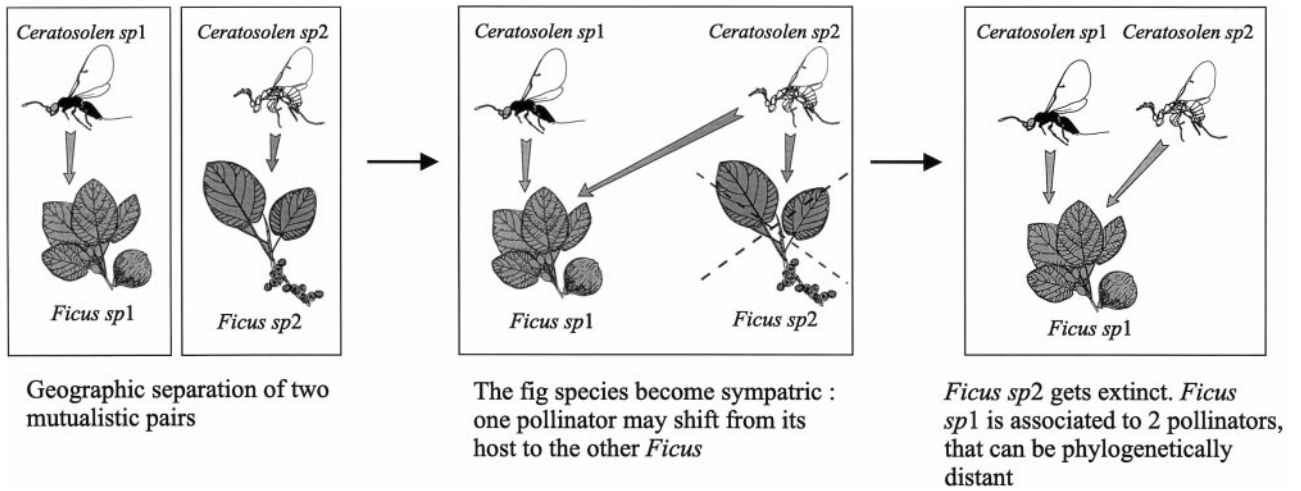


FIG. 6. Schematization of hypothesis (ii). Two fig species evolved independently with their species-specific pollinator in different areas (e.g., *F. sycomorus* and *C. arabicus* in Madagascar, and *F. sp* and *C. galili* in Africa). If one species extends its geographical range and comes in sympatry with the other (e.g., by crossing the Mozambique Channel), subsequent host shift may occur due to biological and/or biochemical affinities of the two hosts. One of the fig hosts is then associated with two phylogenetically distant pollinators (e.g., *F. sycomorus* associated with *C. arabicus* and *C. galili*); the other *Ficus* species might become extinct. See text for details.

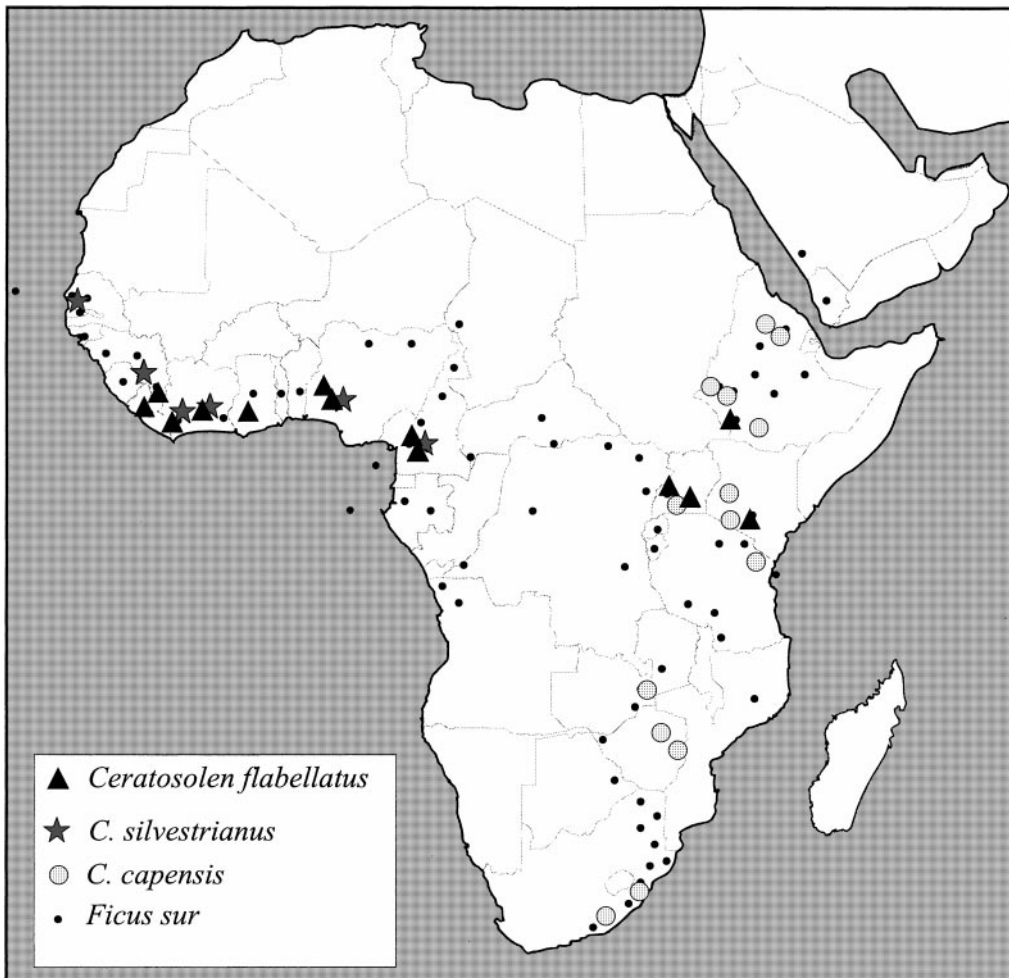


FIG. 7. Geographical distribution of the three pollinators of *F. sur*.

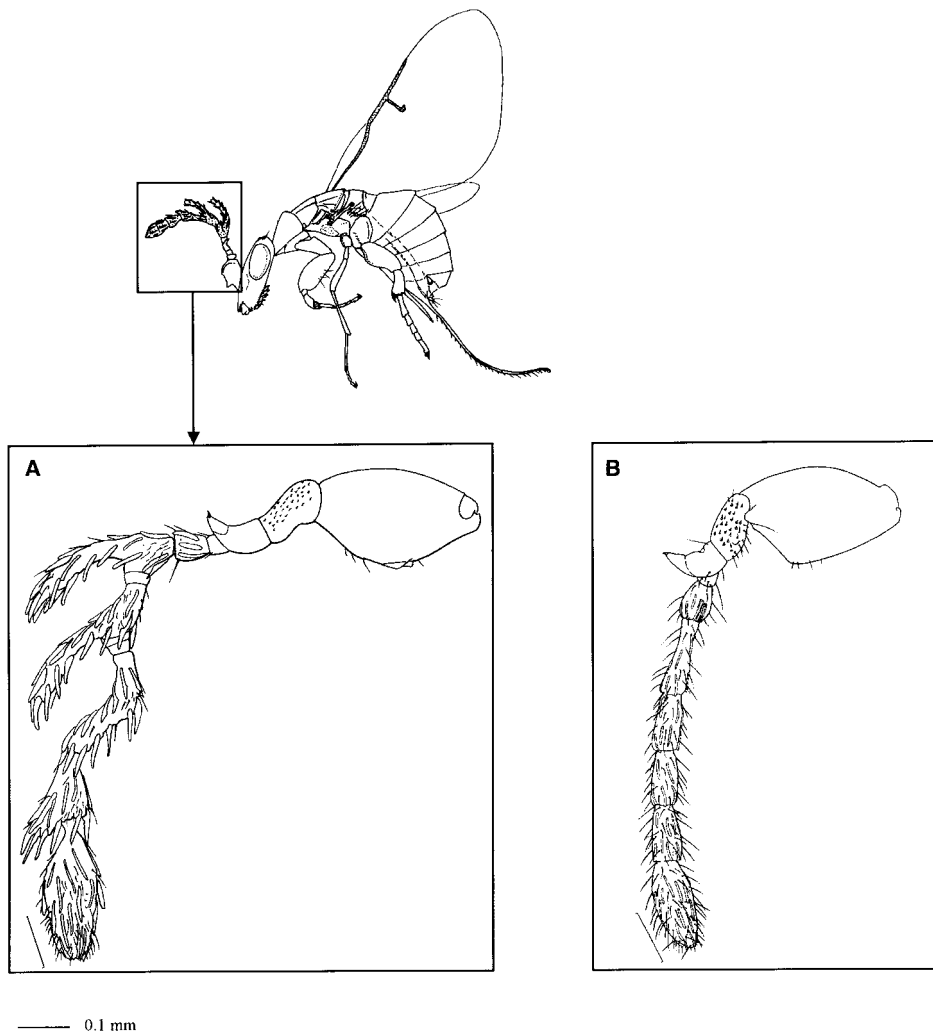


FIG. 8. Antennae of *C. flabellatus* (A) and *C. silvestrianus* (B). (Drawings by E. Barrau).

ii, Fig. 6). However, we cannot rule out the hypothesis of an ancient pollinator speciation (between *C. capensis* and the ancestor of *C. silvestrianus* and *C. flabellatus*) without host speciation (hypothesis i, Fig. 5) when the rift began to form 15 myrs ago, creating a barrier to east-west migrations (Griffiths, 1993; Lovett, 1993). Interestingly, among the 22 African *Ficus* species that are widespread all over Africa, 20 have the same pollinator throughout their whole geographical range and only 2 are not pollinated by the same wasp in eastern and western Africa (namely *F. sur* and *F. ottoniifolia* (Miq) Miq.). The case of *F. sur* is thus the exception rather than the rule and still raises evolutionary as well as biogeographical questions.

C. arabicus and *C. galili*, two species associated with the same hosts. *C. arabicus* and *C. galili* are commonly found in the figs of *F. sycomorus* and *F. mucosa* across all their respective geographical ranges (African mainland and Madagascar). There is now good evidence that *C. arabicus* is the only effective pollinator of

F. sycomorus (Galil and Eisikowitch, 1968, 1969a; Compton *et al.*, 1991) and that the same is most probably true for *F. mucosa* (Wiebes, 1989); *C. galili* does not exhibit any pollination behavior and develops as a cuckoo in respect to the fig-pollinator mutualism. This is the only reported case of loss of pollinating capacity among fig pollinators.

Our results unambiguously show that the two *Ceratosolen* involved belong to clear-cut species groups, as *C. arabicus* is part of the cluster grouping nocturnal species of Madagascar and the Mascarene (cluster 3) and *C. galili* has strong affinities with African *Ceratosolen* species (cluster 1; see Fig. 3). We can definitely reject the hypothesis that the two species arose from a recent speciation event of a common ancestor formerly associated with *F. sycomorus* and *F. mucosa*. On the contrary, we have in that case to invoke a scenario of horizontal host shift (hypothesis ii; Fig. 6) that could be developed as follows.

F. sycomorus and *C. arabicus* probably originated

from Madagascar and secondarily colonized the African mainland where *C. galili* was formerly associated with a distinct African host *Ficus* that has disappeared today. When these two mutualistic pairs became sympatric, *C. galili* could shift to *F. sycomorus* (due to biological and/or biochemical affinities between the two fig species). It was not counterselected when it lost its specific pollination behavior, as seed setting was ensured by *C. arabicus*. It can further be hypothesized that its original host disappeared after *C. galili* lost its pollination capabilities. The occurrence of *C. galili* in Madagascar is probably recent, due to secondarily crossing the Mozambique Channel.

F. mucoso and *F. sycomorus* are two species that are pollinated by the same *Ceratosolen* species, harbor the same 'cuckoo' *Ceratosolen*, and are exploited by the same nonpollinating wasps. Therefore, they also represent an exception to the one-to-one rule in the sense that distinct fig species are associated with the same mutualistic partner. Understanding further this exceptional situation will require a robust evolutionary hypothesis concerning the phylogeny of *Ficus* species, coupled with a fine genetic analysis of *C. arabicus* and *C. galili* populations in order to seek any host race specialization in these species (Bush and Smith, 1997).

CONCLUSION

The study presented here is one of the first attempts to assess the phylogenetic relationships among a genus of *Ficus* pollinators at the species level. It allowed us to erect strong evolutionary hypotheses concerning the possible scenario responsible for the fig-pollinator specificity breakdown cases that were described earlier. We obtained good evidence that one of these exceptional cases is due to speciation of the pollinator without speciation in the host *Ficus*, whereas the other case likely resulted from a horizontal host shift. Such results offer new bases for the understanding of the evolution of fig-pollinator mutualism. Our results also shed light on the biogeographical history of the genus *Ceratosolen* and consequently on that of the subgenus *Sycomorus sensu stricto*. We now need to sample the *Ceratosolen* species associated with Asiatic fig trees to understand further the phylogenetic affinities of African and Asiatic species groups and to propose a global scenario for the distribution of that genus. Moreover, given that Asiatic *Ceratosolen* are associated with dioecious hosts, reconstructing the evolution of the whole genus would allow the creation of hypotheses about the evolution of reproductive strategy in that particular fig lineage.

Finally, morphological characters in Agaonid wasps are very likely to be prone to convergence because of ecological selective forces due to physical features of their associated host. The mitochondrial data set presented here provides a reasonably well resolved phylog-

eny which can be used to critically evaluate the information on morphological characters. It will help clarify which phenotypic traits have evolved under recent ecological pressures and which are shared by common ancestry.

ACKNOWLEDGMENTS

We warmly thank R. Vuattoux, W. Mzirai, L. Rakotovo, and D. Strasberg for providing all facilities for our collecting trips in Côte d'Ivoire, Tanzania, Madagascar, and La Réunion, respectively. We are grateful to J. Kouakou Kouassi, D. Sitoni, and E. Randrianjohany for their technical assistance in the field. We thank the authorities of Côte d'Ivoire, Tanzania, and Madagascar for permission to work in their respective reserves. Many thanks are given to J.-F. Silvain and D. Lachaise for their valuable comments on earlier drafts of the manuscript. J.-F. Silvain also provided us with most useful practical advice and allowed us to use PAUP*. We are grateful to James Cook for his helpful advice on data analyses. We are indebted to E. Barrau for his insect drawings and to S. van Noort, G. Weiblen, and D. McKey for providing us with insect samples. This work was partly financed by a SOFT grant of the French 'Ministère de l'Environnement'. The manuscript was much improved thanks to two anonymous reviewers.

REFERENCES

- Abdurahiman, U. C., and Joseph, K. J. (1976). Observations on the biology and behaviour of *Ceratosolen marchali* Mayr (Agaonidae: Hymenoptera). *Entomon* **1**: 115-122.
- Abdurahiman, U. C., and Joseph, M. (1981). Oviposition behaviour of *Ceratosolen fusciceps* Mayr (Agaonidae) and the mechanism of pollination in *Ficus racemosa* L. *J. Bombay Nat. Hist. Soc.* **15**: 433-442.
- Adachi, J., and Hasegawa, M. (1996). Model of amino acid substitution in proteins encoded by mitochondrial DNA. *J. Mol. Evol.* **42**: 459-468.
- Anstett, M. C., Hossaert-McKey, M., and Kjellberg, F. (1997). Figs and fig pollinators: evolutionary conflicts in a coevolved mutualism. *Trends Ecol. Evol.* **12**: 94-99.
- Begon, M., Harper, J. L., and Townsend, C. R. (1986). "Ecology: Individuals, Populations and Communities," Blackwell Sci., London.
- Berg, C. C. (1989). Classification and distribution of *Ficus*. *Experientia* **45**: 605-611.
- Berg, C. C., and Wiebes, J. T. (1992). "African Fig Trees and Fig Wasps," Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen, Amsterdam.
- Boucek, Z. (1988). "Australian Chalcidoidea (Hymenoptera)," C.A.B. Int., Wallingford.
- Briggs, J. C. (1989). The historic biogeography of India: Isolation or contact? *Syst. Zool.* **38**: 322-332.
- Brown, J. M., Pellmyr, O., Thompson, J. N., and Harrison, R. G. (1994). Mitochondrial DNA phylogeny in the Prodoxidae (Lepidoptera: Incurvarioidea) indicates rapid ecological diversification of *Yucca* moths. *Ann. Entomol. Soc. Am.* **87**: 795-802.
- Bush, G. L., and Smith, J. J. (1997). The sympatric origin of phytophagous insects. *Ecol. Stud.* **130**: 3-19.
- Clary, D. O., and Wolstenhome, D. R. (1985). The mitochondrial DNA molecule of *Drosophila yakuba*: Nucleotide sequence, gene organization, and genetic code. *J. Mol. Evol.* **22**: 252-271.
- Compton, S. G., Holton, K. C., Rashbrook, V. K., van Noort, S., Vincent, S. L., and Ware, A. B. (1991). Studies on *Ceratosolen galili*, a non-pollinating Agaonid fig wasp. *Biotropica* **23**: 188-194.

- Crozier, R. H., and Crozier, Y. C. (1993). The mitochondrial genome of the honeybee *Apis mellifera*: Complete sequence and genome organization. *Genetics* **133**: 97–117.
- Crozier, R. H., and Crozier, Y. C. (1994). The cytochrome *b* and ATPase genes of honeybee mitochondrial DNA. *Mol. Biol. Evol.* **9**: 474–482.
- Crozier, R. H., Dobric, N., Imai, H. T., Graur, D., Cornuet, J. M., and Taylor, R. W. (1995). Mitochondrial-DNA sequence evidence on the phylogeny of Australian jack-jumper ants of the *Myrmecia pilosula* complex. *Mol. Phylogenet. Evol.* **4**: 20–30.
- Eriksson, T. (1997). AutoDecay ver. 2.9.10. Stockholm University, Stockholm.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783–791.
- Fisher, B. L. (1997). Biogeography and ecology of the ant fauna of Madagascar (Hymenoptera: Formicidae). *J. Nat. Hist.* **31**: 269–302.
- Galil, J. (1973). Pollination in dioecious figs. Pollination of *Ficus microcarpa* by *Ceratosolen hewitti*. *Garden's Bull. Singapore* **26**: 303–311.
- Galil, J. (1977). Fig biology. *Endeavour* **1**: 52–56.
- Galil, J., and Eisikowitch, D. (1968). On the pollination ecology of *Ficus sycomorus* in East Africa. *Ecology* **49**: 259–269.
- Galil, J., and Eisikowitch, D. (1969a). Further studies on the pollination ecology of *Ficus sycomorus* L. (Hym., Agaonidae). *Tijdschrift Entomol.* **112**: 1–13.
- Galil, J., and Eisikowitch, D. (1969b). Note on pollen transport, pollination and protection of ovaries in *Ficus sycomorus*. *New Phytol.* **68**: 1243–1244.
- Galil, J., and Eisikowitch, D. (1971). Studies on the mutualistic symbiosis between syconia and sycophilous wasps in monoecious figs. *New Phytol.* **70**: 773–787.
- Galil, J., and Eisikowitch, D. (1974). Further studies on pollination ecology in *Ficus sycomorus*. II. Pocket Filling and emptying by *Ceratosolen arabicus* Mayr. *New Phytol.* **73**: 515–528.
- Garesse, R. (1988). *Drosophila melanogaster* mitochondrial DNA: Gene organization and evolutionary considerations. *Genetics* **118**: 649–663.
- Gibson, G. A. P. (1993). Family Agaonidae. In "Hymenoptera of the World: An Identification Guide to Families" (H. Goulet, and J. T. Huber, Eds.), pp. 610–614. Agriculture Canada Publication, Ottawa.
- Griffiths, C. J. (1993). The geological evolution of East Africa. In "Biogeography and Ecology of the Rain Forests of Eastern Africa" (J. C. Lovett, and S. K. Wasser, Eds.), pp. 9–21. Cambridge Univ. Press, Cambridge, UK.
- Griffiths, C. S. (1997). Correlation of functional domains and rates of nucleotide substitution in cytochrome *b*. *Mol. Phylogenet. Evol.* **7**: 352–365.
- Grissell, E. E. (1995). "Toryminae (Hymenoptera: Chalcidoidea: Torymidae): A Redefinition, Generic Classification, and Annotated World Catalog of Species." Memoirs on Entomology, International, 2, Associated Publishers.
- Harry, M., Solignac, M., and Lachaise, D. (1998). Molecular evidence for parallel evolution of adaptive syndromes in fig-breeding *Lissocephala* (Drosophilidae). *Mol. Phylogenet. Evol.* **9**: 542–551.
- Herre, E. A., Machado, C. A., Bermingham, E., Nason, J. D., Windsor, D. M., McCafferty, S., Van Houten, W., and Bachmann, K. (1996). Molecular phylogenies of figs and their pollinator wasps. *J. Biogeogr.* **23**: 521–530.
- Irwin, D. M., Kocher, T. D., and Wilson, A. C. (1991). Evolution of the cytochrome *b* gene of mammals. *J. Mol. Evol.* **32**: 128–144.
- Janczewski, D. N., Modi, W. S., Stephens, J. C., and O'Brien, S. J. (1995). Molecular evolution of the mitochondrial 12S RNA and cytochrome *b* sequences in the pantherine lineage of Felidae. *Mol. Biol. Evol.* **12**: 690–707.
- Janzen, D. H. (1979). How to be a fig. *Annu. Rev. Ecol. Syst.* **10**: 13–51.
- Jermiin, L. S., and Crozier, R. H. (1994). The cytochrome *b* region in the mitochondrial DNA of the ant *Tetraponera rufoniger*: Sequence divergence in Hymenoptera may be associated with nucleotide contents. *J. Mol. Evol.* **38**: 282–294.
- Kerdelhué, C., Hochberg, M. E., and Rasplus, J. Y. (1997). Active pollination of *Ficus sur* by two sympatric fig wasp species in West Africa. *Biotropica* **29**: 69–75.
- Kerdelhué, C., and Rasplus, J. Y. (1996). Non-pollinating afrotropical fig wasps affect the fig-pollinator mutualism in *Ficus* within the subgenus *Sycomor*. *Oikos* **75**: 3–14.
- Kjellberg, F., Gouyon, P. H., Ibrahim, M., Raymond, M., and Valdeyron, G. (1987). The stability of the symbiosis between dioecious figs and their pollinators: A study of *Ficus carica* L. *Evolution* **41**: 693–704.
- Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo, S., Villablanca, F. X., and Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86**: 6196–6200.
- Kumar, S., Tamura, K., and Nei, M. (1993). MEGA: Molecular Evolutionary Genetic Analysis. Pennsylvania State University, University Park.
- Lachaise, D. (1994). Fig tree-fig wasp assemblage history: The cospeciation versus empty barrel hypothesis. Proc. XIIIth AETFAT Congr. Zomba, Malawi.
- Lachaise, D., Harry, M., and Solignac, M. (1996). Affinités biogéographiques des Drosophilidae de Madagascar et des îles de l'Océan Indien. In "Biogéographie de Madagascar" (W. R. Lourenço, Ed.), pp. 467–478. Editions de l'ORSTOM, Paris.
- Ledje, C., and Arnason, U. (1996). Phylogenetic analysis of complete cytochrome *b* genes of the order Carnivora with particular emphasis on *Caniformia*. *J. Mol. Evol.* **42**: 135–144.
- Lovett, J. C. (1993). Climatic history and forest distribution in Eastern Africa. In "Biogeography and Ecology of the Rainforests of Eastern Africa" (J. C. Lovett, and S. K. Wasser, Eds.), pp. 23–29. Cambridge Univ. Press, Cambridge, UK.
- Machado, C. A., Herre, E. A., McCafferty, S., and Bermingham, E. (1996). Molecular phylogenies of fig pollinator and non-pollinating wasps and the implications for the origin and evolution of the fig-fig wasp mutualism. *J. Biogeogr.* **23**: 531–542.
- Maddison, W. P., and Maddison, D. R. (1992). MacClade: Analysis of Phylogeny and Character Evolution. Sinauer, Sunderland, MA.
- Michaloud, G., Carrière, S., and Kobbi, M. (1996). Exceptions to the one:one relationship between African fig trees and their fig wasp pollinators: Possible evolutionary scenarios. *J. Biogeogr.* **23**: 513–520.
- Mittermeier, R. A., Tattersall, I., Konstant, W. R., Meyers, D. M., and Mast, R. B. (1994). "Lemurs of Madagascar," Conservation International, Washington D.C.
- Normark, B. B., McCune, A. R., and Harrison, R. G. (1991). Phylogenetic relationships of Neopterygian fishes, inferred from mitochondrial DNA sequences. *Mol. Biol. Evol.* **8**: 819–834.
- Pellmyr, O., Thompson, J. N., Brown, J. M., and Harrison, R. G. (1996). Evolution of pollination and mutualism in the *Yucca* moth lineage. *Am. Nat.* **148**: 827–847.
- Ramirez, B. W. (1974). Co-evolution of *Ficus* and Agaonidae. *Ann. Missouri Bot. Garden* **61**: 770–780.
- Rasplus, J. Y. (1996). The one-to-one species-specificity of the *Ficus*-Agaoninae mutualism: How casual? In "The Biodiversity of African plants" (L. J. G. van der Maesen, X. M. van der Burgt, and J. M. van Medenbach de Rooy, Eds.), pp. 639–649. Kluwer Academic, Wageningen, The Netherlands.

- Rasplus, J.-Y., Kerdelhué, C., Le Clainche, I., and Mondor, G. (1998). Molecular phylogeny of fig wasps (Hym. Chalcidoidea): Agaonidae are not monophyletic. *Comptes-Rendus Acad. Sci. (Paris), Série III*. **321**: 517–527.
- Saitou, N., and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual," 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Schatz, G. E. (1996). Malagasy/Indo-Australo-Malesian phylogeographic connections. In "Biogéographie de Madagascar" (W. R. Lourenço, Ed.), pp. 73–83. Editions de l'ORSTOM, Paris.
- Strimmer, K., and Von Haeseler, A. (1997). Puzzle: Maximum likelihood analysis for nucleotide and amino acid alignments. Zoologisches Institut, Universität München, München, Germany.
- Swofford, D. L. (1993). PAUP version 3.1.1. Program and Documentation. Illinois Natural History Museum, Champeigne.
- Swofford, D. L., Olsen, G. J., Waddell, P. J., and Hillis, D. M. (1996). Phylogenetic inference. In "Molecular Systematics," (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.), 2nd ed., pp. 407–514. Sinauer, Sunderland, MA.
- Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). ClustalW: improving sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680.
- Wiebes, J. T. (1964). Fig wasps from Israeli *Ficus sycomorus* and related East African species (Hymenoptera, Chalcidoidea: Agaonidae). *Entomol. Bericht. Amsterdam* **24**: 187–191.
- Wiebes, J. T. (1965). Host specificity of fig wasps (Hymenoptera Chalcidoidea, Agaonidae), Proc. XII Int. Congr. Entomol. London, pp. 95–96.
- Wiebes, J. T. (1968). Fig wasps from Israeli *Ficus sycomorus* and related east African species (Hymenoptera, Chalcidoidea) 2. Agaonidae (Concluded) and Sycophagini. *Zool. Medelingen* **42**: 307–320.
- Wiebes, J. T. (1979). Co-evolution of figs and their insect pollinators. *Ann. Rev. Ecol. Syst.* **10**: 1–12.
- Wiebes, J. T. (1981). The fig insects of La Réunion (Hymenoptera, Chalcidoidea). *Ann. Soc. Entomol. France (N.S.)* **17**: 543–570.
- Wiebes, J. T. (1989). Agaonidae (Hymenoptera Chalcidoidea) and *Ficus* (Moraceae): Fig wasps and their figs, IV. (African *Ceratostenes*). *Proc. Koninklijke Nederlandse Akad. van Wetenschappen (C)* **92**: 251–266.
- Wiebes, J. T. (1994). The Indo-Australian Agaoninae (pollinators of figs). *Verhandelingen der Koninklijke Nederlandsche (Nederlandse) Akademie van Wetenschappen* **92**: 1–208.
- Wiebes, J. T., and Compton, S. G. (1990). Agaonidae (Hymenoptera Chalcidoidea) and *Ficus* (Moraceae): Fig wasps and their figs, VI. (Africa concluded). *Proc. Koninklijke Nederlandse Akad. van Wetenschappen (C)* **93**: 203–222.
- Yoder, A. D., Cartmills, M., Ruvolo, M., Smith, K., and Vilgalys, R. (1996). Ancient single origin for Malagasy primates. *Proc. Natl. Acad. Sci. USA* **93**: 5122–5126.
- Yokohama, J. (1994). Molecular phylogeny and coevolution. *Plant Species Biol.* **9**: 163–167.
- Yokohama, J. (1995). Insect-plant coevolution and speciation. In "Biodiversity and Evolution" (A. Arai, M. Kato, and Y. Doi, Eds.), pp. 115–130. National Science Foundation, Tokyo.