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Geographic structuring into vicariant species-pairs in a wide-ranging, high-dispersal plant–insect mutualism: the case of *Ficus racemosa* and its pollinating wasps

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Abstract *Ficus* and their mutualistic pollinating wasps provide a unique model to investigate joint diversification in a high dispersal system. We investigate genetic structuring in an extremely wide-ranging *Ficus* species, *Ficus racemosa,* and its pollinating wasp throughout their range, which extends from India to Australia. Our samples were structured into four large, vicariant populations of figs and wasps which may correspond to distinct (sub)species, located in India, China-Thailand, Borneo, and Australia. However, the genetically most divergent group was the Indian population for the figs and the China-Thailand population for the wasps, suggesting different evolutionary histories of populations. Molecular dating for the wasps shows that diversification of the pollinator clade is surprisingly old, beginning about 13.6 Ma. Data on both the host fig species and its

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pollinating wasps suggest that strong genetic flow within biogeographic groups over several hundreds of kilometers has limited genetic and morphological differentiation and, potentially, local adaptation. This is probably due to long-distance dispersal of pollinating wasps. The genetic clustering into large geographic units observed in *F. racemosa* and its pollinators is reminiscent of what can be observed in some other high-dispersal organisms characterized by morphology that varies little over huge distances. The implications of strong gene flow for diversification processes and adaptation to different ecological conditions in *Ficus* and their pollinating wasps are just beginning to emerge.

Introduction

A number of highly dispersive organisms present homogeneous morphologies over large expanses, at continental, oceanic or even worldwide scales, suggesting limited genetic differentiation. This is typically the case for some marine organisms whose larvae or adults are planktonic and expected to be passively dispersed by currents (Palumbi 1994; Kinland and Gaines 2003). Terrestrial species that may disperse as aerial plankton (e.g. Bryophytes dispersing as spores) could represent a continental equivalent. Further, even for bryophytes, long distance dispersal may result from dispersal by biotic vectors (Lewis et al. 2014). How speciation and diversification but also how adaptation to local conditions may proceed under such conditions of long distance dispersal is a challenging field of investigation (Palumbi 1994).

In some cases, limited morphological differentiation over huge distances is associated with limited genetic differentiation (Heinrichs et al. 2009; Pokorny et al. 2011). In other cases, molecular studies have revealed situations of separation into large, well-differentiated gene pools corresponding to more or less cryptic species, often allopatric (Thompson et al. 2005; Cunha et al. 2011; Nunes et al. 2011). Such situations of subdivision into very large, morphologically homogeneous units could be explained by (1) morphological stasis associated with lack of genetic drift in very large populations, (2) limited capacity of adaptation to local conditions due to swamping by gene flow and, (3) as a consequence of the two previous points, a possibility of separation into morphologically similar but different species allowing some adaptive divergence among these very large populations, in response to variation in broad regional conditions.

Successful long distance dispersal depends on dispersal capacity *per se* but also on finding favorable habitats for subsequent development, in which competitors (including local populations of the same species), better adapted to local conditions, are absent. For species involved in intricate interspecific interactions this often means finding mutualistic partner organisms (Thompson et al. 2005). There are only limited data on how spatial genetic structure compares within species-pair over large distances ($>10^3$ km) for potentially long-distance dispersing free-living specialized mutualists. In a study on a gobiid fish and a shrimp both presenting pelagic larvae, clear geographic structuring in the fish and lack of geographic structure in the shrimp were documented (Thompson et al. 2005) confirming that similar structuring is not necessarily a prediction in such systems (Alvarez et al. 2010).

In angiosperms, particularly large pollen-dispersal distances have been documented in temperate wind-pollinated trees and, in some cases, effective long-distance fertilization has been confirmed (Kremer et al. 2012). However, these latter cases mainly concern isolated stands, outside the normal range of the species, while within their usual range most pollination occurs at much smaller distances (Buschbom et al. 2011). The current largest average fertilization distance in an angiosperm has been reported in the insect-pollinated genus *Ficus* (Moraceae) (Ashley 2010): a mean pollination distance of 88 km was measured in a population of *Ficus sycomorus* in the Namib Desert (Ahmed et al. 2009). *Ficus*, with 700+ species, constitutes a good model to investigate the consequences of long distance dispersal on adaptation and speciation processes in an obligate mutualism. Long-distance pollen flow is probably frequent in *Ficus*, though not systematic, and is made possible by their mutualistic pollination system (Kjellberg et al. 2005). Indeed, *Ficus* are pollinated by potentially highly dispersive specific wasps that breed exclusively within the urn-shaped inflorescences (called figs or syconia) of their host *Ficus* species. The wasps develop in fig ovules. They become pollen-loaded in their natal fig and leave in search of a tree bearing receptive figs. The wasps are attracted by the scent emitted by receptive figs of their host species (Hossaert-McKey et al. 2010). In many *Ficus* species, due to within-tree reproductive synchrony (Lin et al. 2015), the wasps have to leave their natal tree to find a different tree bearing receptive figs. About half of *Ficus* species are dioecious and are generally shrubs or small trees growing in patches while monoecious *Ficus* trees generally reach the canopy and present more diffuse, less clumped, distributions. In monoecious figs, the freshly emerged wasps ascend above the canopy and let themselves drift in the wind until they encounter the scent of their host species. Then they exit the main wind stream and actively fly upwind to reach the receptive figs (Ware and Compton 1994). On reaching a receptive fig, they enter it, pollinate and oviposit. Such a pollination system allows pollination at low densities and over long distances. Indeed, it combines the long-distance potential of passive dispersal as aerial plankton borne by the wind (Harrison and Rasplus 2006) with the final high-precision delivery ensured by specific insect pollinators. On the other hand pollinators of dioecious figs are less prone to be found above the canopy and hence probably disperse more locally.

Data on local and regional floras of *Ficus* show that all over the tropics, this genus presents high local species diversity compared to other genera but more limited diversity at broader geographic scales, since most species have large distributions, and this is especially true for monoecious *Ficus* species (Harrison 2005). This feature is compatible with the idea that efficient long-distance gene flow may limit local differentiation and hence limit both adaptation to local conditions and local speciation. Indeed data on genetic structuring in South-East China over 1000 km in two dioecious *Ficus* species (*F. pumila* and *F. hirta*) and their pollinators show no sign of genetic isolation by distance (IBD) within species of pollinator and only some IBD in one of the host figs (Liu et al. 2015; Tian et al. 2015). Data at an even larger scale (2800 km) shows the presence of some IBD in the second *Ficus* species (Yu and Nason 2013). Hence, even in dioecious figs, pollinating wasp populations show genetic signatures of widespread dispersal, and this translates into important gene flow in the host figs. Nevertheless the results suggest that in the plant, there is some limitation to gene flow that could allow some local adaptation, while in the insects local adaptation could be largely impeded by gene flow. Parent–offspring dispersal distances of about 200 m were seen in two dioecious fig species in India (Dev et al. 2011). An open question is whether more intense gene flow in monoecious *Ficus* species could limit local differentiation in the plant and hence adaptation to local conditions. Nevertheless genetic data on a monoecious species, *F. insipida*, in the Neotropics, showed some genetic

differentiation between populations located at 500 km distance and even more at 2000 km, but geographic barriers could have facilitated differentiation (Heer et al. 2015).

In most cases, fig pollinating wasps are host-specific (but see Cornille et al. 2012) and co-diversification has been shown to be frequent (Cruaud et al. 2012). Host shifts or non-congruent diversification have also been inferred but mainly between closely related host-species (Cruaud et al. 2012). Nevertheless, accumulating data suggests that some *Ficus* species are pollinated by several more or less cryptic wasp species, often belonging to a single species group (Moe and Weiblen 2010; Chen et al. 2012; Darwell et al. 2014; Yang et al. 2015; Wang et al. 2016). An emerging pattern is that wide-ranging *Ficus* species may be pollinated by different species in different parts of their range (Chen et al. 2012; Moe and Weiblen 2010), or by a different combination of species (Darwell et al. 2014). Strong gene flow within pollinator species may limit adaptation to local conditions. A consequence would be facilitation of the establishment, on a given host, in different parts of its range, of different pollinator species presenting somewhat different physiological requirements, i.e. adaptation to somewhat different conditions (Conchou et al. 2014; Liu et al. 2014; Tian et al. 2015). Such a situation of multiple vicariant pollinator species could explain the narrow physiological plasticity documented in Singapore for a set of pollinators of very widespread *Ficus* species (Jevanandam et al. 2013). With only limited genetic isolation by distance documented in dioecious *Ficus* species, we may wonder whether any isolation by distance will be found in some monoecious *Ficus* species. As their pollinators appear to be much more dispersive, we may wonder whether stronger gene flow in monoecious *Ficus* could lead to a pattern similar to that in pollinators with the establishment of different, vicariant, *Ficus* species constituting cryptic species complexes.

Among monoecious species of fig trees presenting large distributions, *Ficus racemosa* is probably the most studied. It ranges from India to Australia (Berg and Corner 2005). Different sets of biological data suggest both long-distance gene flow and potential separation into different wide-ranging biological units across its range. Indeed, *F. racemosa* pollinators collected in continental South-East Asia between southern China and southern Thailand formed a single population over 1600 km, suggesting potentially strong pollen flow of *F. racemosa* across the region (Kobmoo et al. 2010). In agreement, the scents emitted by receptive figs to attract pollinators did not differ between southern China and southern Thailand, separated by over 800 km. On the other hand, receptive figs from Indian populations, 2700 km away, produced a different scent to attract pollinators (Soler et al. 2011) suggesting that they could be pollinated by different wasp species. Thus, divergence of receptive fig odors could constitute a reproductive barrier if pollinators originating from India and from continental South-East Asia do not recognize the odors produced by *F. racemosa* figs from their non-native region.

Pollinators collected in the Malay Peninsula and in Australia presented substantial sequence divergence suggesting that although they were sister species they may have diverged 16 Ma (Moe and Weiblen 2010). Hence, available data suggest that major ruptures of gene flow may be present for the tree and for its pollinating wasps within the range of *F. racemosa*. Such ruptures may correspond to the separation of *F. racemosa* and its pollinating wasps into distinct vicariant lineages despite long-distance gene flow.

In this work we investigated genetic structuring in *F. racemosa* and its pollinating wasps throughout their range. Our working hypothesis was that, due to long distance gene flow in both pollinators and host trees, genetic diversity would be structured in both species into a set of distinct homogeneous lineages covering large surfaces, constituting a set of vicariant evolutionary units. Such lineages of wasps and tree may abut on the same geographic and climatic barriers leading to similar geographic distributions of the lineages in wasps and

trees. Another prediction was that molecular dating would confirm the old divergence between the different wasp populations throughout the range. A last prediction was that, as there is no theoretical reason why genetic structuring within wasps should mirror genetic structuring within trees (Alvarez et al. 2010), we might expect some major mismatches between genetic histories of the fig tree and its pollinator.

Materials and methods

The study species

Ficus racemosa L., a monoecious species of subsection *Sycomorus*, is a rapidly growing, medium-sized to large tree (25–40 m high), individuals of which produce from 2 to 7 large fruit crops per year (Zhang et al. 2006; Krishnan et al. 2015). It is an abundant species throughout its range and grows mainly along rivers. The pollinator *Ceratosolen fusciceps* Mayr (Hymenoptera, Chalcidoidea, Agaonidae) was first collected on *F. racemosa* at the Bogor Botanical Garden (Java, Indonesia).

Ficus sampling

Ficus racemosa samples were collected throughout its range between February 2005 and July 2006 in six countries from 12 localities for a total of 257 individuals (Fig. 1; Table 1).
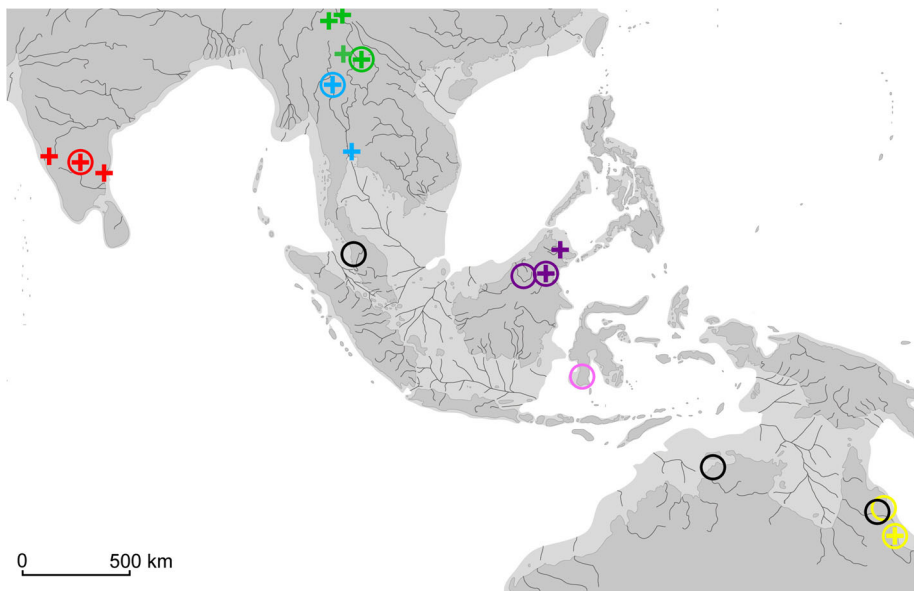

Fig. 1 Sampling locations of *F. racemosa* (*crosses*) and *C. fusciceps* (*circles*) (see Tables 1, 2). Each *color* corresponds to a sampled region (India in *red*, China in *green*, Thailand in *blue*, Borneo in *purple*, Sulawesi in *pink* and Australia in *yellow*). *Black circles* indicate collection locations of the wasp samples sequenced by Moe and Weiblen (2010). The *dark grey background map* indicates currently emerged land. The *light grey zone* shows the extent of emerged land when sea levels were 100 m below current level, i.e. during about 25 % of the last 250,000 years (map after Voris 2000)

Table 1 Sampling locations for *Ficus racemosa* populations

Country	Locality	Coordinates	Sample size
India	Agumbe	13.50°N–75.08°E	29
	Bangalore	12.97°N–77.58°E	19
	Pondicherry	11.93°N–79.83°E	11
China	Dazhuang	24.67°N–98.00°'E	22
	Shitouzhai	24.90° N–98.88°E	23
	Xingfuzhan	22.18°N–100.18°E	23
	Xishuangbanna	21.90°N–101.27°E	64
Thailand	Chiang Mai	18.75°N–98.92°E	16
	Bangkok	13.83°N–100.42°E	5
Borneo/Indonesia	Praktek	03.58°N–116.62°E	12
Borneo/Malesia	Sukau	05.53°N–118.30°E	20
Australia	Townsville	19.32°S–146.75°E	13

We sampled more individuals, and over 100 km distance, in Xishuangbanna, China, than in other localities in order to investigate local spatial genetic structure. Sampled leaves were dried in silica gel and stored dry until DNA extraction.

Wasp sampling

Thirty-two specimens of *Ceratosolen* were included in the study. Among them, 26 *C. fusciceps* specimens were sampled on *F. racemosa* across its entire distribution range (Fig. 1) and one individual each of six related congeneric species on other *Ficus* species (Table 2). Three species from the Chalcidoidea superfamily and belonging to the genera *Ficomila* (Eurytomidae), *Megastigmus* (Torymidae) and *Sycophaga* (Agaonidae: Sycophaginae) were used as outgroups. All material was collected alive and fixed in 95 % EtOH. Morphological species identification was conducted by JYR. A comprehensive list of specimens and sampling localities is given in Table 2.

DNA extraction, PCR amplification and genotyping of plant material

Microsatellite loci were used to analyze fig genetic structuring because microsatellite loci are highly portable, whereas sequence variation is limited in *Ficus*.

Leaves were ground with a Retsch® MM301 mill (Retsch, Haan, Germany) and DNA was extracted with the Qiagen© (Hilden, Germany) DNeasy® plant kit and minikit following the manufacturer's protocol.

Microsatellite primers, both nuclear (24 loci) (Khadari et al. 2001; Vignes et al. 2006; Ahmed et al. 2007) and chloroplastic (24 loci) (Heinze 2007), were tested and those that were most easily scored were selected (9 nuclear, 9 chloroplastic). PCR amplifications were done combining primers using the Qiagen Multiplex© Mix. PCR runs were programmed on a MJ Research Inc.© (Hercules, CA, USA) PTC-100 thermocycler divided in several steps: denaturation at 95 °C during 15 min, then 35 cycles of: 30 s at 94 °C, then 90 s of polymerization and one min of elongation at 72 °C, followed by a last step of

Table 2 List of *Ceratosolen* and outgroup species included in this study: voucher numbers, taxonomic information, host *Ficus* species, and locality data

Voucher number	Genus	Species	Locality	Latitude	Longitude	Host *Ficus* species
0659_23w01x	*Ceratosolen*	*coecus*	La Réunion: Route de Salazie	−21.01303°	55.560597°	*F. mauritiana*
0417_22w01x	*Ceratosolen*	*flabellatus*	Ivory Coast: Lamto	6.222197°	−5.024070°	*F. sur*
1810_01w01x	*Ceratosolen*	*flabellatus*	Burkina Faso: Kourouma	11.612073°	−4.791639°	*F. sur*
1209_01w01a	*Ceratosolen*	*fusciceps*	Indonesia: Kalimantan	3.583187°	116.583337°	*F. racemosa*
1209_01w01b	*Ceratosolen*	*fusciceps*	Indonesia: Kalimantan	3.583187°	116.583337°	*F. racemosa*
1211_04w013	*Ceratosolen*	*fusciceps*	Malaysia: Sarawak: Long San	3.307421°	114.786213°	*F. racemosa*
1211_04w01a	*Ceratosolen*	*fusciceps*	Malaysia: Sarawak: Long San	3.307421°	114.786213°	*F. racemosa*
1426_04w013	*Ceratosolen*	*fusciceps*	Australia: Queensland: Port Douglas	−16.48323°	145.464058°	*F. racemosa*
1426_04w01a	*Ceratosolen*	*fusciceps*	Australia: Queensland: Port Douglas	−16.48323°	145.464058°	*F. racemosa*
2028_01w011	*Ceratosolen*	*fusciceps*	Indonesia: Sulawesi: Pangia	−5.056495°	119.717657°	*F. racemosa*
2028_01w01a	*Ceratosolen*	*fusciceps*	Indonesia: Sulawesi: Pangia	−5.056495°	119.717657°	*F. racemosa*
2701_01w01c	*Ceratosolen*	*fusciceps*	India: Bangalore	12.974131°	77.592896°	*F. racemosa*
2702_01w01c	*Ceratosolen*	*fusciceps*	India: Bangalore	12.974131°	77.592896°	*F. racemosa*
2705_01w01c	*Ceratosolen*	*fusciceps*	India: Bangalore	12.974131°	77.592896°	*F. racemosa*
2706_01w01c	*Ceratosolen*	*fusciceps*	India: Bangalore	12.974131°	77.592896°	*F. racemosa*
2707_01w01c	*Ceratosolen*	*fusciceps*	India: Bangalore	12.974131°	77.592896°	*F. racemosa*
2709_01w01c	*Ceratosolen*	*fusciceps*	India: Bangalore	12.974131°	77.592896°	*F. racemosa*
2714_01w01x	*Ceratosolen*	*fusciceps*	China: Xishuangbanna	21.919967°	101.267083°	*F. racemosa*
2715_01w01x	*Ceratosolen*	*fusciceps*	China: Xishuangbanna	21.938717°	101.249367°	*F. racemosa*
2716_01w01y	*Ceratosolen*	*fusciceps*	China: Xishuangbanna	21.938717°	101.249367°	*F. racemosa*
2717_01w01x	*Ceratosolen*	*fusciceps*	China: Xishuangbanna	21.928058°	101.256908°	*F. racemosa*
2721_01w01x	*Ceratosolen*	*fusciceps*	Thailand: Chiang Mai	18.427450°	98.675367°	*F. racemosa*
2724_01w01y	*Ceratosolen*	*fusciceps*	Thailand: Chiang Mai	18.916050°	98.971167°	*F. racemosa*
2728_01w01y	*Ceratosolen*	*fusciceps*	Thailand: Chiang Mai	18.876617°	98.978700°	*F. racemosa*
2729_01w01a	*Ceratosolen*	*fusciceps*	Thailand: Chiang Mai	19.295833°	98.962733°	*F. racemosa*

Table 2 continued

Voucher number	Genus	Species	Locality	Latitude	Longitude	Host *Ficus* species
2800_01w01c	*Ceratosolen*	*fusciceps*	Australia: Cairns	−16.88614°	145.741019°	*F. racemosa*
2800_01w01d	*Ceratosolen*	*fusciceps*	Australia: Cairns	−16.88614°	145.741019°	*F. racemosa*
2802_01w01b	*Ceratosolen*	*fusciceps*	Australia: Townsville, Cranbrook Park	−19.30651°	146.759748°	*F. racemosa*
2803_01w01d	*Ceratosolen*	*fusciceps*	Australia: Townsville, Riverside Gardens	−19.31872°	146.746648°	*F. racemosa*
1929_01w013	*Ceratosolen*	*megacephalus*	Cameroon: Route Bafang-Baha	4.946767°	10.740467°	*F. vallis-choudae*
1929_01w01x	*Ceratosolen*	*megacephalus*	Cameroon: Route Bafang-Baha	4.946767°	10.740467°	*F. vallis-choudae*
2459_01w013	*Ceratosolen*	*stupefactus*	Madagascar: Ranomafana	−21.23889°	47.425530°	*F. tiliifolia*
1987_02	*Ficomila*	sp.	Malaysia: Sarawak: Pa'Lungan	3.784198°	115.534916°	*F. variegata*
1259_03	*Megastigmus*	sp.	New Caledonia: Maré	−21.59887°	167.938708°	*F.virgata sessilis*
2510_02	*Sycophaga*	sp.	Solomon Islands: Gatokae: Peava	−8.788952°	158.219801°	*F. dissipata*

elongation at 60 °C during 30 min. Nine nuclear microsatellite markers amplified successfully. Eight of these were polymorphic. Of the nine amplified chloroplast microsatellite markers, one was polymorphic.

Post-PCR products were analyzed using an Applied Biosystems (Foster City, CA, USA) ABI 3130 Prism® capillary sequencer. The microsatellite sequences obtained were analyzed and manually checked with ABI Prism® Genemapper™ 3.0.

DNA extraction, PCR amplification and sequencing of wasps

Sequences were used for the pollinating wasps because portability of microsatellite loci is limited in wasps while sequencing is easy and the genes are sufficiently polymorphic.

To infer phylogenetic relationships between species, we combined two nuclear protein-coding genes [F2 copy of elongation factor-1a (*EF1a*, 516 bp), Wingless (*Wg*, 403 bp)]; two mitochondrial protein-coding genes [Cytochrome c Oxidase subunit I (*COI*, 1483 bp), Cytochrome b (*Cyt b*, 745 bp)]; and two ribosomal genes [28S rRNA (D2–D3 and D4–D5 expansion regions, 1301 bp), 18S rRNA (variable regions V3–5, 753 bp)]. Ninety-three sequences were obtained from de novo sequencing for the purpose of this study. Other sequences were obtained from previous work (Cruaud et al. 2010, 2011, 2012).

Genomic DNA was isolated using the Qiagen DNeasy kit (Hilden, Germany) following the manufacturer's protocol without destruction of the specimens. Vouchers were deposited at CBGP, Montferrier-sur-Lez, France. Amplification and sequencing protocols followed Cruaud et al. (2012) and all sequences were deposited in GenBank (Table S1).

Statistical analyses of microsatellite data on *Ficus racemosa*

Within-locality deviations from Hardy–Weinberg equilibrium were tested using exact tests implemented in GENEPOP 4 (Raymond and Rousset 1995). We adjusted for multiple tests using the False Discovery Rate (Benjamini and Hochberg 1995). We calculated expected (H_{exp}) and observed (H_{obs}) heterozygosity using GENALEX 6 (Peakall and Smouse 2006). We calculated the number of alleles for each locality for each locus, and the number of private alleles for each locality. We tested for global and pairwise genetic differentiation between localities using exact tests (G-test on F_{st} values) implemented in GENEPOP 4. We also used ρ statistics (R_{st}) implemented in SPAGeDi v1.2b (Hardy and Vekemans 2002). Significance of ρ statistics was tested by permutations using SPAGeDi. R_{st} values take into account variation in allele length and thus allow categorization of which localities belong to different gene pools (R_{st} larger than F_{st}) (Hardy et al. 2003).

For the cytoplasmic markers, variation was distributed in such a way that no statistical tests could be implemented: the subdivision between large geographic entities was discrete, and the variation among geographic entities was not structured in a way that would have allowed calculating distances.

For the largest sample (from Xishuangbanna, China) we calculated the regression of the pairwise Loiselle kinship coefficients over distance and calculated the significance of the regression using 5000 permutations under SPAGeDi v1.2b.

In order to detect large biogeographic genetic entities, we grouped individual genotypes into genetic clusters without a priori information on origin using the Bayesian approach implemented in the software STRUCTURE v2.3.1 (Pritchard et al. 2000). We used a model without admixture, with a burn-in period of 100,000 and a run length of 1,000,000 varying K from K = 2 to K = 10. For each value of K, ten runs were done. To determine the most relevant number of clusters, we plotted ΔK as described in Evanno et al. (2005).

The best K corresponds to the highest value of ΔK, and alternative K values are adjacent to this maximum. Furthermore, we also constructed an unweighted neighbor-joining tree of all genotypes (Saitou and Nei 1987) using DARwin v5 (Perrier and Jacquemoud-Collet 2006).

Phylogenetic analyses of the wasps

Alignments of protein-coding genes were straightforward due to a lack of length variation. 28S and 18S RNA were aligned with MAFFT 6.864 (Katoh et al. 2005) using the L-INS-i option. Alignments of protein-coding genes were translated to amino acids using Mega 4.0.2 (Tamura et al. 2007) to detect frame-shift mutations and premature stop codons, which may indicate the presence of pseudogenes.

Phylogenetic trees were estimated using maximum likelihood (ML) and Bayesian methods. All the analyses were conducted on a 150 cores Linux Cluster at CBGP. The data were partitioned into mitochondrial and nuclear gene regions. The best-fitting model for each partition was identified using the Akaike information criterion (Akaike 1973) as implemented in MrAIC.pl 1.4.3 (Nylander 2004). We performed ML analyses as in Cruaud et al. (2012) except that we used RAxML version 7.2.8-ALPHA.

The Bayesian analyses follow Cruaud et al. (2012) with the following modifications. The version 3.1.2. of MrBayes was used. For the Metropolis-coupled Markov Chain Monte Carlo (MCMC), 5 million generations sampled every 500 generations were used and finally convergence was also evaluated, using Tracer v1.5 (Drummond and Rambaut 2007). The three *COI* sequences for pollinators of *F. racemosa* obtained by Moe and Weiblen (2011) were retrieved from GenBank and placed within the *COI* phylogeny (RAxML analysis with 1000 bootstrap replicates).

Molecular dating analyses of the wasps

The times of divergence events were estimated using the uncorrelated lognormal relaxed clock method implemented in BEAST 1.7.4. (Drummond and Rambaut 2007). The same modelling strategies as for MrBayes and RAxML were used, and clock models were unlinked. We assumed a Yule tree prior, and used default priors for all other parameters. Two runs of 20 million generations were performed with sampling every 2000 generations. The two separate runs were then combined using LogCombiner 1.7.4. We ensured convergence using Tracer 1.5 (Drummond and Rambaut 2007). Following the removal of 10 % burn-in, the sampled posterior trees were summarized using TreeAnnotator 1.7.4 to generate a maximum clade credibility tree and to calculate the mean ages, 95 % highest posterior density intervals (95 % HPD) and PP. The BEAST chronogram was visualized using FigTree 1.3.1 (Rambaut 2006). Following Cruaud et al. (2012), we used the following calibration constraints:

1. The ingroup node was modelled with a normal distribution with a mean of 38.0 Ma and a standard deviation of 6.5 (95 % highest posterior density intervals (HPD) = 48.7–27.3 Ma).
2. The split between *Ceratosolen stupefactus* and *C. coecus* was assigned a normal prior distribution with a mean of 17.5 Ma and a standard deviation of 3.5 (95 % HPD = 23.3–11.7 Ma).

Results

Genetic differentiation of *Ficus racemosa*

Over all loci and localities, 54 nuclear microsatellite alleles were identified, corresponding to 6.75 alleles per locus (Table S2). The Indian sample from Agumbe presented the largest number of alleles (31) and the Bornean sample from Sukau the lowest number (15). The average number of alleles per locality was 25.5 (Table S3). The number of private alleles was nine (17 % of the total number of alleles) (Table S3).

Heterozygote deficits were heterogeneous among biogeographic regions (Table S2). Indeed, seven out of 15 cases of significant deficits of heterozygotes corresponded to samples from India, suggesting the presence of null alleles. This was reflected by the inbreeding coefficients, which were particularly strong for Indian populations. The inbreeding coefficients were also high for Praktek on Borneo (Table S3), a feature which in that situation was associated with the presence of three monomorphic loci, suggesting local founder effects.

Pairwise kinship coefficients (Loiselle et al. 1995) were independent of geographic distance over 100 km in Xishuangbanna, revealing lack of spatial genetic structure at that scale. Indeed, the coefficients of determination of the linear and logarithmic regressions were very low (respectively 0.000009 and 0.0000004) and non-significant (respectively $p = 0.9424$ and $p = 0.905$).

Global and pairwise F_{st} tests (Table 3) showed that the different sampling locations were genetically differentiated (global test: $p < 0.001$). Eight pairs of populations did not present significant genetic differentiation (out of a total of 66 pairs): all the comparisons between the three Indian sampling locations (three pairwise comparisons), as well as Xishuangbanna-Chiang Mai, Chiang Mai-Bangkok, Bangkok-Xishuangbanna, Bangkok-Xingfuzhan and Bangkok-Dazhuang comparisons. The Bangkok sample was present in half of these pairs, probably owing to its low sample size. Pairwise F_{st} values between localities showed a clear-cut pattern (Fig. 2a). All pairwise comparisons within India and within continental South-East Asia provided low F_{st} values (<0.07, except for comparisons involving the low population size Bangkok sample for which $F_{st} < 0.13$), without any correlation between F_{st} values and geographical distance. All other pairwise comparisons provided uniformly high F_{st} values (>0.32). As a result the relationship of pairwise F_{st} values and geographic distance presented a stepped pattern. The only exception was in Borneo, with high F_{st} value (0.45) between the two samples.

Pairwise R_{st} values were similar to pairwise F_{st} values for all comparisons that did not involve Indian populations. On the other hand, all comparisons involving a population from India and a population from elsewhere provided high R_{st} values, significantly higher than F_{st} values (compare Fig. 2a, b). Coupled F_{st} and R_{st} results suggest that we have two different major gene pools: India and all other samples. Further, within the second group F_{st} values separate continental South-East Asia, the set of two populations within Borneo, and Australia, without any hint in favor of a finer grouping of populations.

The unweighted neighbor-joining tree (Saitou and Nei 1987) grouped individuals according to their geographic origin (Fig. 3). An Indian cluster included all individuals originating from India and no non-Indian individual. A Chinese–Thai cluster included all the individuals from all Chinese and Thai localities. Only two individuals originating from other geographical regions were mixed into that latter cluster: one from Australia and one from Borneo. Individuals from the two localities from Borneo were separated from each

Table 3 Pairwise F_{st} and R_{st} below the diagonal and above the diagonal, significance of the pairwise genetic differentiation (G-test) and significance of the R_{st} values

		Agumbe, India	Bangalore, India	Pondicherry, India	Dazhuang, China	Xingfuzhan, China	Shitouzhai, China
Agumbe, India	F_{st}		NS	NS	***	***	***
	R_{st}		NS	NS	**	***	**
Bangalore, India	F_{st}	−0.0023		NS	***	***	***
	R_{st}	−0.0011		NS	**	***	**
Pondicherry, India	F_{st}	0.0001	0.0104		***	***	***
	R_{st}	−0.0225	−0.0111		*	*	**
Dazhuang, China	F_{st}	0.4060	0.4069	0.4444		***	***
	R_{st}	0.7388	0.6623	0.7634		NS	NS
Xingfuzhan, China	F_{st}	0.4173	0.4178	0.4554	0.0388		***
	R_{st}	0.6997	0.6311	0.6968	0.0617		NS
Shitouzhai, China	F_{st}	0.3526	0.3653	0.4088	0.0688	0.0451	
	R_{st}	0.7148	0.6422	0.7249	−0.0036	0.0029	
Xishuangbanna, China	F_{st}	0.4126	0.4054	0.4281	0.0141	0.0182	0.0652
	R_{st}	0.7133	0.6502	0.6871	0.0045	0.0109	−0.0096
Chiang Mai, Thailand	F_{st}	0.3239	0.3367	0.3616	0.0121	0.0413	0.0367
	R_{st}	0.6309	0.5629	0.6324	0.0086	0.0460	−0.0005
Bangkok, Thailand	F_{st}	0.3266	0.3489	0.3843	0.0722	0.0880	0.1227
	R_{st}	0.6453	0.5635	0.6829	0.0428	−0.0510	−0.0410
Praktek, Borneo	F_{st}	0.4624	0.4830	0.5500	0.4644	0.5136	0.3927
	R_{st}	0.7092	0.6148	0.7337	0.3239	0.3385	0.3068
Sukau, Borneo	F_{st}	0.5090	0.5102	0.6010	0.5796	0.5991	0.5220
	R_{st}	0.8693	0.8139	0.9198	0.7806	0.7755	0.7619
Queensland, Australia	F_{st}	0.4407	0.4534	0.5136	0.4376	0.4604	0.3819
	R_{st}	0.7447	0.6709	0.7975	0.4324	0.5106	0.4564

Table 3 continued

		Xishuangbanna, China	Chiang Mai, Thailand	Bangkok, Thailand	Praktek, Borneo	Sukau, Borneo	Queensland, Australia
Agumbe, India	F_{st}	***	***	***	***	***	***
	R_{st}	**	***	**	***	**	*
Bangalore, India	F_{st}	***	***	***	***	***	***
	R_{st}	*	***	**	**	**	*
Pondicherry, India	F_{st}	***	***	***	***	***	***
	R_{st}	*	***	***	***	**	*
Dazhuang, China	F_{st}	***	*	NS	NS	NS	***
	R_{st}	NS	NS	NS	NS	NS	NS
Xingfuzhan, China	F_{st}	*	*	NS	NS	NS	***
	R_{st}	NS	NS	NS	NS	NS	NS
Shitouzhai, China	F_{st}	***	*	**	***	***	***
	R_{st}	NS	NS	NS	NS	NS	NS
Xishuangbanna, China	F_{st}		NS	NS	***	***	***
	R_{st}		NS	NS	NS	NS	NS
Chiang Mai, Thailand	F_{st}	−0.0045		NS	***	***	***
	R_{st}	0.0031		NS	NS	NS	NS
Bangkok, Thailand	F_{st}	0.0340	0.0115		***	***	***
	R_{st}	−0.0381	−0.0518		NS	NS	NS
Praktek, Borneo	F_{st}	0.4292	0.3753	0.5310		NS	***
	R_{st}	0.2695	0.1846	0.3315		NS	NS
Sukau, Borneo	F_{st}	0.5081	0.5152	0.6305	0.4544		***
	R_{st}	0.6821	0.7020	0.8822	0.7700		NS
Queensland, Australia	F_{st}	0.3869	0.3384	0.4636	0.3966	0.5612	
	R_{st}	0.3980	0.3252	0.5597	0.3355	0.6682	

NS not significant

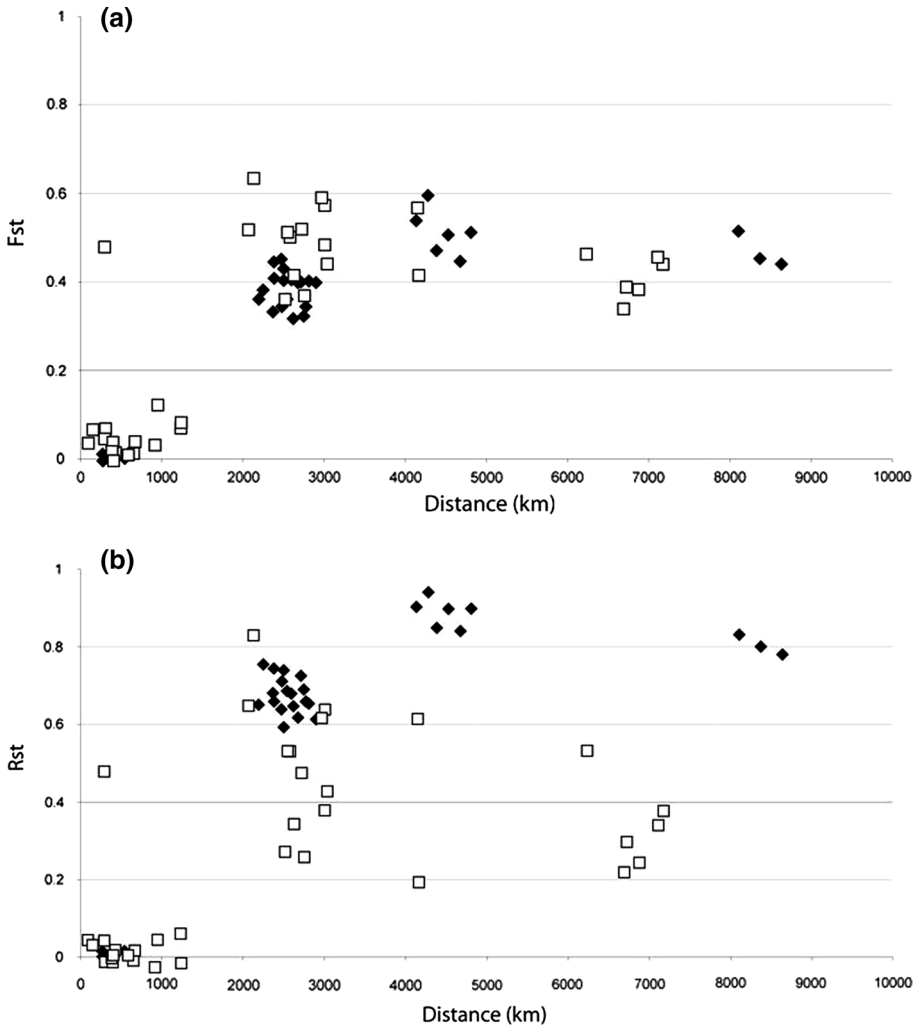*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Fig. 2 Pairwise F_{st} (**a**) and R_{st} (**b**) values between populations of *F. racemosa* according to geographic distance (*squares* for comparisons excluding Indian populations and *diamonds* for comparisons including Indian populations). R_{st} values are significantly higher than F_{st} values for all pairs of populations involving an Indian population and a population from outside India (*diamonds*, for distances above 1500 km). Note the higher variance of R_{st} values comparatively to F_{st} values

other but formed jointly a single group distinct from other localities. One Australian individual was grouped with the individuals from Borneo. Globally we observed three geographic clusters of individuals: India, Continental South-East Asia (China–Thailand population) and Insular South-East Asia plus Australasia (Borneo and Australian populations).

The Bayesian clustering analysis (Pritchard et al. 2000) gave the same configuration. The highest ΔK value was achieved for K = 2 (Fig. S1), and produced in most runs two perfectly separated geographic groups: the individuals from India versus all other
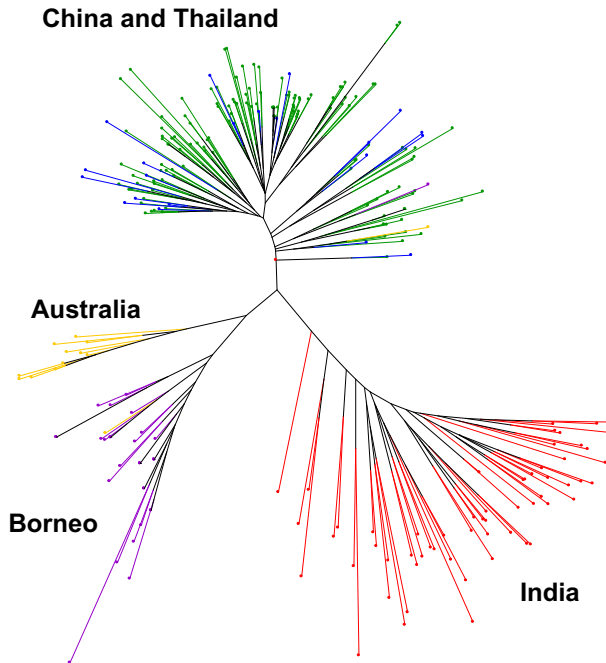
Fig. 3 Unrooted unweighted Neighbor-joining tree of the analyzed genotypes of *Ficus racemosa*. The same color code is used as in Fig. 1 (India in *red*, China in *green*, Thailand in *blue*, Borneo in *purple* and Australia in *yellow*)

individuals (Fig. S1). In some runs, some individuals from Borneo clustered with the Indian group. The alternative number of clusters (K = 3) separated the individuals into three geographic groups: the individuals from India, the individuals from the China–Thailand group and finally the individuals from Borneo–Australia (Fig. S1).

Finally, the polymorphic chloroplast locus (CCMP2 in Heinze 2007) confirmed this genetic differentiation in four clusters and presented mainly a single specific allele characterizing each geographic group: allele 242 in India, allele 250 in China–Thailand, allele 249 in Borneo, and allele 252 in Australia. There were a few exceptions, namely two individuals from Xishuangbanna (China), one presenting the Indian allele and the other presenting the Borneo allele, and one individual from Bangalore (India) presenting the Chinese–Thai allele. We detected three rare alleles: one was obtained twice in Bangalore, India (allele 251); one was obtained once in Agumbe, India (allele 216) and the last one once in Chiang Mai, Thailand (allele 238).

Genetic differentiation of the pollinating wasps

Alignment of protein-coding genes revealed no stop codons or frame shifts. Models chosen by MrAIC for each partition were as follows: GTR + Γ (mitochondrial), GTR + I + Γ (nuclear). Given that α and the proportion of invariable sites cannot be optimized independently from each other (Gu 1995) and following Stamatakis's personal recommendations (RAxML manual), we used GTR + Γ with four discrete rate categories for both partitions.
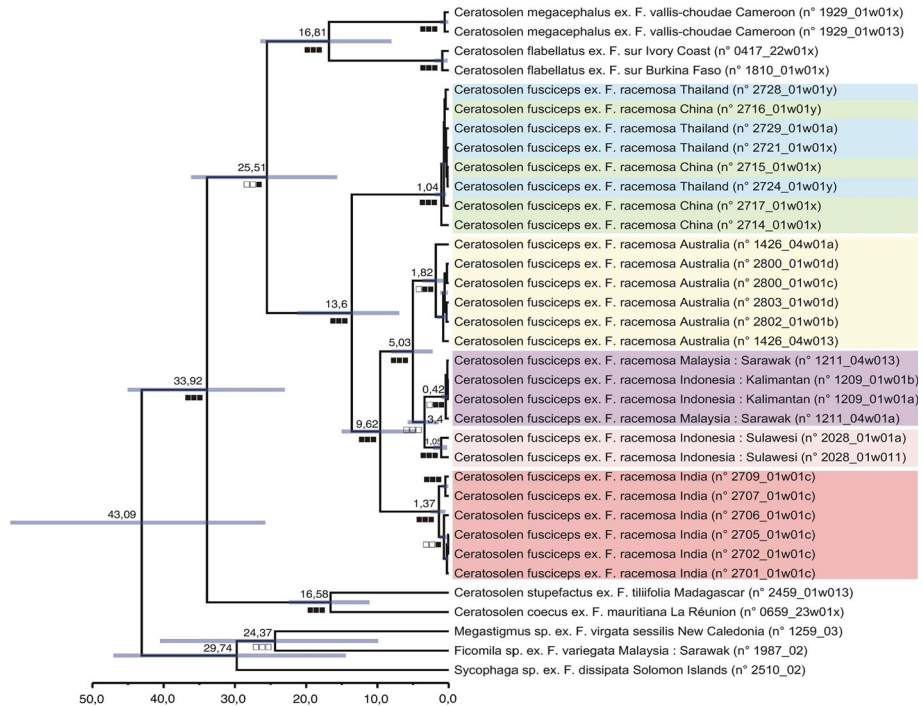
Fig. 4 BEAST chronogram showing the timing of evolution of the pollinating wasps associated with *Ficus racemosa*. *Grey bars* around node ages (Ma) indicate the 95 % highest posterior density (HPD) intervals. The geological time-scale is shown at the *bottom*. *Squares* correspond to node supports and are respectively given for RAxML, MrBayes and BEAST analyses. *Black squares* highlight bootstrap values >70 or posterior probability >0.95. The same color code is used as in Fig. 1 (India in *red*, China in *green*, Thailand in *blue*, Borneo in *purple*, Sulawesi in *pink* and Australia in *yellow*)

For each partition, BEAST returned a 95 % credible interval for the coefficient of variation of rates that was not abutting against zero, suggesting among-branch rate heterogeneity (i.e. Rejection of the molecular clock). Furthermore, the covariance statistics showed no strong evidence of autocorrelation of rates in the two combined phylogenies (covariance values spanning zero). RAxML, MrBayes and BEAST topologies were similar and we chose to map node support values on the BEAST chronogram (Fig. 4).

The samples from China and Thailand, about 500 km distant, grouped together. Furthermore, the sequence from continental Malaysia obtained by Moe and Weiblen (2011), 2800 km away from the Chinese samples, was nested within these samples (Fig. S2). The samples from Sarawak and Kalimantan on Borneo, about 200 km distant, grouped together, and the samples from Australia, about 350 km apart, clustered in a monophyletic group including the two Australian samples sequenced by Moe and Weiblen (2011). We observed that the wasps from China–Thailand were sister to all other wasps. Then, the wasps originating from India separated from the other ones, and finally the wasps from Borneo and Sulawesi were closer to each other than either was to the wasps of Australia. The estimated mean age of the stem leading to the pollinators of *F. racemosa* was 25.5 Ma and the diversification of extant pollinators began 13.6 Ma, while the youngest separation between biogeographic groups was estimated at 3.4 Ma.

Discussion

This is the first study of genetic differentiation of a very wide-ranging *Ficus* species and its associated pollinating wasps over their whole distribution covering several subcontinents and continents. It is one of the first studies to demonstrate similar genetic structuring at such a large scale in a highly dispersive obligate mutualistic association. Nevertheless, despite global co-diversification between *Ficus* and fig-pollinating wasps (Cruaud et al. 2012), the evidence suggests different histories of diversification in plant versus wasp.

Ficus racemosa is structured into four biogeographic units: India, China–Thailand, Borneo and Australia. Each of these units is characterized by a different main chloroplastic haplotype. We observe lack of spatial genetic structure at the scale of a 100 km, and at even wider scales within India and within China–Thailand, as evidenced by low F_{st} values and lack of correlation with distance within each of these two regions subcontinents (Table 2; Fig. 2a). There is more variation between the two collections from Borneo, a feature that could be due to a local founder effect at Praktek, as suggested by low genetic diversity. Because of the high genetic differentiation into four biogeographic units at nuclear microsatellite loci and the presence of distinct chloroplastic haplotypes, *F. racemosa* is probably best considered as a species complex including genetically well-defined allopatric entities that could be ranked as subspecies or species. Allele size distributions show that the populations from India are the most divergent from the others. Indeed, for pairwise genetic comparisons between populations, excluding India, we observe (Fig. 2) (1) similar F_{st} and R_{st} values and (2) the predicted higher variance of R_{st} estimates. For all pairwise comparisons involving an Indian population and a population from elsewhere in the area of distribution, R_{st} values are significantly higher than F_{st} values. Hence, the Indian populations constitute a highly distinct gene pool while the other biogeographic units constitute separate but less distinct gene pools. The Bayesian assignation method (STRUCTURE) confirms this major division into two main entities. Further, it groups together all individuals from Borneo and Australia when three groups are imposed, a grouping confirmed by neighbor-joining reconstruction (Fig. 3). We therefore conclude that the populations from Borneo are genetically closer to those of Australia than to those of continental Asia. Hence, all the results indicate the presence of two main gene pools: India versus all other regions. The other regions are subdivided into two subgroups: Continental South-East Asia versus insular South-East Asia plus Australia. It must be noted, however, that all the wasps and fig trees sampled in India were restricted to the southern peninsula; it is highly possible that wasps and fig trees sampled in north/north-east India will fall within the China–Thailand cluster.

The very limited genetic differentiation of *F. racemosa* within continental South-East Asia is also observed for its mutualistic pollinator, *C. fusciceps* sensu lato, based on microsatellite data (Kobmoo et al. 2010) and on the sequence data presented here. We may propose that *F. racemosa* in continental South-East Asia corresponds to a single population of trees pollinated by a single population of mutualistic wasps spanning well over 1500 km. Hence, the situation very much resembles what can be observed in some oceanic highly dispersive organisms. What is unique in our study is the demonstration of similar lack of genetic structuring over large expanses in a pair of free-living obligate mutualists presenting very different generation times. Nevertheless, we observe a major difference between the biogeographic pattern evidenced for *F. racemosa* and for its pollinating wasps. For the wasps, the most divergent genetic entity corresponds to China–Thailand and not India, while the higher proximity of the Bornean and Australian samples observed for the

tree is confirmed for the pollinator (Fig. 4). The use of microsatellite markers for the plant precludes dating of the separation into different units, so we cannot compare the timing of differentiation of plants and wasps. Further, microsatellite markers reflect global genome differentiation for neutral genes, while gene sequences used for the wasps reflect the history of individual genes. Next Generation Sequencing will probably allow to overcome these limitations in a near future.

Similar to the data on the fig tree, data on the pollinators suggest genetic homogeneity within each biogeographic region and strong differentiation among regions. The divergence between the different lineages of wasps pollinating *F. racemosa* is old comparatively to other pollinating fig wasps, the first split going back to 13.6 Ma. The age of the group of wasps pollinating *F. racemosa* would be about 25.5 Ma, a figure consistent with the estimated age of the branch leading to *F. racemosa* (Cruaud et al. 2012). We may compare these results with the current estimates of the age of American fig wasps for instance. Two genera of fig pollinating wasps are present in, and endemic to, the Americas. One diversified 15.9 Ma and the other 18.6 Ma (Cruaud et al. 2012) so the diversification of the wasps pollinating *F. racemosa* is almost as old as the diversification of all American wasps. One may speculate that further sampling may increase the age of these crown groups. However, the estimated length of the stems leading to the genera (62.1 and 43.5 Ma for the American genera, 25 Ma for the pollinators of *F. racemosa*) is probably less subject to sampling bias and still suggests a surprising age of the wasp species group pollinating the *F. racemosa* species complex.

Hence *F. racemosa* and its pollinating wasps provide an image quite similar to what has been observed in some wide-ranging organisms: despite morphological homogeneity across a very large range (from India to Australia), we evidence the presence of well-differentiated wide-ranging genetic entities within that range (Thompson et al. 2005). Further, as observed in the paleontological record for wide-ranging morphospecies (Jablonski and Roy 2003; Hopkins 2011), the wide ranging *F. racemosa* is an old lineage that did not diversify morphologically. Similar ages of morphospecies have been calculated for some American tropical trees with an estimated age of 6.4–13.9 Ma for *Chrysophyllum argenteum* (Dick et al. 2013), suggesting that our findings for *F. racemosa* could be observed in other tree genera.

We may propose as a working hypothesis that *F. racemosa* is subdivided into a set of very large populations (some spanning more than 1500 km), each corresponding to a subspecies (or a species) and that each large population is pollinated by a single population of wasps corresponding to a species. Interestingly, the genetic separation between the China–Thailand pollinator populations (species) and the Borneo populations (species) has been maintained despite the presence of a land bridge connecting Borneo to the continent 40 % of the time during the last 250,000 years (Voris 2000). We may suggest that a transient geographic barrier (the Sunda shelf sea) limiting gene flow between populations of *F. racemosa* has been sufficient to allow ecological differentiation through adaptation to different average climatic conditions, a feature which would have facilitated a speciation process (Tian et al. 2015). Such situations of very large populations have important implications on how co-evolutionary processes play out, as local adaptation cannot occur due to gene flow: there is no geographic mosaic of coevolution (Thompson 2005) for this association. This opposes *F. racemosa*, and probably many *Ficus* species, to some other, low-dispersal obligate mutualisms, such as the ant–plant association involving *Leonardoxa africana africana* and the ant *Petalomyrmex phylax*, in which highly similar genetic structuring into well-defined populations is documented, with evolutionary processes played out over periods of tens to thousands of years rather than millions of years (Léotard

et al. 2009; Alvarez et al. 2010). The *Yucca-Tegeticula* mutualistic association provides an intermediate situation in which the plant, with its poorly dispersing seeds, is much more spatially structured than the free-roaming pollinating moths and in which structuring is played out over a period of 100,000–200,000 years (Smith et al. 2011).

Ficus racemosa is a large tree, a feature that should facilitate long-distance dispersal of pollinators, and its large figs are consumed by large bats that disperse seeds over long distances (Shilton et al. 1999; Oleksy et al. 2015); these bats include the world's largest fruit bats, the *Pteropus* flying foxes. In comparison, *F. pumila*, a creeping dioecious species, presented a clear geographical structuration over 1000 km in southern China (Liu et al. 2015) and *F. hirta*, a small understory dioecious fig tree presenting a continuous distribution, presented modest but significant differentiation with distance over 3000 km of continuous continental habitat (Yu and Nason 2013). Hence some limited genetic differentiation with distance over continuous stretches of habitat could be frequent in dioecious *Ficus*. Genetic differentiation for cytoplasmic markers suggests limited seed dispersal associated with longer-distance pollinator dispersal. However, because interspecific transfers of cytoplasm occur in *Ficus*, results should be interpreted with caution (Renoult et al. 2009).

The lack of genetic isolation by distance pattern observed for *F. racemosa*, at least in South China–Thailand and India, differs from the one observed for dioecious figs in which (1) some isolation by distance was present within continents in the plants and (2) different pollinators are present in different parts of the range, apparently without a similar discontinuity in genetic differentiation in the plant's range (Chen et al. 2012; Liu et al. 2015; Tian et al. 2015). It is an open question whether the patterns observed for *F. racemosa* can be generalized to other wide-ranging monoecious *Ficus* species. Indeed for *F. burkei*–*F. natalensis* in Southeast Africa and for *F. rubiginosa* in Eastern Australia, we observe a latitudinal (and hence climatic) gradient in which different pollinator species or different pollinator populations are present while no major geographic barrier seems to explain this variation (Cornille et al. 2012; Sutton et al. 2016). In these two cases, spatial genetic structure has not been investigated in the host plant. We predict that the situation will turn out to be similar to that observed in dioecious *Ficus* with some genetic isolation by distance in the plants. Our suggestion is that *Ficus* morphospecies will provide a continuum of situations ranging from lack of any genetic isolation by distance, with vicariant subspecies or species in different parts of the range and lack of local adaptation to some spatial genetic structure associated with some adaptation to local conditions. The challenge now is to analyze for a set of species, the implications of different pollinator dispersal traits, e.g. flight height, which varies with pollinator genus (see Harrison and Rasplus 2006 for details) on how ecological diversification proceeds in the plants and in the insects.

The implications of studies on the *Ficus*–fig pollinating wasp model on our understanding of patterns of adaptation to local conditions, speciation and diversification in wide-ranging-high gene flow species and in mutualistic and more generally plant–pollinator–herbivore–parasitoid interaction systems are just beginning to become apparent (Borges 2015; Kjellberg and Proffit 2016). Much progress may be expected in the coming years.

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References

Ahmed S, Dawson DA, Compton SG et al (2007) Characterization of microsatellite loci in the African fig *Ficus sycomorus* L. (Moraceae). Mol Ecol Notes 7:1175–1177

Ahmed S, Compton SG, Butlin RK et al (2009) Wind-borne insects mediate directional pollen transfer between desert fig trees 160 kilometers apart. Proc Natl Acad Sci USA 106:20342–20347

Akaike H (1973) Information theory and an extension of the maximum likelihood principle. In: Petrov PN, Csaki F (eds) Second international symposium on information theory. Akad, Kiado, pp 267–281

Alvarez N, McKey D, Kjellberg F et al (2010) Phylogeography and historical biogeography of obligate specific mutualisms. In: Morand S, Krasnov BR (eds) The biogeography of host-parasite interactions. Oxford University Press, Oxford, p 277

Ashley MV (2010) Plant parentage, pollination, and dispersal: how DNA microsatellites have altered the landscape. Crit Rev Plant Sci 29:148–161

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B Methodol 57:289–300

Berg CC, Corner EJH (2005) Moraceae (*Ficus*). In: Nooteboom HP (ed) Flora Malesiana. National Herbarium Nederland, Leiden

Borges RM (2015) How to be a fig wasp parasite on the fig–fig wasp mutualism. Curr Opin Insect Sci 8:34–40

Buschbom J, Yanbaev Y, Degen B (2011) Efficient long-distance gene flow into an isolated relict oak stand. J Hered 22:484–493

Chen Y, Compton SG, Liu M et al (2012) Fig trees at the northern limit of their range: the distributions of cryptic pollinators indicate multiple glacial refugia. Mol Ecol 21:1687–1701

Conchou L, Cabioch L, Rodriguez LJV et al (2014) Daily rhythm of mutualistic pollinator activity and scent emission in *Ficus septica*: ecological differentiation between co-occurring pollinators and potential consequences for chemical communication and facilitation of host speciation. PLoS ONE 9:e103581

Cornille A, Underhill JG, Cruaud A et al (2012) Floral volatiles, pollinator sharing and diversification in the fig–wasp mutualism: insights from *Ficus natalensis*, and its two wasp pollinators (South Africa). Proc R Soc B 279:1731–1739

Cruaud A, Jabbour-Zahab R, Genson G et al (2010) Laying the foundations for a new classification of Agaonidae (Hymenoptera: Chalcidoidea), a multilocus phylogenetic approach. Cladistics 26:359–387

Cruaud A, Jabbour-Zahab R, Genson G et al (2011) Phylogeny and evolution of life-history strategies in the Sycophaginae non-pollinating fig wasps (Hymenoptera, Chalcidoidea). BMC Evol Biol 11:178–192

Cruaud A, Rønsted N, Chantarasuwan B et al (2012) An extreme case of plant-insect co-diversification: figs and fig-pollinating wasps. Syst Biol 61:1029–1047

Cunha RL, Blanc F, Bonhomme F et al (2011) Evolutionary patterns in pearl oysters of the genus *Pinctada* (Bivalvia: Pteriidae). Mar Biotechnol 13:181–192

Darwell CT, Al-Beidh S, Cook JM (2014) Molecular species delimitation of a symbiotic fig-pollinating wasp species complex reveals extreme deviation from reciprocal partner specificity. BMC Evol Biol 14:189

Dev SA, Kjellberg F, Hossaert-McKey M et al (2011) Fine-scale population genetic structure of two dioecious Indian keystone species, *Ficus hispida* and *Ficus exasperata* (Moraceae). Biotropica 43:309–316

Dick CW, Lewis SL, Maslin M et al (2013) Neogene origins and implied warmth tolerance of Amazon tree species. Ecol Evol 3:162–169

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214–221

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Gu X (1995) Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. Mol Biol Evol 12:546–557

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620

Hardy OJ, Charbonnel N, Fréville H et al (2003) Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. Genetics 163:1467–1482

Harrison RD (2005) Figs and the diversity of tropical rainforests. Bioscience 55:1053–1064

Harrison RD, Rasplus J-Y (2006) Dispersal of fig pollinators in Asian tropical rain forests. J Trop Ecol 22:631–639

Heer K, Kalko EKV, Albrecht L, Garcia-Villacorta R, Staeps FC, Herre EA, Dick CW (2015) Spatial scales of genetic structure in free-standing and strangler figs (*Ficus*, Moraceae) inhabiting Neotropical forests. PLoS ONE 10:e0133581

Heinrichs J, Hentschel J, Feldberg K et al (2009) Phylogenetic biogeography and taxonomy of disjunctly distributed bryophytes. J Syst Evol 47:497–508

Heinze B (2007) A database of PCR primers for the chloroplast genomes of higher plants. Plant Methods 3:4

Hopkins MJ (2011) How species longevity, intraspecific morphological variation, and geographic range size are related: a comparison using late Cambrian trilobites. Evolution 65:3253–3273

Hossaert-McKey M, Soler C, Schatz B et al (2010) Floral scents: their roles in nursery pollination mutualisms. Chemoecology 20:75–88

Jablonski D, Roy K (2003) Geographical range and speciation in fossil and living mollusks. Proc R Soc B 270:401–406

Jevanandam N, Goh AGR, Corlett RT (2013) Climate warming and the potential 16 extinction of fig wasps, the obligate pollinators of figs. Biol Lett 9:20130041

Katoh K, Kuma K, Toh H et al (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res 33:511–518

Khadari B, Hochu I, Santoni S et al (2001) Identification and characterization of microsatellite loci in the common fig (*Ficus carica* L.) and representative species of the genus *Ficus*. Mol Ecol Notes 1:191–193

Kinland BP, Gaines SD (2003) Propagule dispersal in marine and terrestrial environments: a community perspective. Ecology 84:2007–2020

Kjellberg F, Proffit M (2016) Tracking the elusive history of diversification in plant-herbivorous insect-parasitoid food webs: insights from figs and fig-wasps. Mol Ecol 25:843–845

Kjellberg F, Jousselin E, Hossaert-McKey M et al (2005) Biology, ecology, and evolution of fig-pollinating wasps (Chalcidoidea, Agaonidae). In: Raman A, Schaefer W, Withers TM (eds) Biology, ecology and evolution of gall-inducing arthropods. Science Publishers Inc, Enfield (NH) USA, Plymouth, pp 539–572

Kobmoo N, Hossaert-McKey M, Rasplus J-Y et al (2010) *Ficus racemosa* is pollinated by a single population of a single agaonid wasp species in continental South-East Asia. Mol Ecol 19:2700–2712

Kremer A, Ronce O, Robledo-Arnuncio JJ et al (2012) Long-distance gene flow and adaptation of forest trees to rapid climate change. Ecol Lett 15:378–392

Krishnan A, Ghara M, Kasinathan S et al (2015) Plant reproductive traits mediate tritrophic feedback effects within an obligate brood-site pollination mutualism. Oecologia 179:797–809

Léotard G, Debout G, Dalecky A et al (2009) Range expansion drives dispersal evolution in an equatorial three-species symbiosis. PLoS ONE 4:e5377

Lewis LR, Rozzi R, Goffinet B (2014) Direct long-distance dispersal shapes a New World amphitropical disjunction in the dispersal-limited dung moss *Tetraplodon* (Bryopsida: Splachnaceae). J Biogeogr 41:2385–2395

Lin S-Y, Chou L-S, Di Giusto B et al (2015) Sexual specialization in phenology in dioecious *Ficus benguetensis* and its consequences for the mutualism. Bot stud 56:32

Liu M, Zhao R, Chen Y et al (2014) Competitive exclusion among fig wasps achieved via entrainment of host plant flowering phenology. PLoS ONE 9:e97783

Liu M, Compton SG, Peng F-E et al (2015) Movements of genes between populations: are pollinators more effective at transferring their own or plant genetic markers? Proc R Soc B 282:20150290

Loiselle BA, Sork VL, Nason J et al (1995) Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). Am J Bot 82:1420–1425

Moe AM, Weiblen GD (2010) Molecular divergence in allopatric *Ceratosolen* (Agaonidae) pollinators of geographically widespread *Ficus* (Moraceae) species. Ann Entomol Soc Am 103:1025–1037

Moe AM, Weiblen GD (2011) Development and characterization of microsatellite loci in dioecious figs (*Ficus*, Moraceae). Am J Bot Notes Prot 98:e25–e27

Nunes FLD, Norris RD, Knowlton N (2011) Long distance dispersal and connectivity in amphi-atlantic corals at regional and basin scales. PLoS ONE 6:e22298

Nylander JAA (2004) MrAIC.pl. In: Nylander JAA (ed) Evolutionary Biology Centre. Uppsala University, Uppsala

Oleksy R, Racey PA, Jones G (2015) High-resolution GPS tracking reveals habitat selection and the potential for long-distance seed dispersal by Madagascan flying foxes *Pteropus rufus*. Glob Ecol Conserv 3:678–692

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. Ann Rev Ecol Syst 25:547–572

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Perrier X, Jacquemoud-Collet JP (2006) DARwin software. http://darwin.cirad.fr/darwin

Pokorny L, Oliván G, Shaw AJ (2011) Phylogeographic patterns in two Southern Hemisphere species of *Calyptrochaeta* (Daltoniaceae, Bryophyta). Syst Bot 36:542–553

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Rambaut A (2006) FigTree. http://tree.bio.ed.ac.uk/software/figtree/

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Renoult JP, Kjellberg F, Grout C et al (2009) Cyto-nuclear discordance in the phylogeny of *Ficus* section *Galoglychia* and host shifts in plant-pollinator associations. BMC Evol Biol 9:248

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Shilton LA, Altringham JD, Compton SG et al (1999) Old World fruit bats can be long-distance seed dispersers through extended retention of viable seeds in the gut. Proc R Soc B 266:219–223

Smith CI, Tank S, Godsoe W et al (2011) Comparative phylogeography of a coevolved community: concerted population expansions in Joshua trees and four yucca moths. PLoS ONE 6:e25628

Soler C, Hossaert-McKey M, Buatois B et al (2011) Geographic variation of floral scent in a highly specialized pollination mutualism. Phytochemistry 72:74–81

Sutton TL, Riegler M, Cook JM (2016) One step ahead: a parasitoid disperses farther and forms a wider geographic population than its fig wasp host. Mol Ecol 25:882–894

Tamura K, Dudley J, Nei M et al (2007) MEGA4: molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thompson JN (2005) The geographic mosaic of coevolution. University of Chicago Press, Chicago

Thompson AR, Thacker CE, Shaw EY (2005) Phylogeography of marine mutualists: parallel patterns of genetic structure between obligate goby and shrimp partners. Mol Ecol 14:3557–3572

Tian E, Nason JD, Machado CA et al (2015) Lack of genetic isolation by distance, similar genetic structuring but different demographic histories in a fig-pollinating wasp mutualism. Mol Ecol 24:5976–5991

Vignes H, Hossaert-McKey M, Beaune D et al (2006) Development and characterization of microsatellite markers for a monoecious *Ficus* species, *Ficus insipida*, and cross-species amplification among different sections of *Ficus*. Mol Ecol Notes 6:792–795

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J Biogeogr 27:1153–1167

Wang G, Cannon CH, Chen J (2016) Pollinator sharing and gene flow among closely related sympatric dioecious fig taxa. Proc R Soc B 283:20152963

Ware AB, Compton SG (1994) Dispersal of adult female fig wasps. 2. Movements between trees. Entomol Exp Appl 73:231–238

Yang L-Y, Machado CA, Dang X-D et al (2015) The incidence and pattern of copollinator diversification in dioecious and monoecious figs. Evolution 69:294–304

Yu H, Nason JD (2013) Nuclear and chloroplast DNA phylogeography of *Ficus hirta*: obligate pollination mutualism and constraints on range expansion in response to climate change. New Phytol 197:276–289

Zhang G, Song Q, Yang D (2006) Phenology of *Ficus racemosa* in Xishuangbanna, Southwest China. Biotropica 38:334–341