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Genetic diversity, geographical range and origin of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Indian Ocean Ms

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Abstract

The whitefly *Bemisia tabaci* is a pest vector of begomoviruses on crops worldwide. *Bemisia tabaci* is composed of a complex of cryptic species which barely interbreed. An exception is the Ms from the South West Indian Ocean (SWIO), which crosses in low proportions with the exotic B. The Ms, together with B and Q is part of the same phylogenetic clad. To infer the genetic structure, the geographical range and putative origin of this putative species, microsatellite data and mitochondrial DNA (cytochrome oxydase I) sequences were analysed on an extensive sample set, including all the islands of the region and samples from mainland Africa. Only B and Ms populations were detected across these islands. The exotic B was found only on the islands of Réunion and Mauritius, whereas the Ms is found on all the SWIO islands. Very high isolation by distance was found for the Ms populations between islands of the SWIO, suggesting a long period of presence in this region. Ms populations from mainland Africa had a higher COI diversity than the Ms of the SWIO islands. This diversity is correlated with size and geological ages of the SWIO islands. The population genetic data obtained are in accordance with an origin of Ms in Africa, followed by its expansion and evolution across the SWIO islands prior to human arrival, confirming the status of Ms as indigenous in the SWIO islands.

Keywords: *Bemisia tabaci*, microsatellite, insular environment, genetic diversity, whiteflies invasions, origin of Ms, indigenous status

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Introduction

The consequences of increasing globalization, due to elevated trade and passenger traffic between different continents, are becoming evident worldwide. One particularly noticeable aspect is the increased movement of species beyond their native ranges. Invasions by non-indigenous (alien, exotic) species such as pests can have spectacular impacts on their new environment. Jones & Kitching (1981) define a pest as an organism that damages crops, destroys products, transmits or causes disease, is annoying or in other ways conflicts with human needs or interests. International concern about preserving biodiversity further extends the definition of a pest to a species that can either cause native species decline or alters the structure and function of natural ecosystems (Worner, 2002). More recently, pest invasions have been recognized as an important cause of loss of biodiversity, but pests as 'vectors' can also be considered as factors of emergence or re-emergence of viruses. For example, *Begomoviruses* (family: *Geminiviridae*), only vectored by the whitefly *Bemisia tabaci*, are considered as an emerging disease in many

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Table 1. *Bemisia tabaci* samples repartition by country, town (locality), host plants, number of individuals tested (*n*) and accession number.

Country	Locality	Host plant	*B. tabaci*	*n*	Accession N°
Madagascar	Antsiranana	Eggplant	Ms	6	
	Antsiranana	Cabbage	Ms	2	
	Antsiranana	Tomato	Ms	67	Madagascar 1_ HQ622840
	Tolanaro	Eggplant	Ms	7	
	Tolanaro	Bean	Ms	29	Madagascar 2_ HQ622841
	Miandrivazo	Eggplant	Ms	7	
	Miandrivazo	Cotton	Ms	2	
	Miandrivazo	Tomato	Ms	71	Madagascar 3_ HQ622842
	Morondava	Cabbage	Ms	11	
	Morondava	Cucumber	Ms	24	Madagascar 4_ HQ622843
	Morondava	Tomato	Ms	21	
	Toamasina	Annual Poinsettia	Ms	10	
	Toamasina	Cassava	Ms	1	
	Toamasina	Chili	Ms	17	Madagascar 5_ HQ622844
	Toamasina	Tomato	Ms	53	Madagascar 6_ HQ622845
	Antananarivo/ Itasy	Tomato	Ms	8	
	Antananarivo	Eggplant	Ms	3	
	Antananarivo	Cabbage	Ms	8	
	Antananarivo	Courgette	Ms	8	
	Antananarivo	Tomato	Ms	18	Madagascar 7_ HQ622846
	Toliara	Eggplant	Ms	7	
	Toliara	Bean	Ms	20	Madagascar 8_ HQ622847
	Toliara	Tomato	Ms	22	Madagascar 9_ HQ622848
Grande Comore	Inrape	Cassava	Ms	10	
	Malondja	Cotton	Ms	2	
	Malondja	*Leptadenia*	Ms	11	
	Moroni	Cassava	Ms	23	
	Moroni	Tobacco	Ms	29	
Anjouan	Nord	Tomato	Ms	7	Ajouan 1 – 3_HQ622858, HQ622859, HQ622860
Mayotte	Kahani	Tomato/Chili	Ms	3	
	Malamani	Tomato	Ms	17	Mayotte 1 – 3_ HQ622861, HQ622862, HQ622863
	Combani	Tomato	Ms	8	
	Tzoundzou	Tomato	Ms	11	
	Marembere	Tomato	Ms	6	
	Dzoumonie	Tomato	Ms	17	Mayotte 4 – 5_ HQ622864, HQ622865
	Moya	Lantana	Ms	15	Mayotte 6_HQ622866
	Kangani	Tomato	Ms	4	
	Mronbeja	*Indigofera*	Ms	16	Mayotte 7 – 9_ HQ622867, HQ622868, HQ622869
	Dembeni	Tomato	Ms	8	
	Mtsamboro	Lantana	Ms	8	
Seychelles	Mahe	Cassava	Ms	28	Seychelles 1 – 4_ HQ622849, HQ622850, HQ622851, HQ622852
	Mahe	Tomato	Ms	2	
	La Digue	Cassava	Ms	24	Seychelles 5 – 9_HQ622853, HQ622854, HQ622855, HQ622856, HQ622857
Réunion	Cambuston	Annual Poinsettia	Ms	15	Réunion Ms 1 – 3_HQ622819, HQ622820, HQ622821
	Grande Chaloupe	Annual Poinsettia	Ms	21	Réunion Ms 4 – 6_HQ622822, HQ622823, HQ622824
	Manapany	Annual Poinsettia	Ms	3	
	Piton sainte Rose	Tomato	Ms	7	Réunion Ms 7 – 9_HQ622825, HQ622826, HQ622827
	Souris chaude	Lantana	Ms	20	Réunion Ms 10 – 12_HQ622828, HQ622829, HQ622830
	Saint Gilles	Annual Poinsetia	Ms	2	Réunion Ms 19 – 20_HQ622837, HQ622838
	Saint Philippe	Annual Poinsetia/ Bohemeria	Ms	28	Réunion Ms 13 – 18_HQ622831, HQ622832, HQ622833, HQ622834, HQ622835, HQ622836
	Manapany	Tomato	B	14	Réunion B 1 – 7_HQ622879, HQ622880, HQ622881, HQ622882, HQ622883, HQ622884, HQ622885
	Saint Gilles	Tomato	B	23	Réunion B 8 – 14_HQ622886, HQ622887, HQ622888, HQ622889, HQ622890, HQ622891, HQ622892
	Souris chaude	Lantana	B	4	
Mauritius	Poste de Flacq	Tomato	Ms	1	
	Solitude	Tomato	Ms	3	
	Trou d'eau douce	Cucumber/Eggplant	B	17	Mauritius B 1 – 5_HQ622894, HQ622895, HQ622896, HQ622897, HQ622898
	Poste de Flacq	Tomato	B	3	
	Riambel	Tomato	B	5	
	Petitraffray	Eggplant	B	6	
	Solitude	Tomato	B	1	
	Plaisance	Eggplant	B	16	Mauritius B 6 – 10_HQ622899, HQ622900, HQ622901, HQ622902, HQ622903
Tanzania	Morogoro	Tomato	Ms	4	Tanzania 1 – 4_ HQ622870, HQ622871, HQ622872, HQ622873
	Arusha	Tomato	Ms	5	Tanzania 5 – 9_HQ622874, HQ622875, HQ622876, HQ622877, Q622878
Controls		Cabbage	B	11	Réunion B 15_HQ622893
		Annual Poinsetia	Ms	11	Réunion Ms 21_HQ622839

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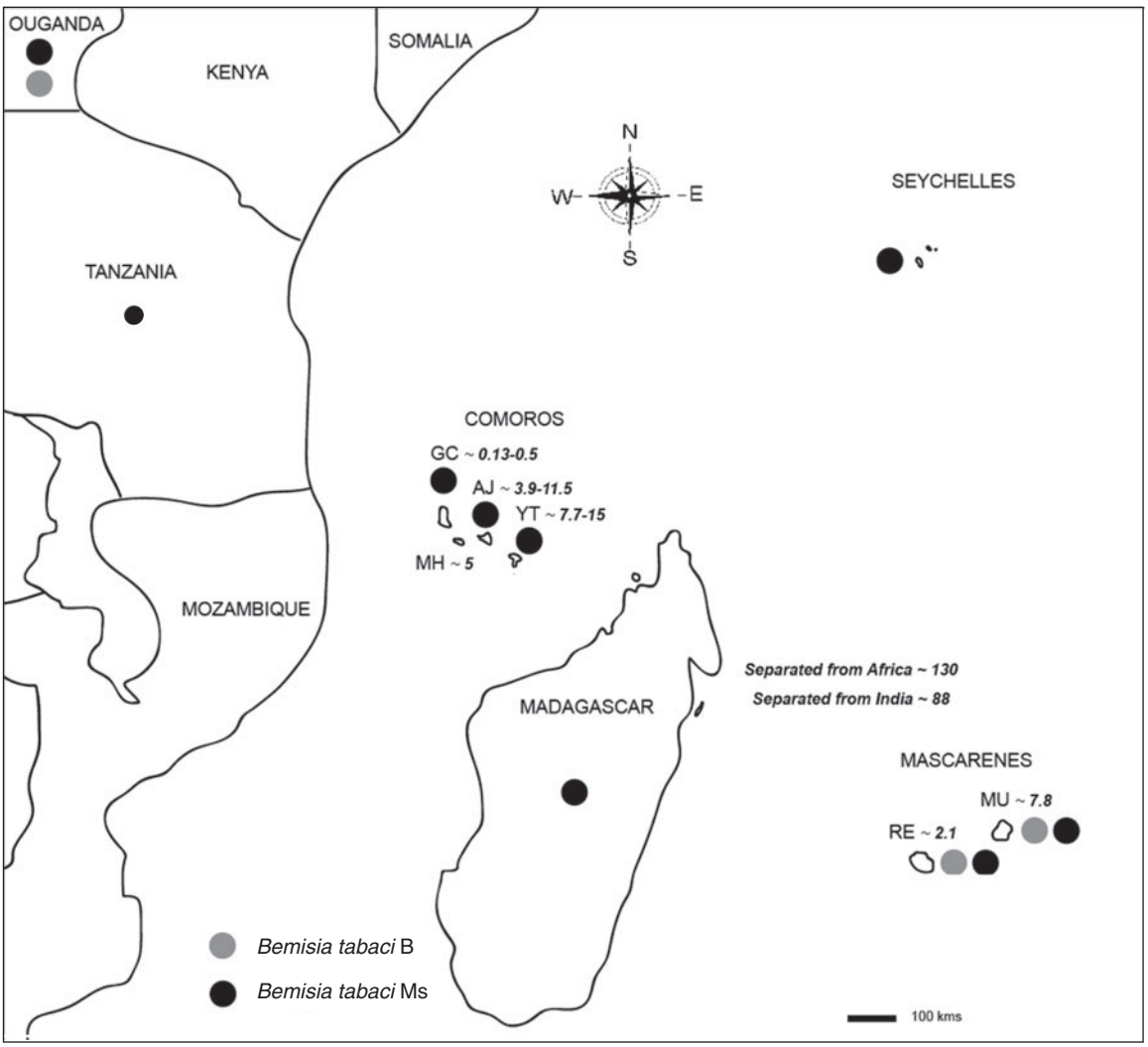

Fig. 1. Map of the sub-region of the southwest part of the Indian Ocean with the repartition of *Bemisia tabaci* B and Ms according to this study and Sseruwagi *et al*., 2005. Geological times are also indicated for each island, in million years (from Warren *et al*., 2005). Beside Seychelles and Madagascar, which are continental islands, the other islands are of volcanic origin. GC, Grande Comore; MH, Mohéli; AJ, Anjouan; YT, Mayotte; RE, Réunion; Mu, Mauritius.

countries (Anderson *et al*., 2004). *B. tabaci* is vector of more than 100 begomoviruses (Jones, 2003). Emergence of begomoviruses is considered to cause severe yield losses, but is often linked to the vector, more precisely, to the introduction of a new population (or putative species according to De Barro *et al*., 2011) of *B. tabaci* with an increased fitness or a wider host range, compared to the indigenous ones (Delatte *et al*., 2009, 2007). In South America, new indigenous begomoviruses have been described after the introduction of the B species so-called *B. tabaci* 'biotype B', and declared severe epidemics; in this case, the exotic B was described as more polyphagous than the indigenous ones (Ribeiro *et al*., 2003). *B. tabaci* was formerly thought to be a unique species composed of several well-differentiated groups, and recently those groups had been referred as species, and *B. tabaci* is now considered as composed of a complex of 24 cryptic species which barely interbreed and form different phylogenetic clades (Dinsdale *et al*., 2010; Xu *et al*., 2010; De Barro *et al*., 2011). Among the different clades found in the general phylogeny of Boykin *et al*. (2007), one is identified as comprising the 'invasive' biotypes/species, including B, Q and Ms. Nevertheless, only B and Q are known worldwide as invasive. The Ms, presumed to be the Indian Ocean putative species (Delatte *et al*., 2005b), has never been considered as invasive so far. Its presumed indigenous status is supported by the presence of several monophyletic endemic begomoviruses, only vectored by *B. tabaci* (Delatte *et al*., 2005a; Lefeuvre *et al*., 2007) and documented on some of the islands of the region (Mayotte, Madagascar and Seychelles). The biology and genetics of this putative species was studied in Réunion, together with the invasive B which recently invaded the island (Delatte *et al*., 2006, 2009). In Delatte *et al*. (2006), for the first time, apparently fertile hybrids (presence of hybrids after multiple generations) between B and Ms were found in the field in Réunion. Nevertheless, they were found at low frequency, with a majority of pure B or pure Ms individuals. Furthermore, no extensive sampling in the

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other islands was performed. To assess the Ms diversity presumed to be indigenous to this region, we infer the genetic structure and the geographical range of Ms using microsatellite data and mitochondrial DNA (cytochrome oxydase I) sequences from all the main islands of the region and samples from mainland Africa.

Materials and methods

Sample collection

Wild samples of *B. tabaci* were collected from most of the larger islands of the SWIO: Madagascar (2001), Réunion (2005), Mauritius (2004), Seychelles (2003), Grande Comore (2005), Anjouan (2005) and Mayotte (2005), and also from one country of mainland Africa, Tanzania (2009) (table 1, fig. 1). All samples were obtained as ethanol-preserved adults.

Microsatellite analysis

Male whiteflies are haploid, and females are diploid. Each field-captured whitefly, therefore, was sexed under a binocular microscope, and only females were used for DNA extraction due to their diploid state (see Delatte *et al.*, 2006). A total of 882 females were analysed in this study. Nine microsatellite markers with fluorescent labels were used in this study (P5, P7, P53, P62, P11, P32, P59 (Delatte *et al.*, 2006), Ms145 (Dalmon *et al.*, 2008) and Bem25 (De Barro *et al.*, 2003)). Primer sequences and methods used for DNA extraction, amplification, electrophoresis and allele scoring followed (De Barro *et al.*, 2003; Delatte *et al.*, 2006). The genotyping phase was conducted in an ABI PRISM 3100 (©Applied Biosystem) automated sequencer.

Mitochondrial DNA

The mitochondrial cytochrome oxidase I (*COI*) gene, known as diagnostic for whitefly biotypes/species, was amplified and sequenced for 76 of the 882 wild SWIO individuals already typed for microsatellites, as well as for nine individuals collected in Tanzania. We used primers COI-F-C1: CATCTAATCAGCAGTGAGGCTGG and COI-R-C1: AAAAGTTAAATTTACTCCAAT. The PCR was conducted in a final volume of 25 µl with 10× PCR Optibuffer (Eurogentec), 0.2 mM dNTPs (New England Biolabs), 1.5 mM $MgCl_2$, 400 nM of each primer, 1 unit of DAp GoldStar®, (Eurogentec) and 10 ng of insect DNA extract. The PCR programme was a cycle of 35 times: 1 min at 94°C, 30 s at 55°C and 1 min at 72°C, then a final step at 72°C for 7 min.

Data analysis

Genetic diversity within each cluster was quantified by the number of alleles per locus, the observed heterozygosity (H_o) and gene diversity (H_e). FREEna software was used to estimates null allele frequencies for each locus and population analysed following the Expectation Maximization (EM) algorithm (Chapuis & Estoup, 2007) with 1000 bootstrap iterations. For the two clusters separately, F_{st} using the ENA correction described in Chapuis & Estoup (2007) were given by FREEna; Weir & Cokerham (1984) estimates of F_{is} within localities were calculated using Genepop 3.3 (Raymond & Rousset, 1995). The null hypotheses of Hardy-Weinberg frequencies within populations, and lack of population structure, were tested with

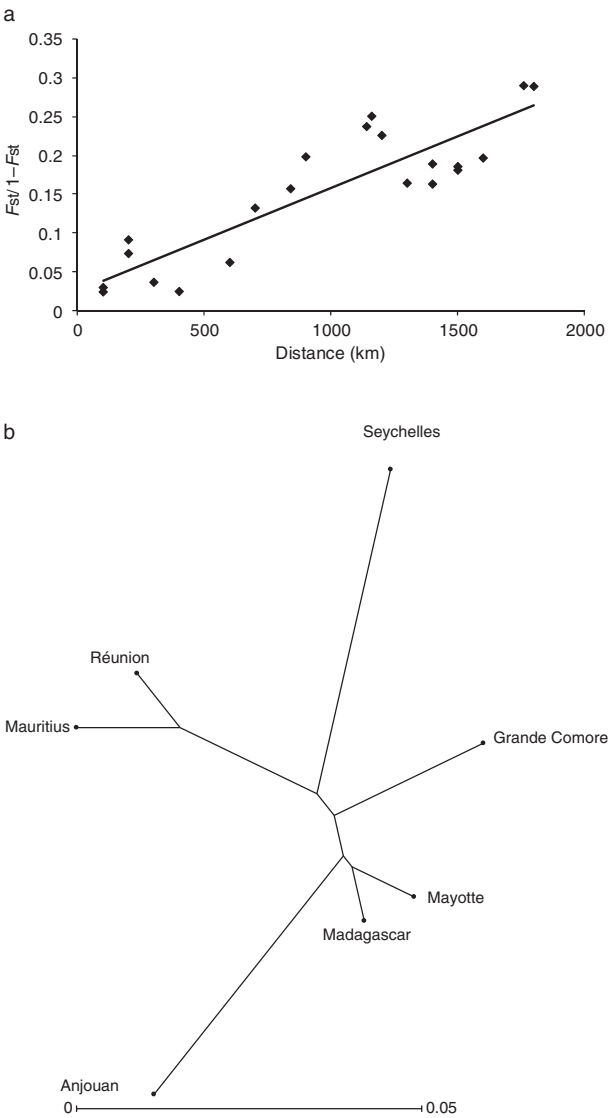

Fig. 2. (a) Plot of geographic and genetic distances for *Bemisia tabaci* Ms ($r^2 = 0.71$), using all the individuals of the study. (b) Neighbour-joining tree constructed on a distance matrix for the different populations of *B. tabaci* Ms (using microsatellite data, with Cavallis-Forza distance).

exact tests using Genepop 3.3. Allelic richness was estimated using F_{stat} V9.3.2 (Goudet, 2001) based on minimum sample size of 30 diploid individuals per country for Ms populations (both countries Mauritius and Anjouan were not included in the analysis due to the smaller sample size). A hierarchical analysis of molecular variance (AMOVA) was obtained using the software Arlequin (Excoffier *et al.*, 2005), partitioning the genetic variance into three components: (i) within-site within-cluster, (ii) among-sites within cluster, and (iii) between clusters. Gene flow (Nm) between populations was estimated with Genepop 3.3 and the method of Barton & Slatkin (1986).

The species distribution was checked with the Structure software (Pritchard *et al.*, 2000) as described in Delatte *et al.* (2006). This software differentiates mixed populations based on allele frequency at each locus. To use Structure,

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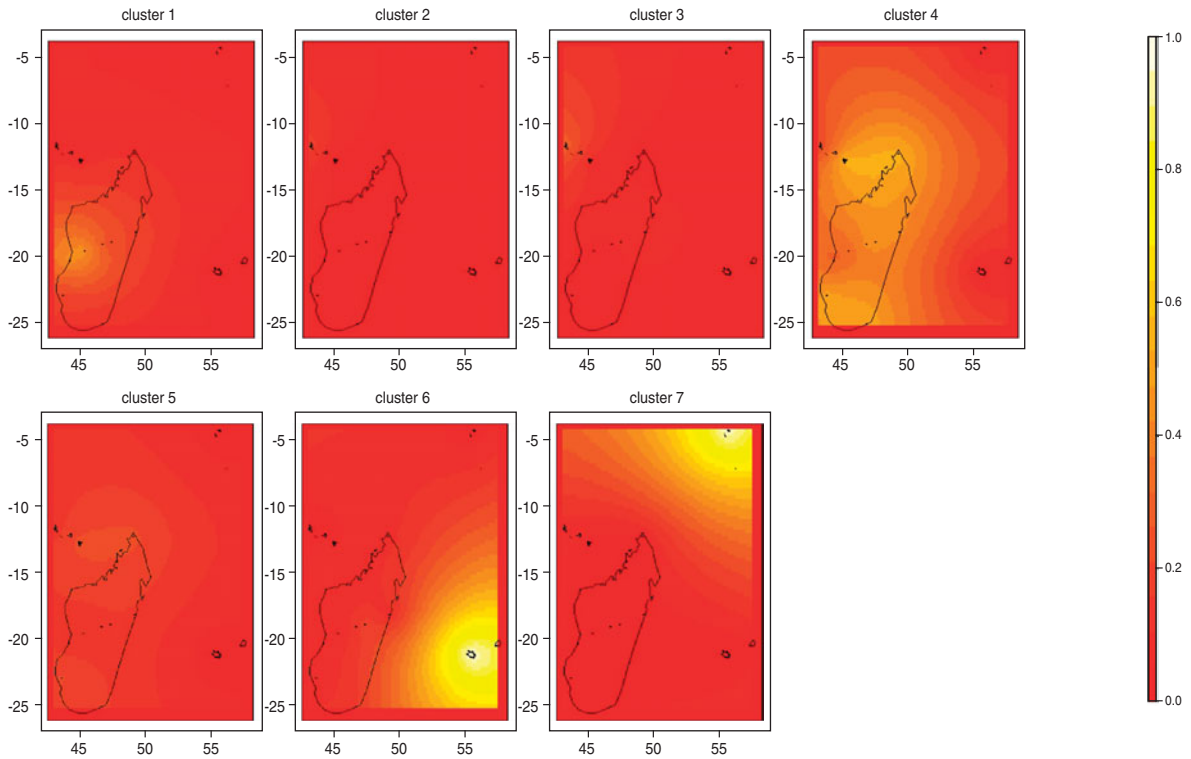

Fig. 3. Posterior predictive maps of admixture proportions as resulting from the interpolation of coancestry coefficients and the geographic distribution (restricted to the Indian Ocean region) of the seven Ms *Bemisia tabaci* populations obtained in TESS (each cluster corresponding to a K population found by TESS). Cluster 1, includes Madagascar; cluster 2, Grande Comore; cluster 3, 4 & 5, Madagascar, Comoros archipelago and Mayotte; cluster 6, Réunion and Mauritius; and cluster 7: Seychelles islands.

Hardy-Weinberg (HW) and linkage equilibrium are assumed within each group. Both hypotheses were tested *a posteriori* on each cluster using exact tests implemented in Genepop 3.3 (Raymond & Rousset, 1995). Nevertheless, it has recently been proven that the bias linked to HWE in assignment tests, such as the ones implemented in Structure, lead only to a slight reduction in the power of the test (between 0.2 and 1.0% units) and could not affect the results (Carlsson, 2008). The software TESS (Chen *et al*., 2007) also infers population structure, making use of geographical data. It was used for our dataset of Ms across islands excluding B. Analyses in TESS were run for 100,000 sweeps (the first 10,000 discarded as burn-in, with K ranging from 1 to 25 and 100 iterations for each value of K). Twenty percent of the 100 runs, representing the best Deviance Information Criterion, was kept. The range of K which best explained the data was inferred by plotting the average values of the DIC for each K and following the recommendations of Chen *et al*. (2007). For each K, CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007) was used to summarise the posterior estimates of cluster memberships of the 20 runs with the lowest DIC. We used the Large K Greedy algorithm with random input order and 1000 permutations to align the runs and the G′ pairwise matrix similarity statistics. Admixture proportions of samples and individuals were visualised using DISTRUCT v1.1 (Rosenberg, 2004). The admixture models implemented in TESS allowed us to predict expected admixture proportions on every point of the SWIO map. Each element of the matrix found represented the depth value of a particular location on the map (Chen *et al*., 2007). The admixture proportions resulting from TESS (K = 7) were interpolated with the geographic distribution of individuals using the 'maps' and 'fields' libraries of R http://www.r-project.org/ with a universal kriging function to obtain posterior predictive maps of admixture proportions. The R script was modified to include only the SWIO region (see R Script/krigAdmixProportions). A correspondence analysis (COA) was performed using Genetix 4.01 (Belkhir *et al*., 1996–2004) in order to visualize the major axes of genetic variation within the sample. Isolation by distance was tested within Ms populations with the correlation between genetic and geographical distance, tested by the regression of $F_{st}(1–F_{st})^{-1}$ on the logarithm of geographical distance (Rousset, 1997). A neighbour-joining tree was constructed from the Cavallis-Forza distance matrix given by Genetix 1.01 (Belkhir *et al*., 1996–2004) and drawn in Darwin 5.0.132 (Perrier *et al*., 2003).

Sequences obtained from *COI* sequencing were aligned with software DNAMAN version 5.2.2 (Lynnon BioSoft, Quebec, Canada) and MEGA4 (Tamura *et al*., 2007). Sequences were then analysed under the module APE of R (R Development Core Team, 2004), including sequence alignment by ClustalX. The model of sequence evolution best fitting the data was then selected using PhyML. Clade support was evaluated by bootstrapping, using 2000 pseudo-replicates and performed under PhyML. Genetic distances were estimated using the method of Nei & Gojobori (1986) for synonymous and nonsynonymous substitutions, and diversity indices were calculated using the software DnaSP

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version 4.10 (Rozas *et al*., 2003). Genetic variation was assessed by calculating the average number of pairwise nucleotide differences among the sequences (π).

Results

A total of 882 *B. tabaci* were analysed, and individuals with more than 33% of missing microsatellite loci were discarded (only nine individuals were discarded from the study, two from Seychelles and seven from Madagascar). Overall, an average of 18 alleles per locus were observed for the populations of Madagascar ($n=422$) with an allelic richness of 9.5, five alleles for the Seychelles ($n=54$) with an allelic richness of 4.4, 12 alleles for Mayotte ($n=113$) with an allelic richness of 8.6, five for Anjouan ($n=7$), three for Mauritius Ms ($n=5$), seven for Réunion Ms ($n=96$) with an allelic richness of 5.7 and 12 for Grande Comore ($n=75$) with an allelic richness of 10.1. The highest diversity was found in Madagascar Ms populations, albeit with a higher number of samples used.

Species differentiation

The complete dataset was analysed with Structure, which identified two clusters according to the Evanno *et al.* (2005) method, including the B and Ms controls of the laboratory strains, respectively. Across our dataset, nine hybrids between B and Ms were found on Réunion, and these were discarded from the dataset. No hybrids were found on the other islands. A second check was made by sequencing the *COI* fragment of nine to 21 individuals randomly chosen from each species and each island. Species assessment by both techniques was similar. As a result of its useful allele size pattern, locus Ms 145 was revealed as a diagnostic locus to differentiate B from Ms. Indeed, allele sizes ranged from 170 to 200 for Ms, and from 210 to 225 for B. It has also shown utility in differentiating B from Q (Dalmon *et al*., 2008).

Our results show that Ms was found on all the sampled islands of the SWIO: Seychelles (Mahé), Grande Comore, Anjouan, Mayotte, Madagascar, Réunion and Mauritius (fig. 1, table 1), whereas B was only found on Réunion and Mauritius. Furthermore, B populations were only found on crops, whereas the Ms was found on both crops and weeds in all islands where it occurred. On Réunion, Ms was found mostly on weeds (table 1).

Isolation by distance

The regression of $F_{st}(1–F_{st})^{-1}$ calculated across all loci on geographical distance was assessed to determine if there is a relationship between genetic distances and geographical distances. The regression was correlated with a highly significant Mantel test (Pearson r = 0.883; Mantel $P=0.002$, with unilateral test done on 1000 permutations) for the Ms (fig. 2a).

A neighbour-joining unweighted tree was constructed for the microsatellite data using the Cavallis-Forza distance matrix for the different island populations. This illustrates the large divergence between Ms populations, especially between the Seychelles population and all the others (fig. 2b). Réunion and Mauritius Ms populations are little diverged, as are Madagascar and Mayotte Ms populations.

TESS analyses implementing genetic and geographic coordinates, based on Bayesian analyses, showed the presence of seven populations (lowest DIC value) among the Ms population. The seven clusters found, plotted with a kriging function on the SWIO map, showed well-defined groups, largely reflecting the geographical distances between landmasses (fig. 3). The seven clusters are comprised of individuals from the following islands: cluster 1, Madagascar; cluster 2 Grande Comore; clusters 3, 4 and 5, Madagascar and the closest islands of the Comoros archipelago (Anjouan and Mayotte), respectively; cluster 6, Réunion and Mauritius; cluster 7, the Seychelles islands. This last analysis is congruent with the previous linear regression, showing strong genetic structure with geographic distance.

Table 2. Genetic diversity of *Bemisia tabaci* B and Ms within and among sites of all the islands of the Indian Ocean. The observed heterozygosity (H_o), the expected heterozygosity (H_e) and the fixation indices (F_{is} and F_{st} using the ENA correction described in Chapuis & Estoup (2007)) of Weir & Cockerham (1984) were given by the softwares Genepop and FreeNA. Hardy-Weinberg tests are indicated together with F_{is} values. Significant *P*-values are presented by an *. The *P*-values were combined over all loci using Fisher's method. Estimate of null allele frequency (r) was also implemented.

Locus	H_o	H_e	F_{is} (W&C)	F_{st} (W&C)	r
Ms					
P5	0.43	0.57	0.17*	0.106	0.043
P7	0.41	0.59	0.26*	0.068	0.168
P53	0.44	0.56	0.17*	0.100	0.077
P62	0.55	0.45	–0.28	0.069	0.029
Ms145	0.37	0.63	0.39*	0.072	0.112
P11	0.33	0.67	0.39*	0.265	0.097
P32	0.47	0.53	0.08*	0.073	0.075
P59	0.42	0.58	0.20*	0.087	0.061
Bem25	0.45	0.55	0.08*	0.161	0.039
Average	**0.43**	**0.57**	**0.14***	**0.16**	
B					
P5	0.51	0.49	–0.04	0.004	0.000
P7	0.47	0.53	0.11	0.036	0.034
P53	0.51	0.49	–0.05	0.026	0.028
P62	0.61	0.39	–0.6	–0.002	0.000
Ms145	0.44	0.56	0.18*	–0.013	0.001
P11	0.49	0.51	–0.01	0.002	0.000
P32	0.54	0.46	–0.21	0.021	0.021
P59	0.49	0.51	–0.01	0.073	0.013
Bem25	0.43	0.57	0.17*	–0.008	0.065
Average	**0.50**	**0.50**	**–0.11**	**0.025**	0.034

Genetic structuring

For eight loci of Ms and five of B (table 2), the average observed heterozygosity (H_o) was lower than the average expected heterozygosity (H_e). The average within-population heterozygote deficiencies were higher for the Ms (0.14 and –0.11, respectively; table 2). The Ms showed genetic substructure between sampling sites, as illustrated by high values of corrected F_{st} for null alleles (table 2) and previous results. F_{st} values were highest for the Ms. Low proportions of null alleles were detected through our dataset for B and Ms (rMs < 0.17 and rB < 0.03; table 2). Cases of linkage disequilibrium were detected for Ms, not for B (see Appendix 1).

The hierarchical AMOVA tests between species showed most variation to be within individuals within populations (66.84%) and among species (21.6%) (table 3a). The genetic differences between the B and Ms account for much more genetic variance (21.23%, $F_{ct}=0.216$, $P<0.001$) than those

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Table 3. Analysis of molecular variance computed by the method of Excoffier *et al.* (2005) on samples of *Bemisia tabaci* B and Ms from the Indian Ocean Islands. (a) *F*-statistics of genetic differentiation between B and Ms biotypes and among sampling sites of *B. tabaci* (populations). (b) *F*-statistics of genetic differentiation between Ms populations of Madagascar and all the other islands of the SWIO and among sampling sites.

(a)

	Source of variation								
	Among population			Among individuals within population			Within individuals within population		
	V	%	F_{ct}	V	%	F_{sc}	V	%	F_{st}
Ms vs. B	0.912	21.63	0.216*	0.486	11.53	0.147*	2.819	66.84	0.331*
Ms	0.375	10.51	0.105*	0.278	7.81	0.087*	2.909	81.67	0.183*
B	0.040	1.96	0.0196	–0.021	–1.01	–0.010	2.026	99.05	0.009

(b)

	Source of variation								
	Among population			Among individuals within population			Within individuals within population		
	V	%	F_{ct}	V	%	F_{sc}	V	%	F_{st}
Madagascar/ Seychelles	0.726	18.19	0.182*	0.187	4.68	0.057*	3.078	77.13	0.229*
Madagascar/ Mayotte	0.159	4.53	0.045*	0.239	6.82	0.071*	3.113	88.65	0.114*
Madagascar/ Maurice	0.584	14.71	0.147*	0.197	4.95	0.058*	3.190	80.33	0.197*
Madagascar/ Grande Comore	0.186	5.19	0.052*	0.292	8.14	0.086*	3.110	86.66	0.133*
Madagascar/ Réunion	0.417	11.37	0.114*	0.192	5.23	0.059*	3.056	83.4	0.166*

* stands for significant values.

Table 4. Average number of migrants (Nm)* of *Bemisia tabaci* Ms and B within the South Islands of the Indian Ocean.

B. tabaci Ms	Seychelles	Anjouan	Mauritius	Réunion	Grande Comore	Mayotte
Madagascar	1.04	9.73	1.66	1.67	3.86	7.01
Seychelles		0.78	0.64	0.62	0.88	0.82
Anjouan			1.39	1.29	7.68	9.51
Mauritius				2.98	1.21	1.27
Réunion					1.16	1.30
Grande Comore						3.12
B. tabaci B			*Mauritius*			
Réunion			8.71			

* Nm, $(1–F_{st})(4 \times F_{st})^{-1}$ estimation of genetic flow between pairs of populations (Genetix).

among populations within the same species (11.53%, $F_{sc}=0.147$, $P<0.001$). The differentiation among populations is present both within the B and within the Ms. However, due to low sample numbers for the B, no *F*-statistics are significant for the B.

The second hierarchical test for the Ms populations (between pairs of populations of the different islands) showed significant differences between all pairs analysed (table 3b). The highest F_{ct} was found between Madagascar and Mauritius (($F_{ct}=0.147$, $P<0.001$), between Madagascar and Réunion ($F_{ct}=0.114$, $P<0.001$) and between Madagascar and the Seychelles ($F_{ct}=0.182$, $P<0.001$). The lowest F_{ct} values were found for Madagascar and two of the populations of the Comoros archipelago (Mayotte and Grande Comore).

The matrix of Nm estimates presented in table 4 shows that the Ms populations of the Seychelles were the only ones with values less than 1 (0.62 to 0.82) for all the tested populations except with Madagascar (1.04).

The Ms population pairs of: Madagascar/Anjouan, Mayotte/Madagascar, Mayotte/Anjouan and Grande Comore/Anjouan had the highest gene flow estimation (Nm). A high gene flow between B was also estimated between the islands of Mauritius and Réunion (8.71).

Table 5. Genetic diversities calculated from a partial *mtCOI* fragment of 428 nucleotides among *Bemisia tabaci* B and Ms.

Origin	*B. tabaci*	*n*	S	π
Uganda	Ms	9	9	0.00584
Tanzania	Ms	9	1	0.00052
Madagascar	Ms	9	6	0.00312
Seychelles	Ms	9	4	0.00325
Réunion	Ms	21	7	0.00261
Mayotte	Ms	9	1	0.00052
Anjouan	Ms	3	1	0.00156
Réunion	B	15	1	0.00033
Mauritius	B	10	0	–

n, number of sequences used; S, the number of segregating sites; π, the nucleotide diversity.

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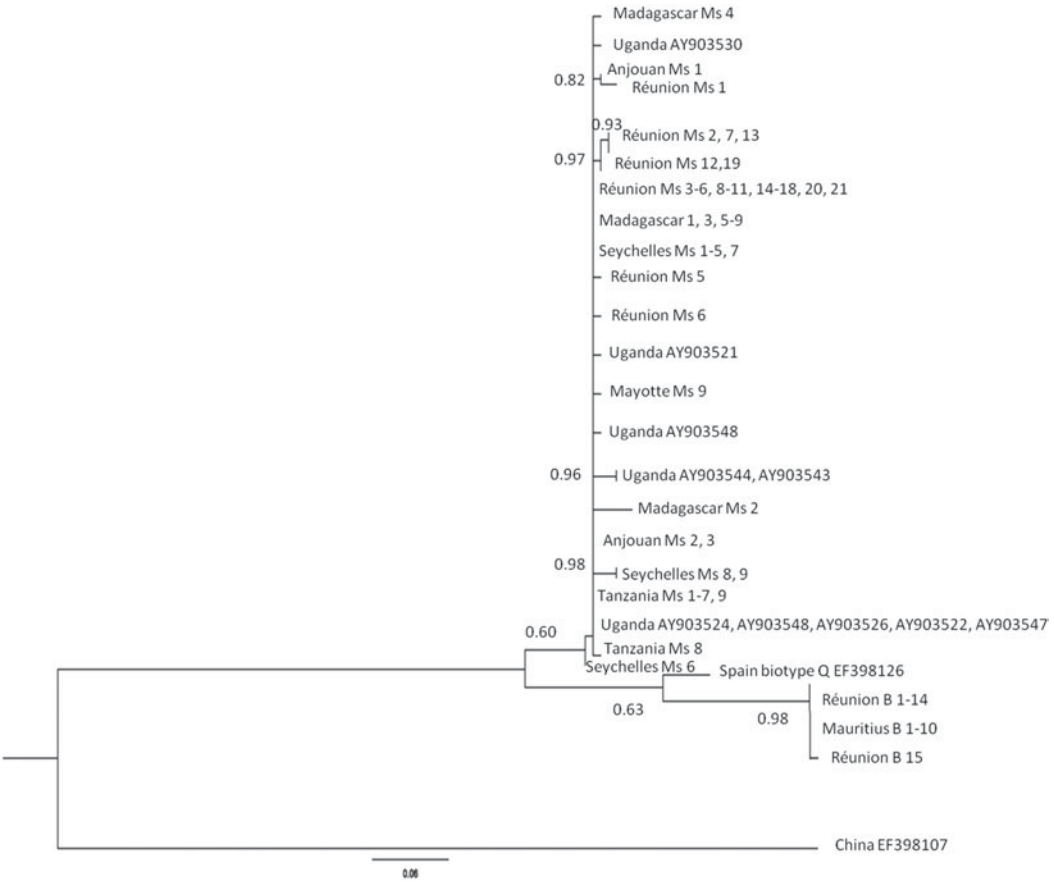

Fig. 4. Maximum likelihood analysis of the mitochondrial *COI* dataset for all samples used in table 5 of *Bemisia tabaci*. Clade support was evaluated by bootstrappings, using 2000 pseudoreplicates and performed under PhyML. Boostraps above 0.6 are indicated.

Mitochondrial DNA analysis

A total of 85 individuals was sequenced for a portion of 490 nucleotides of the mitochondrial *COI* gene, of which 428 nucleotides were readable and used in this study. Among those individuals, 51 belonged to the Ms, while the rest belonged to the B (table 5). Sseruwagi *et al.* (2005) demonstrated the presence of *B. tabaci* Ms in Uganda, and the nine sequences from this study deposited in Genbank were added to our analysis (AY903521, AY903522, AY903524, AY903526, AY903530, AY903543, AY903544 AY903547 and AY903548). Overall, nucleotide diversity (π) among the Ms populations ranged from 0.00052 in Mayotte/Tanzania to 0.00584 in Uganda. The lowest diversities were recorded for the B, despite an equivalent pool of sequences studied compared to the number of localities sequenced. A phylogeny was reconstructed with those sequences and the GTR+I model was selected as the best-fitting model of DNA substitution for the data set. A lack of monophyly for island Ms populations was observed (fig. 4, table 5). However, the deepest divergence involves haplotypes from the Seychelles from all other haplotypes.

Discussion

Three lines of evidence strongly support the action of natural processes in the divergence of the Ms within the SWIO region. Since patterns of genetic divergence in introduced species are often highly incompatible with natural processes, the patterns observed in the Ms support its status as indigenous to the SWIO region.

The first line of evidence supporting this status is that on all the islands supporting indigenous begomoviruses – Madagascar, Mayotte and the Seychelles (Delatte *et al.*, 2005a; Lefeuvre *et al.*, 2007) – no species of *B. tabaci* other than Ms was found. Begomoviruses are viruses only transmitted by *B. tabaci*; and, on the basis of previous studies, the virus species present in the SWIO represent a distinct monophyletic group with close relationships to monopartite and bipartite African begomoviruses.

The second line of evidence is the strong correlation between nucleotide diversity of the Ms and island age and size. If introduced, one might not expect the pattern to be so clear. Within the SWIO islands, the highest diversity within the Ms was observed within the population collected on Madagascar and the Seychelles. This is consistent with the Ms populations having radiated from these islands to most of the islands. Nucleotide diversity further appears to be related to both the age and size of islands; Madagascar and the granitic Seychelles are continental blocks formed by the separation of Gondwana (Rabinowitz *et al.*, 1983), while the Mascarenes and Comoros are much more recent archipelagos with islands having formed within the last 0.13–15 Ma (Warren *et al.*, 2005). A strong correlation ($R^2 = 0.75$) is obtained

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between the nucleotide diversity of each group of Ms from the different islands of the SWIO and the age of these islands (see Appendix 2). Furthermore, despite a lack of monophyly for island populations, the COI phylogeny demonstrated that the deepest splits in the Ms concern the divergence of one haplotype only found on the oldest islands from the principal grouping (Seychelles). In other words, based on *COI* sequencing for 21 individuals from Réunion, the second youngest volcanic island in the region, all Réunion haplotypes fall within the most recently diverged clade (fig. 4).

The third line of evidence is that the diversity observed between populations is strongly correlated with the distance between landmasses. The presence of seven different populations within Ms, strongly related to the island of collection (significant isolation by distance, and TESS structuring) suggests that the radiation occurred as a result of natural processes prior to human arrival in the region. Ancestral expansion of this species from Madagascar and subsequent radiation is supported by gene flow observed between Madagascar and all the other islands, except the Seychelles. Indeed, the levels of gene flow are clearly too low between the Seychelles and the other islands to have homogenised the gene pool of Ms. Isolation by distance appears to limit gene flow, such that Nm is usually less than one.

Despite the fact that our data support an indigenous status for Ms in the SWIO islands, the highest nucleotide diversity observed for Ms populations, for *COI*, comes from individuals from mainland Africa (Uganda). This suggests that Ms population present in all the islands of the SWIO, might originate from mainland Africa. Up to now, no other sequences from the Ms are available from other African countries, but obviously we suspect that in the future more Ms individuals could be found in the Eastern part of mainland Africa. In previous studies, *B. tabaci* (Delatte *et al*., 2005b; Sseruwagi *et al*., 2005: fig. 4), B, Q and Ms have been found to be monophyletic. B was originally believed to have originated in the Mediterranean countries (Guirao *et al*., 1997), while population genetic studies have since shown that the B probably originated in the Middle Eastern/North African region (Frohlich *et al*., 1999). Those two species have sympatric regions especially in northern Africa, such as Morocco (Tahiri *et al*., 2006). Delatte *et al*. (2005b) showed that for *COI*, B and Q are genetically closer to each other than the Ms is to either of them, indicating that the most ancient divergence is of Ms from B and Q. The possibility to have field hybrid populations between B and Ms (Delatte *et al*., 2006), is also suggesting that both may have a recent common ancestor. Up to now, no references are available showing any fit hybrids between B and Q populations. As a result, we can imagine that these three species originate from a common ancestor on the African continent and subsequently diverged through evolutionary time. Furthermore, genetic differentiation among the Ms populations between islands indicate that the species differentiation could have occurred before human arrival, differences in environment (such as host plants and climate) and corresponding selection pressures occurring after island colonization may account for species divergence.

The analysis of a broad sample of whiteflies in the Indian Ocean provides strong evidence that the B is confined to the islands of Réunion and Mauritius. The very low *COI* nucleotide diversities observed for the B compared to the Ms suggests a shorter period of presence in the SWIO and, therefore, that it may well have an exotic status in this region. Furthermore, our data demonstrate that the B of Mauritius and Réunion originated from the same population and have undergone little divergence (with no significant departure from HWE between islands and very low null allele frequency observed). The high number of migrants observed with microsatellite data may reflect the recent genetic link between both populations and may not result from ongoing gene flow. Since no B or any other species were found on islands other than Mauritius or Réunion, two new exclusive hypotheses are proposed: (i) the colonisation of B was hindered by distance; (ii) B has different ecological constraints than Ms, and it is unable to become established on the other islands. In the case of the first hypothesis, if B reaches one of the other islands, it is likely to be extremely damaging due to the numerous, and not yet emergent, indigenous begomoviruses present on those islands.

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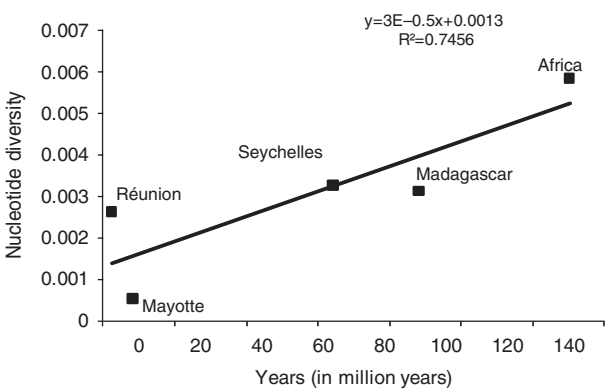
Appendix 1. Linkage disequilibrium table for *Bemisia tabaci* Ms and B.

Bemisia tabaci Ms	*P5*	*P7*	*P53*	*P62*	*Ms145*	*P11*	*P59*	*P32*
P7	*							
P53	NS	*						
P62	*	*	*					
Ms145	*	*	*	*				
P11	*	*	*	*	*			
P59	*	*	*	*	NS	*		
P32	*	*	NS	*	*	*	*	
Bem25	*	*	*	*	*	*	*	*
Bemisia tabaci B	*P5*	*P7*	*P53*	*P62*	*Ms145*	*P11*	*P59*	*P32*
P7	NS							
P53	NS	NS						
P62	NS	NS	NS					
Ms145	NS	NS	NS	NS				
P11	NS	NS	NS	NS	NS			
P59	NS	NS	NS	NS	NS	NS		
32	NS	NS	NS	NS	NS	NS	NS	
Bem25	NS	NS	NS	NS	NS	NS	NS	NS

NS, not significant.

Appendix 2. Plot between nucleotide diversity (π) of the partial *COI* gene of the *Bemisia tabaci* Ms and ages (million years) of the different islands of the Indian Ocean region.



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