MOLECULAR ECOLOGY

Molecular Ecology (2015) **24**, 5976–5991 doi: 10.1111/mec.13438

Lack of genetic isolation by distance, similar genetic structuring but different demographic histories in a fig-pollinating wasp mutualism

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Abstract

Historical abiotic factors such as climatic oscillations and extreme climatic events as well as biotic factors have shaped the structuring of species' genetic diversity. In obligate species-specific mutualisms, the biogeographic histories of the interacting species are tightly linked. This could be particularly true for nuclear genes in the *Ficus*-pollinating wasp mutualistic association as the insects disperse pollen from their natal tree. In this study, we compare spatial genetic structure of plant and pollinator for the *Ficus hirta–Valisia javana* association throughout southeast China including Hainan Island, for both nuclear and cytoplasmic markers. We show that dispersal of the insect leads to plant and insect presenting similar signatures of lack of genetic isolation by distance for nuclear genes on the continent over a distance of 1000 km. But we also show that the demographic histories of plant and insect are strikingly different. This is in agreement with extreme climatic events leading to transient regional extinctions of the insects, associated with local survival of the plants. We also observe evidence of genetic differentiation for both wasps and fig-tree between the continent and Hainan Island, although the Qiongzhou Strait is only on average 30 km wide, suggesting that geographic isolation by itself has not been sufficient to generate this differentiation. Hence, our results suggest that in highly dispersive mutualistic systems, isolation-by-dispersal limitation across a geographic barrier could be supplemented by isolation by adaptation, and maybe by coevolution, allowing further genetic divergence. In such systems, species may frequently be composed of a single population.

Keywords: *Ficus hirta*, mutualism, phylogeography, *Valisia javana*

Received 26 June 2015; revision received 23 October 2015; accepted 26 October 2015

Introduction

Climatic oscillations and extreme climatic events driving range contractions and expansions of species have strongly impacted geographical distributions of the Earth's biota. The rate at which a species is able to expand its geographical range following a climate change is influenced by attributes of the organism, including its behaviour (Hazell *et al.* 2010; Sinsch & Leskovar 2011), physiological capacity (Liu *et al.* 2003; Wiens & Donoghue 2004; McKechnie & Wolf 2010) and other life-history traits (Morrison & Hero 2003; Maraldo & Holmstrup 2009; Walther *et al.* 2009). Less well understood, however, is how a species' range expansion is influenced by interactions with other organisms. In predator–prey and host–parasite systems, for example, range expansion in prey or host species may be enhanced by escape from predators or parasites (Ramanantoanina *et al.* 2011), whereas range expansion

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in these antagonists may be constrained if their preferred hosts possess characteristics that result in slow rates of range expansion (Kubisch *et al.* 2014; Svenning *et al.* 2014). Mutualistic interactions can also influence rates of range expansion, particularly where interactions between symbionts are species specific. In these specialized, often highly coevolved systems, successful range expansion involves both symbionts with one species potentially constraining the range expansion of the other (McCoy *et al.* 2005; Léotard *et al.* 2009; Borer *et al.* 2012).

Range expansion and associated dispersal and colonization events are expected to have significant impacts on the spatial distribution of a species' genetic variation (Avise 2000; Hewitt 2000, 2004). In turn, these processes can be inferred by the signatures they leave in a species' population and phylogeographic genetic structure. Vicariance events and strong barriers to gene flow may be inferred from significant spatial discontinuities in genetic variation (Avise *et al.* 1987; Bowen & Grant 1997; Avise 2000), whereas range expansion is typically indicated by an isolation-by-distance pattern of interpopulation relatedness coupled with reductions in genetic diversity and increased evidence of genetic bottlenecks along the axis of range expansion (Nason *et al.* 2002; Toju & Sota 2006; Léotard *et al.* 2009; Garrick *et al.* 2013; Yu & Nason 2013). While genetic evidence of range expansion has been documented for numerous animal and plant species (*e.g.* McLachlan *et al.* 2005; Baenfer *et al.* 2006; Excoffier *et al.* 2009; Murtskhvaladze *et al.* 2010; Yan *et al.* 2012; Yu & Nason 2013), comparative genetic studies of interacting species have primarily focused on macroevolutionary patterns of co-speciation (*e.g.* Cruaud *et al.* 2010), and phylogeographic patterns of variation in community composition (Stone *et al.* 2012), with few microevolutionary studies examining how species interactions have influenced the process of range expansion and genetic structuring (Althoff & Thompson 2001; Nieberding *et al.* 2004; Brandt *et al.* 2007). Ecologically interacting species are often assumed to exhibit nonindependent histories that could promote congruent patterns of genetic diversity (Nason *et al.* 2002; Smith *et al.* 2008, 2011; Garrick *et al.* 2013; Yu & Nason 2013). Nonetheless, our understanding of how biotic interactions, such as antagonism and cooperation, enhance or constrain species distributions and patterns of genetic diversity remains limited (Garrick *et al.* 2013).

Obligate nursery pollination mutualisms involving flowering plants and their insect symbionts represent particular systems in which the mutualism may facilitate range expansion of the host plants. Indeed, the insects will seek out plants of their host species facilitating long distance pollination of colonizers. Further, the insects often do not feed as adults and the plants constitute their breeding habitat so that plants colonizing new locations should facilitate the colonization of these locations by their pollinators. In these typically species-specific systems, such as the yucca–yucca moth mutualism, the fig–fig wasp mutualism or the *Glochidion–Epicephala* moth mutualism, the insect relies on host plant ovules to support larval development while the egg-laying female provides the host with pollination services (Wei *et al.* 2014). In such systems, plants signal their receptivity by producing specific odours that will enable their specific pollinators to locate receptive inflorescences from a distance (Hossaert-McKey *et al.* 2010). Efficient attraction of pollinators from a distance probably facilitates pollination at low densities and over long distances. This may explain why, in opposition to the paradigm that taxa with specialized biotic interactions should not be able to get established on islands, remote Pacific islands have been colonized by *Glochidion* which are pollinated by mutualistic *Epicephala* moths (Hembry *et al.* 2012). Similarly, *Ficus prolixa* ranges from New Caledonia to the Marquise islands across 6000 km of Pacific Ocean, while maintaining the association with its pollinator and remaining morphologically homogeneous (Florence 1997). This observation suggests that long-distance dispersal of pollinators results in long-distance pollen transfer, homogenizing host populations. Indeed, in another *Ficus* species, *F. pumila*, while pollinator species varied geographically, no genetic structuring was discovered within a pollinator species in southeast China over a 1000-km transect. On the other hand, *F. pumila* presented clinal variation in gene frequencies (Liu *et al.* 2015).

How diversification and co-diversification proceed in such large gene flow mutualistic systems requires phylogeographic investigations of genetic structuring and co-structuring to get insights into the first steps of codivergence and the intraspecific population dynamics leading to it (Espíndola *et al.* 2014). A current challenge is to understand how such high dispersion mutualisms fit 'the geographic mosaic theory of coevolution' championed by Thompson (2005).

In this study, we compare the spatial genetic structure of plant and pollinator for the *Ficus hirta–Valisia javana* association throughout southeast China, for both nuclear and cytoplasmic markers. *Ficus hirta* has been shown to present extremely limited spatial genetic structure from south Thailand to southeast China (Yu & Nason 2013). We add here data on the pollinator. Our working hypotheses are (i) that plant and insect have present similar signatures of lack of genetic isolation by distance for nuclear genes and (ii) that at least the insects have present no signature of isolation by distance for cytoplasmic genes, but (iii) that we would detect genetic signature of differences in the

demographic histories of plant and insect. Indeed, the insects present larger population sizes and shorter generation times and may also be more sensitive than the plants to extreme climatic events such as drought (Liu *et al.* 2015). Our sampling included two wasp populations in Hainan Island that were included to detect whether a limited obstacle to gene flow (a 30-km-wide straight), associated with somewhat different ecological conditions, was sufficient to allow population differentiation.

Materials and methods

Study material

Ficus hirta (family Moraceae, subgenus *Ficus*, section *Eriosycea*, sub-section *Eriosycea*; Berg 2003) is a dioecious shrub or small tree (1.5–2.5 m) distributed from tropical South-East Asia (Indonesia) to tropical and subtropical northeast India and south China (Zhou & Gilbert 2003). It is pollinated throughout its range by *Valisia javana sensu lato* (super-family Chalcidoidea, family Agaonidae; Cruaud *et al.* 2010). In southeast China, it is pollinated by *V. javana* subspecies *hillii* (Wiebes 1994) whose range extends throughout southeast China and into north Vietnam (H. Yu, unpublished data).

Characteristic of all ~750 figs, flowers are borne within a specialized urn-shaped inflorescence – the fig – internally lined with numerous female florets and some male ones. Pollen-bearing female fig wasps are attracted to receptive figs via the production of species-specific volatile cues by receptive figs (Hossaert-Mckey *et al.* 1994; Grison-Pigé *et al.* 2002); the wasps enter the fig and pollinate the uni-ovulate female florets while also ovipositing into a subset of them. Only a limited number of foundresses enter each fig (1.7 females on average, Yu *et al.* 2008). The larvae gall the flowers of their host and, at maturity, males exit into the fig cavity and mate the females within their natal figs. Mated females then emerge into the fig cavity, collect pollen and disperse in search of new receptive figs. Because of low numbers of foundresses and of local matings (local mate competition, Hamilton 1967), the wasps are expected to be inbred.

In *F. hirta*, as in other functionally dioecious figs, female trees produce female figs containing only female flowers, which produce seeds and no wasp offspring. At maturity, the small, red and sweet female fruit are attractive to a variety of birds, which are the primary seed dispersers (Corlett 2006). Male trees, in contrast, serve as hosts for fig wasps, producing 'male' figs containing pollen-bearing male flowers and female flowers that are each capable of supporting a single developing fig wasp but generally produce no seeds. The succession of generations in *V. javana* is thus linked specifically to the reproductive phenology of male *F. hirta* trees. In *F. hirta*, the production of young receptive figs is continuous throughout the year (Yu *et al.* 2006) as opposed to some other co-occurring dioecious *Ficus* species such as *Ficus pumila* which presents well-defined crops on male trees (Liu *et al.* 2014).

Sample populations

Between 2006 and 2011, we sampled *V. javana* from 11 locations throughout the eastern part of its range, in southern mainland China (nine locations) and Hainan (two locations), a 33,920 km^2 island, south of China, and separated from the continent by a 30-km straight (Table 1 and Fig. 1). The sampling spanned a distance of over 1200 km and includes the northernmost range of this species in eastern China. In each location, 10–30 mature male figs (D phase, Galil & Eisikowtich 1968) were collected from a set of plants. These figs were placed individually in fine-mesh bags to allow the wasps to emerge naturally from the figs. The pollinating fig wasps that emerged were preserved in 95% ethanol and stored at −20 °C until DNA extraction. A single female wasp per fig was used for genetic analyses (for the exact distribution of the origin of these females within and among plants on the sites for which the data were recorded, see Microsatellite genotype data in Data accessibility Supporting information). Genetic data from nuclear microsatellites (nSSR) and the mitochondrial cytochrome oxidase c subunit I gene (mtCOI) were collected from a total of 208 and 110 individuals, respectively (Table 1). From each location, an average of 18.8 individuals (range 10–27) was genotyped with microsatellites, and the mtCOI gene was amplified and sequenced in an average of 10 individuals (range 6–20).

Table 1 Sampled populations with their abbreviations, geographical coordinates and rotated latitudinal distance (D_{RL}), the number (N_{nSSR} and N_{mtDNA}) of *Valisia javana* samples used in microsatellite genotyping and mitochondrial COI gene sequencing for each population

Pop.	Lat.(N)	Lon.(E)	D_{RL}(km)	N_{nSSR}	N_{mtDNA}
Ning	26.66417	119.5489	1746.6	20	10
Sha	26.41858	117.81847	1670.6	27	7
Sui	26.47639	114.23917	1549.5	20	11
Xiang	22.42444	114.30639	1268.7	16	8
Huo	23.17031	113.37267	1293	15	20
DHS	23.16638	112.54278	1265.8	24	14
Hu	25.57083	111.9456	1405.3	20	7
Sand	25.98356	107.87428	1278.7	18	6
Nan	22.78659	108.38871	1101.9	19	9
Ding	19.69722	110.3283	964.3	19	8
Wan	18.795	110.3908	891.8	10	10
Total				208	110

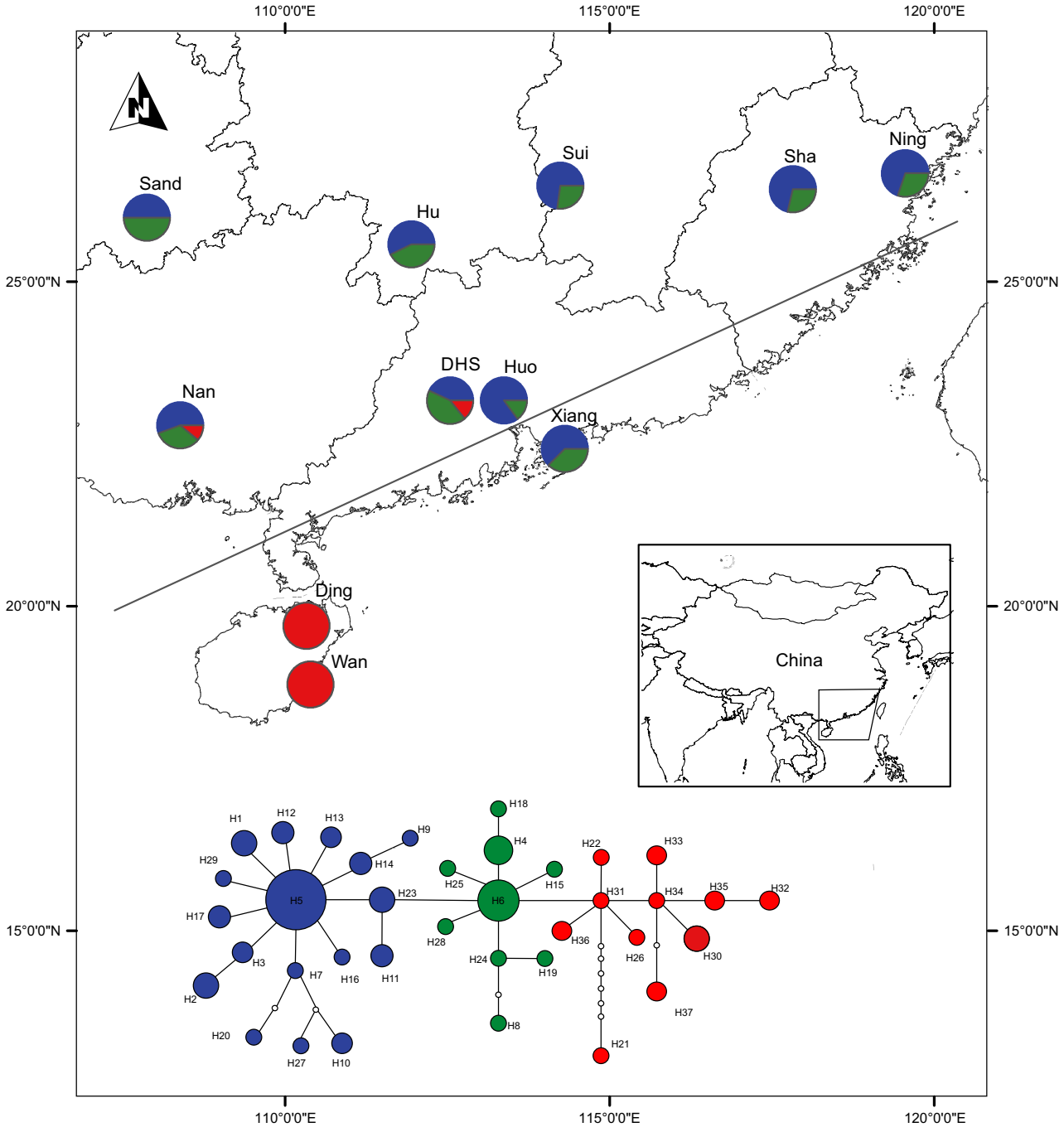

Fig. 1 Sampling sites and mitochondrial DNA (mtDNA) nested haplotype clade distribution for *Valisia javana*. The grey line represents the rotated measure of latitude implemented following Yu & Nason (2013).

DNA extraction, amplification and sequencing

Genomic DNA was extracted from the whole body of each female fig wasp using the EasyPure Genomic DNA Extraction Kit (TransGen, Beijing, China). Two hundred and eight individuals were genotyped at nine unlinked microsatellite loci (1-78, 1-141, A34, A80, A99, B30, C25, F17, H33) that had been previously developed for this fig wasp species (Tian *et al.* 2011). The PCR protocols for microsatellite amplification were performed as described in Tian *et al.* (2011). The fragment sizes of the PCR products were determined on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems, Foster City, CA) using GENOTYPER 4.0 and LIZ 500 (Applied Biosystems) as the internal size standard.

A 685-bp fragment of the mtCOI gene was amplified in 110 individuals using the universal primer pair Jerry-Pat (Jerry:5′-CAACATTTATTTTGATTTTTTGG,Pat:5′-TCCAATGCACTAATCTGCCATATTA; Simon *et al.* 1994) and a species-specific primer pair designed for this species (COI (JX)-F: 5′-ATTTGGGTTAGTTTCTCA-3′, R: 5′-CTCTTCATTTAATTTT GG-3′) using standard conditions with 48–50 °C annealing temperature. PCR amplification of COI was carried out in a 20-μL reaction volume using 10 × buffer, 2 mM Mg^{2+}, 0.25 mM each dNTP, primer 0.6 μM, Taq polymerase (Takara) 2U and DNA 150 ng. The reaction was optimized and programmed on a MJ Thermal Cycler (PTC 200) as one cycle of denaturation at 95 °C for 5 min, 34 cycles of 30 s denaturation at 94 °C, 1 min at a primer-specific annealing temperature (50–54 °C) and 45 s extension at 72 °C, followed by 8 min extension at 72 °C. All amplified PCR products were purified using QIAquick spin columns (Qiagen) and were sequenced in an ABI 3730xl capillary sequencer using BIGDYE TERMINATOR V 3.1 chemistry (Applied Biosystems).

We checked for indications of pseudogenes such as multiple peaks in chromatograms, stop codons or frameshift mutations according to the procedure suggested by Song *et al.* (2008). The COI sequences were translated using the invertebrate mitochondrial genetic code using MEGA 5.1(Tamura *et al.* 2011) and blasted in GenBank using MEGABLAST. No signs of pseudogenes were detected. Consensus sequences were aligned using muscle implemented in MEGA 5.1 (Tamura *et al.* 2011) with manual corrections.

Haplotype sequences have been deposited in GenBank under Accession No: KR873011-KR 873047.

Nuclear microsatellite analyses

We used Genepop 4.0 (Raymond & Rousset 1995) to analyse the nSSR loci testing for departures from Hardy–Weinberg equilibrium using the Markov chain method (settings: dememorization: 1000; batches: 100; iterations per batch: 1000). *P*-values were adjusted using the Bonferroni correction (Rice 1989). Tests of linkage disequilibrium for every pair of loci across all populations were conducted using GENEPOP 4.0. Genetic diversity, number of alleles (*Na*), observed (*Ho*) and expected heterozygosities (*He*) per population were estimated using GENALEX version 6.1 (Peakall & Smouse 2006). We calculated Wright's *F*-statistics F_{IS} (Weir & Cockerham 1984) using ARLEQUIN 3.0 (Excoffier *et al.* 2005), with significance determined by permutation (1000 replicates).

Molecular analysis of variance (AMOVA) was conducted in ARLEQUIN 3.0 (Excoffier *et al.* 2005) to quantify microsatellite variation among populations (φ_{st}) with 999 permutations used for tests of significance. Presence of isolation by distance (IBD) was evaluated using GENALEX 6.1 with all populations and with only continental populations (excluding the Hainan populations) separately. The significance of the correlation between genetic distance and the log-transformed geographic distance was evaluated with a Mantel test with 10 000 permutations (Mantel 1967). Genetic distances between populations were estimated for nSSRs using $F_{ST}/(1-F_{ST})$ (Rousset 1997) estimated with GENEPOP 4.0 (Raymond & Rousset 1995).

Allelic richness (*Ar*) and private allelic richness (*PAr*) were estimated for each population at a minimum allele sample size of 20 using the rarefaction procedure in HP-Rare (Kalinowski 2005). Private alleles are typically found at low frequency (Slatkin 1985), and their loss may be a good indicator of a process of range expansion. We regressed allelic richness (*Ar*) and private allelic richness (*PAr*) on latitude using JMP10 (SAS Institute, NC, USA). Given that *F. hirta* is distributed from the southwest (SW) to the northeast (NE) of China (Yu & Nason 2013), we also conducted these analyses using a rotated measure of latitude that may more accurately reflect the direction of range expansion. We adopted the method described by Yu & Nason (2013) to create a reference line. The reference line is represented as a grey line in Fig. 1. We then determined the distance (km) of populations along this reference line, with zero corresponding to ST, the southernmost population in Yu & Nason (2013). Estimates of *Ar* and *PAr* were then also regressed against the rotated latitudinal distance using JMP 10 (SAS Institute, NC, USA).

Multilocus nSSR genotypes were assigned to population clusters using the software STRUCTURE 2.2 (Pritchard *et al.* 2000) with the Bayesian MCMC assignment method. The admixture ancestry and correlated allele frequencies model (Falush *et al.* 2003) was used with five independent runs each of 500 000 MCMC iterations and 500 000 burn-in steps. We ran STRUCTURE setting K (the number of clusters) between 1 and 10 with the number of natural clusters in the data inferred from a plateau in the estimated posterior probability of K (Falush *et al.* 2003).

Grouping of the populations was also analysed using a principal coordinate analysis (PCoA) performed on Nei's genetic distance matrix, using the program GENALEX 6.1 (Peakall & Smouse 2006). The first two axes from this analysis were plotted.

Mitochondrial DNA analyses

Polymorphism indices of the mtCOI sequences were calculated using the software DNASP 5.0 (Librado & Rozas 2009). Polymorphism indices included the number of haplotypes (*K*), the number of segregating sites

(S), nucleotide diversity (π) and haplotype diversity (Hd). For comparison, relationships among haplotypes were also estimated using a statistical parsimony network approach, implemented in TCS 1.21 (Clement *et al.* 2000) with loops in the network resolved following Crandall & Templeton (1993).

Using the same software as for the microsatellite data, we used AMOVA to quantify and test the significance of cpDNA differentiation (φ_{ST}) among populations (999 permutations) and we tested for IBD by conducting a Mantel test based on population permutation of the correlation between log-transformed N_{ST} (calculated using ARLEQUIN 3.0; Excoffier *et al.* 2005) and log-transformed geographical distance for all population pairs (999 permutations). N_{ST} takes into account differences between populations in haplotype frequency and sequence homology and is expected to increase with geographic distance, and so we employed a one-tailed test of significance. To avoid negative values of N_{ST}, 0.2 was added to all N_{ST} estimates before taking the natural logarithm.

We estimated latitudinal patterns of variation in haplotype diversity by regressing mtCOI haplotype diversity (Hd) against the latitude and rotated latitudinal distance using JMP 10 (SAS Institute, NC, USA). Given a hypothesis of decreasing diversity with increasing latitude, one-tailed tests of significance were employed. Hd was calculated using DNASP 5.0 (Librado & Rozas 2009) including all the 11 populations (6–20 sequences).

Presence of signals consistent with historical population expansions were inferred using Tajima's D (1989), Fu and Li's D (1993) and Fu and Li's F (1993) tests using DNASP 5.0. All values of these statistics are expected to equal zero under selective neutrality with constant population size. A significantly negative value of those statistics indicates a recent population size expansion, a selective sweep or purifying selection, whereas a significantly positive value indicates a decrease in population size or balancing selection (Simonsen *et al.*1995).

To further evaluate the evidence suggesting population expansion, we conducted a mismatch analysis of mtDNA sequence differences using DNASP 5.0. Populations that have experienced a sudden demographic expansion are expected to display a unimodal and smooth distribution of mismatches (Slatkin & Hudson 1991; Rogers & Harpending 1992). The raggedness index (r) of the observed distribution can be used to quantify the smoothness of mismatch distribution, as defined by Harpending (1994). Low raggedness represents a population which has experienced sudden expansion, whereas higher values of the raggedness index suggest stationary or bottlenecked populations (Harpending *et al.* 1993; Harpending 1994). We calculated the raggedness index of *V. javana* for all populations and for continent populations only according to the demographic expansion model implemented in ARLEQUIN 3.0.

Genetic differentiation between continental haplotypes and Hainan haplotypes was analysed using the pairwise Kimura-2-parameter (K2P) as implemented in MEGA 5.1.

Comparison with genetic structuring in the host tree, Ficus hirta

Genetic data from a broader geographic range was previously published for *F. hirta* (Yu & Nason 2013). We restricted that set of data to cover the same geographic range as for the pollinator *V. javana*, to allow comparison of genetic structuring at the same geographic scale using the same statistics. While populations did not systematically coincide, the geographic distribution of populations was similar. Sampling locations and sample sizes for the plant are given in Table S1 (Supporting information).

Results

Nuclear microsatellite analysis

For *Valisia javana*, the number of alleles amplified at each location across loci ranged from 5.1 to 8.2 per locus, with an average of 6.3 (Table 2). As predicted, most of the observed heterozygosities (Ho) were lower than expected (He), with an average $Ho = 0.405$ and

Table 2 Estimates of the number of alleles (Na), allelic richness (Ar), private allelic richness(PAr), and observed (Ho) and expected (He) heterozygosity, inbreeding coefficients (Fis) for 11 *Valisia javana* populations assayed over nine microsatellite loci

Locations abbr.	*Na*	*Ar*	*PAr*	*Ho*	*He*	*Fis*
Ning	5.3	4.418	0.05	0.483*	0.528	0.109
Sha	5.6	4.234	0.07	0.393*	0.562	0.312
Sui	5.7	4.595	0.15	0.340*	0.551	0.395
Xiang	5.1	4.316	0.19	0.229*	0.494	0.559
Huo	6.4	5.565	0.51	0.367*	0.632	0.444
DHS	6.9	5.212	0.55	0.447*	0.589	0.252
Hu	6.7	5.230	0.32	0.472*	0.591	0.216
Sand	7.1	5.312	0.27	0.412*	0.683	0.371
Nan	6.7	5.232	0.56	0.538*	0.557	0.061
Ding	8.2	6.527	1.50	0.430*	0.651	0.353
Wan	5.2	5.222	0.50	0.344*	0.539	0.406
Average	6.3	5.079	0.425	0.405*	0.580	0.316

*Indicates significant departure from HWE for each population over loci ($P < 0.05$).

He = 0.580 (Table 2). Forty-three (46.7%) of the 92 locus–population comparisons indicated significant deviations from Hardy–Weinberg expectations ($P < 0.0001$), remaining significant after sequential Bonferroni correction for multiple tests (Rice 1989). In agreement, the estimates of inbreeding for each population significantly departed from panmixis (Table 2), total F_{IS} over all loci was 0.316 with the highest F_{IS} value reaching 0.559 ($P < 0.001$) for the Xiang population and the lowest 0.061 ($P = 0.794$) for the Nan population. Tests of linkage disequilibrium (*LD*) conducted using GENEPOP were not significant for most pairs of loci over populations except in two pairs (1-78/A34; 1-78/F17), and after sequential Bonferroni correction, none of the 36 informative locus pairs showed significantly LD.

AMOVA revealed significant microsatellite variation among populations ($P < 0.0001$; Table 3, $\varphi_{st} = 0.121$). Compared to *Ficus hirta* (Yu & Nason 2013), a higher fraction of the variance was explained by differentiation among populations in *V. javana*. Including all populations, tests of IBD (Fig. 2) were significant for both *V. javana* ($R^2 = 0.147$, $P = 0.002$ Fig. 2A) and *F. hirta* ($R^2 = 0.105$, $P = 0.004$ Fig. 2B). However, significance of the spatial genetic structure tests was due to the Hainan island populations. When these populations were excluded, no isolation by distance was detected for *V. javana* ($R^2 = 0.027$, $P = 0.167$, Fig. 2A), and for *F. hirta* ($R^2 = 0.000$, $P = 0.259$, Fig. 2B).

A Mantel test revealed a significant decline in microsatellite allelic richness (*Ar*) and private allelic richness (*PAr*) with latitude and with rotated latitudinal distance for *V. javana* but not for *F. hirta* (Fig. 3A, B; Table 4) (Fig. 3C, D; Table 4). The same results were obtained when including only the continental populations in the analysis (Fig. S1C, D, Supporting information; Table 4).

Using STRUCTURE, the estimated posterior probability plateau for *V. javana* was $K = 3$. However, only two clear-cut clusters were observed, one including all individuals from Hainan and another including almost all individuals from continental populations (Fig. 4). Indeed, individuals from any continental populations could be assigned to one or another cluster with variable assignation probabilities.

For *V. javana*, the principal component analysis revealed a similar pattern with populations assigned into two separate clusters (Fig. S2, Supporting information). The first dimension separated the two Hainan populations from the nine continental ones and contributed 54.75% of the total variation, while the second dimension (vertical axis) contributed only 14.83% of the total variance. The two preceding tests, based on the assignation of individuals, were not performed on *F. hirta* because of too low sample size in Hainan.

Cytoplasmic DNA analyses

From the 110 mtDNA COI sequences (685 bp), 37 different haplotypes were identified on the basis of nucleotide substitutions at 38 sites. Twenty of the 38 polymorphic sites were singletons, which were responsible for the high level of private haplotypes in each population: only 17 haplotypes were shared across individuals, and 16 haplotypes were found in more than one population. Similar to other Hymenoptera mtDNA (Simon *et al.* 1994), overall base composition was highly A + T biased (73.6%) with no insertions and deletions. Haplotype diversity (*Hd*) of mtCOI averaged over all populations was 0.921, ranging from 0.571 (*Hu*) to 0.964 (Xiang). The mean nucleotide diversity (π) was 0.00421, ranging from 0.00167 (*Hu*) to 0.00649 (Nan) (Table 3).

AMOVA revealed haplotype variation among populations to be very high ($\varphi_{st} = 0.262$, $P < 0.001$; Table S2,

Table 3 Polymorphism of mtDNA COI gene in 11 populations of *Valisia javana*. Sample size (*N*), number of haplotypes (*K*), haplotype diversity (*Hd*), nucleotide diversity (π) and name of haplotypes (*H*) with Number of individuals

Pop.	*N*	*K*	*Hd*	π	H (Number of individuals)
Ning	10	7	0.911	0.00344	H2 (2), H3 (1), H5 (1), H6 (3), H12 (1), H13 (1), H14 (1)
Sha	7	5	0.905	0.00403	H1 (1), H2 (2), H3 (1), H4 (2), H5 (1)
Sui	11	8	0.945	0.00356	H1 (2), H4 (1), H5 (2), H6 (1), H10 (1), H11 (2), H13 (1), H15 (1)
Xiang	8	7	0.964	0.00391	H5 (1), H6 (2), H7 (1), H8 (1), H9 (1), H10 (1), H11 (1)
Huo	20	9	0.789	0.00216	H1 (1), H5 (9), H6 (2), H12 (1), H14 (1), H23 (3), H27 (1), H28 (1), H29 (1)
DHS	14	9	0.912	0.00330	H4 (2), H5 (4), H6 (2), H14 (1), H22 (1), H23 (1), H24 (1), H25 (1), H26 (1)
Hu	7	2	0.571	0.00167	H5 (4), H6 (3)
Sand	6	4	0.800	0.00273	H5 (1), H6 (3), H12 (1), H16 (1)
Nan	9	7	0.917	0.00649	H5 (1), H6 (1), H17 (3), H18 (1), H19 (1), H20 (1), H21 (1)
Ding	8	5	0.786	0.00182	H30 (1), H31 (1), H32 (1), H33 (1), H34 (4)
Wan	10	6	0.867	0.00276	H30 (3), H31 (1), H34 (1), H35 (3), H36 (1), H37 (1)
Total	110	37	0.921	0.00421	

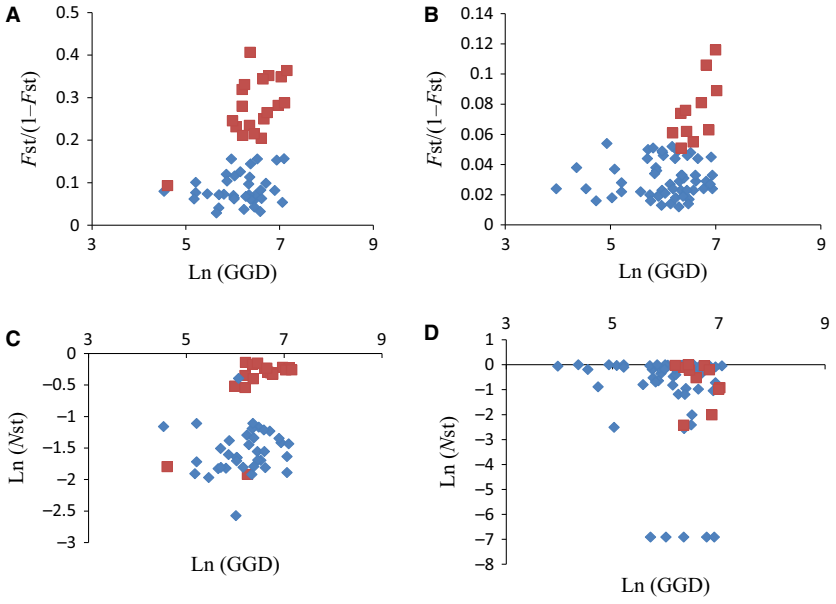

Fig. 2 Pairwise genetic differentiation among populations according to geographic distance in *Valisia javana* (A, C) and *Ficus hirta* (B, D) including all populations (red squares: comparisons involving the Hainan populations; blue diamonds: comparisons between pairs of continental populations) for microsatellite data A, B and cytoplasmic DNA sequence (C: mt DNA for *V. javana* and D: cpDNA for *F. hirta*). (A) Pairwise genetic distance $Fst/(1- F_{ST})$ according to the natural logarithm of geographic distance (*GGD*; km) in *V. javana*. (B) Pairwise genetic distance $F_{ST}/(1-F_{ST})$ according to the natural logarithm of geographic distance (km) of *F. hirta*. (C) Logarithm of genetic distance (*Nst*) according to the natural logarithm of geographic distance (km) of *V. javana*. (D) Logarithm transform of genetic distance (N_{ST}) according to the natural logarithm of geographic distance (km) in *F. hirta*.

Supporting information). The correlation between ln (N_{ST}) and the natural log of geographic distance was significant for *V. javana* ($R^2 = 0.1336$, $P = 0.029$) (Fig. 2C), but not significant for *F. hirta* ($R^2 = 0.0199$, $P = 0.109$) (Fig. 2D) with all populations included, and was not significant for both *V. javana* ($R^2 = 0.0309$, $P = 0.08$) and *F. hirta* ($R^2 = 0.0201$, $P = 0.383$) without Hainan populations.

Haplotype diversity (*Hd*) of *V. javana* ranged from 0.571 to 0.964 over populations (Table 3). The regression slope of *Hd* for *V. javana* against both latitude ($R^2 = 0.003$, $P = 0.863$) and rotated latitudinal distance ($R^2 = 0.016$, $P = 0.353$) was not significant. The regression slope of *Hd* for *F. hirta* against both latitude ($R^2 = 0.010$, $P = 0.377$) and rotated latitudinal distance ($R^2 = 0.055$, $P = 0.232$) was not significant.

Tajima's *D* ($D = -1.84749$, $P < 0.05$), Fu and Li's *D* ($D = -3.62811$, $P < 0.02$) and Fu and Li's *F* ($F = -3.49254$, $P < 0.02$) (Table 5) were all significantly negative, thereby suggesting recent population expansion in *V. javana* within the whole sampling region. Excluding the Hainan populations, Tajima's *D* ($D = -1.93452$, $P < 0.05$), Fu and Li's *D* ($D = -3.29051$, $P < 0.02$) and Fu and Li's *F* ($F = -3.30931$, $P < 0.02$) remained significantly negative, thereby suggesting recent population expansion in *V. javana* on the continent. In addition, the mismatch distribution of *V. javana* for both the total populations and continental populations only were unimodal with low Harpending's *r*, also indicative of population expansion (Fig. S3A, B, Supporting information; Table 5).

For *F. hirta*, we obtained nonsignificant values of Tajima's *D*, Fu and Li's *D* and Fu and Li's *F* (Table 5) including all individuals and continental individuals only. The mismatch distributions were in both cases multimodal with much higher values of Harpending's *r* than for the pollinator (Fig. S3C,D, Supporting information; Table 5). These results suggest that *F. hirta* has not experienced a recent population expansion. These results were obtained in a context of higher haplotypic diversity in *V. javana* (37 haplotypes), than in *F. hirta* (only 12 haplotypes obtained over a wider range (Yu & Nason 2013) and of lower genetic differentiation among populations for cytoplasmic markers in *V. javana* than in *F. hirta* (Fig. 2).

For *Valisia javana*, the COI Kimura-2-parameter genetic distance between mainland haplotypes and Hainan Island haplotypes was 0.0072 ± 0.002, while among mainland populations, the distance was 0.0055 ± 0.0013, and between Hainan Island populations, the distance was 0.0035 ± 0.0012.

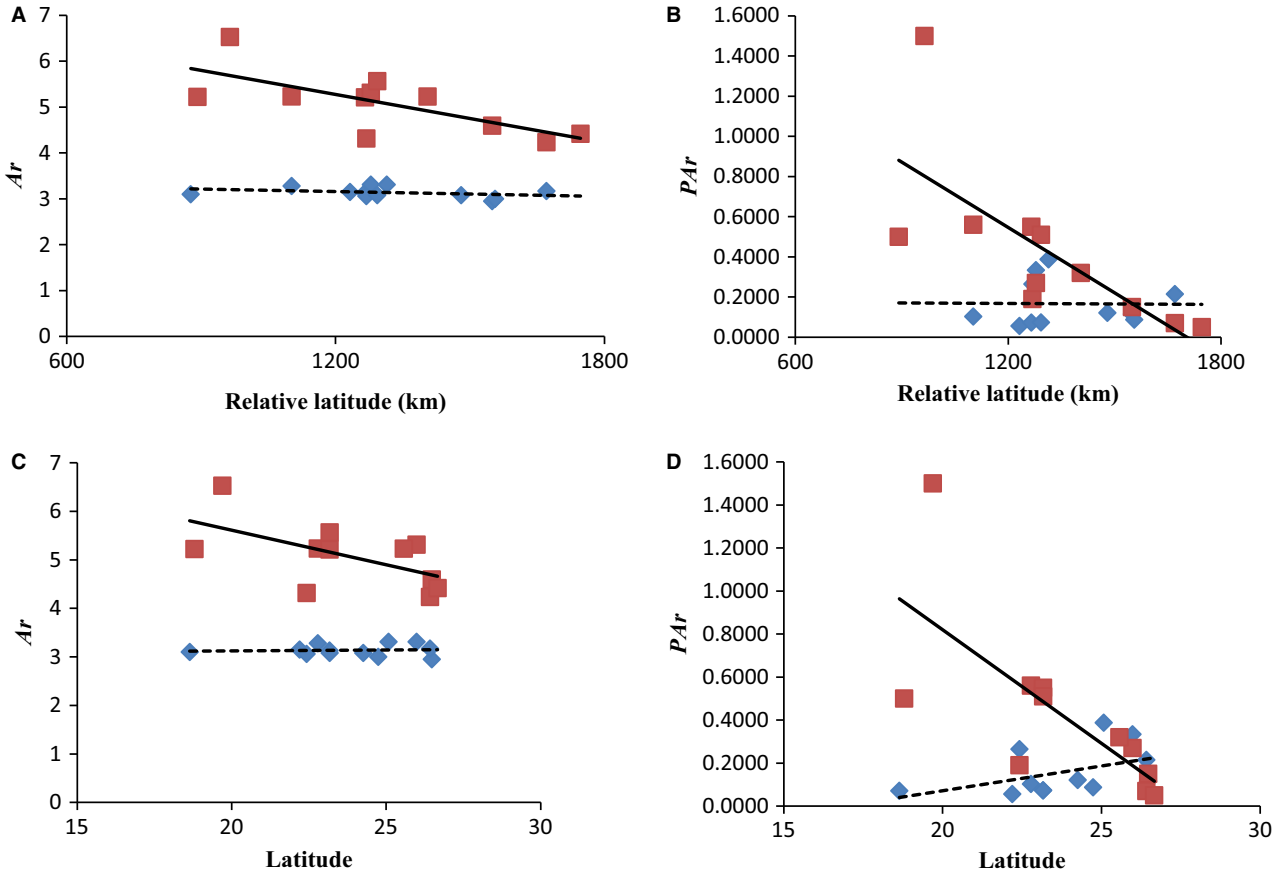

Fig. 3 Decreasing microsatellite diversity with relative latitude in *Valisia javana* (red squares) but not in *Ficus hirta* (blue diamonds), including all populations. For *V. javana*, both allelic richness (*Ar*) and private allelic richness (*PAr*) decreased significantly with latitude. (A) *Ar* with relative latitude; (B) *PAr* with relative latitude; (C) *Ar* with latitude; (D) *PAr* with relative latitude.

Table 4 Significance of the decrease in allelic richness (*Ar*) and private allele richness (*PAr*) with relative latitude and with latitude in *Valisia javana* and *Ficus hirta* based on microsatellite data, including (i) all populations and (ii) only continental populations

	With relative latitude				With latitude			
	Ar		*PAr*		*Ar*		*PAr*	
	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
V. javana								
All populations	**0.502**	**0.007**	**0.525**	**0.006**	**0.346**	**0.029**	**0.517**	**0.006**
Continental populations	**0.445**	**0.025**	**0.690**	**0.003**	0.131	0.169	**0.536**	**0.012**
F. hirta								
All populations	0.107	0.149	0.022	0.324	0.005	0.414	0.205	0.07
Continental populations	0.183	0.053	0	0.484	0	0.498	0.172	0.102

Significant values ($P < 0.05$) are in shown in bold.

Discussion

We observe some striking similarities in genetic structuring between *Valisia javana* and *Ficus hirta*, two species engaged in an obligate species-specific nursery pollination mutualism, but we also observe important differences. The main genetic signal shared between mutualists is (i) lack of genetic IBD on the continent for both nuclear and cytoplasmic markers and (ii) genetic isolation of Hainan populations for nuclear and

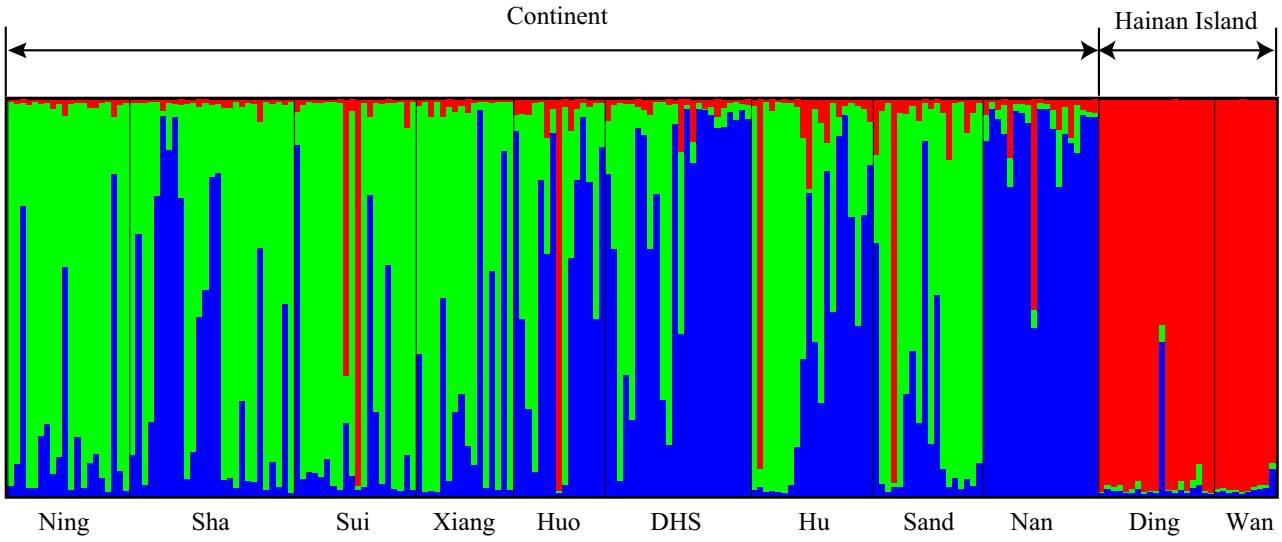

Fig. 4 The STRUCTURE analysis of *Valisia javana* nuclear microsatellite genotypes for $K = 3$ clusters separates Hainan individuals. Black lines separate individuals of different populations. Population names are labelled under the figure, with their regional affiliations (continent and Hainan Island) above it.

Table 5 Neutrality tests and mismatch analyses under a sudden and a spatial expansion model for *Valisia javana*, based on COI. *RAG*: Harpending's Raggedness index estimated under demographic expansion model

	Tajima's *D*	Fu and Li's *D*	Li's *F*	*RAG*
Valisia javana				
All populations	−1.84749*	−3.62811*	−3.49254*	0.022
Continental populations	−1.93452*	−3.29051*	−3.30931*	0.036
Ficus hirta				
All populations	−0.04321	1.41979	1.07244	0.166
Continental populations	1.45611	1.11763	1.45345	0.181

*Indicates significance of the likelihood-ratio tests ($P < 0.05$).

cytoplasmic markers in both species (Fig. 2). Important differences are (i) a signal of recent population expansion in the pollinator but not in its host (Table 5), (ii) stronger differentiation among populations for cytoplasmic genes in the host than in its pollinator leading to frequent signals of genetic isolation among populations (Fig. 2C and D) and (iii) decreased genetic diversity in the pollinator but not in its host close to the northern limit of the distribution range of the association (Fig. 3).

Limited differentiation among insect populations on the continent for both cytoplasmic and nuclear genes and lack of IBD for both nuclear and cytoplasmic markers is suggestive of substantial migration among populations of mated pollen-loaded female wasps. Such migration of pollinators should lead to substantial pollen flow among populations for the plant, in agreement with genetic results on *F. hirta* and with the data presented by Yu *et al.* (2010) and Yu & Nason (2013). They observed unusually low nuclear differentiation among populations and only very limited spatial genetic structure in a study of *F. hirta* spanning much larger distances. These results are in agreement with the high local species diversity of *Ficus* throughout the tropics associated with limited species diversification at broader geographic scales, suggesting that the mutualistic mode of pollination of *Ficus* leads to strong pollen flow, limiting local differentiation and hence limiting speciation (Harrison 2005). As noted by Yu *et al.* (2010), strong differentiation among plant populations for cytoplasmic markers is suggestive of more limited gene flow through seed dispersal than through pollen dispersal. This strong differentiation among populations on the continent makes it difficult to decide whether Hainan populations even more different from continental populations as a single plant population was sampled on Hainan. Even though some creeping species of *Ficus* show stronger spatial genetic structure than *F. hirta* (*F. tikoua*: Chen *et al.* 2011; *F. pumila*: Liu *et al.* 2015), this spatial genetic structure has not been sufficient to lead to species diversification: *F. tikoua* and *F. pumila* do not form species complexes.

A major difference between the host plant and the pollinator is a signal of recent population expansion in the insect but not in the plant. This fits a general picture of relatively frequent regional extinctions for pollinating fig wasps. Indeed, extreme climatic events may result in transient regional extinctions of pollinating wasps while

the plants survive, followed by subsequent recolonization by the wasps (Southern France: Joseph 1958; Florida: Bronstein & Hossaert-McKey 1995; Borneo: Harrison 2000). This should result in a decoupling of wasp and host plant genetic structure as suggested by Alvarez *et al.* (2010) and Liu *et al.* (2015). The signal of recent demographic expansion in pollinators and not the plant fits this hypothesis. A similar signature of recent demographic expansion was observed in the only other fig-pollinating wasp in which gene sequence variation was investigate, the black coloured pollinator of *Ficus septica* in Taiwan (Lin *et al.* 2008). We may also expect extreme climatic events to be more frequent at the limit of the range of *F. hirta*, a feature which could explain the somewhat reduced genetic diversity in the pollinator at the northeast limits of its distribution range. Alternatively, the cytoplasmic signal of recent demographic expansion could result from a selective sweep, which for instance, could be induced by *Wolbachia* (Haine & Cook 2005).

We document on the continent a lack of spatial genetic structure for neutral genes over distances of more than 1000 km. Over such distances, environmental conditions change substantially. For instance between Nanping and Nanning, yearly average high and yearly average low temperatures vary by more than 1.5 °C. In *F. hirta*, presence of structuring for cytoplasmic genes suggests some limitation to gene flow that could allow local adaptation. In *V. javana* however, lack of genetic structuring for nuclear and cytoplasmic genes suggests stronger gene flow limiting the possibility of local adaptation. A similar lack of genetic differentiation for nuclear markers was observed in a transect of over 1500 km from Yunnan to south Thailand for *Ceratosolen fusciceps* the pollinator of *F. racemosa* (Kobmoo *et al.* 2010), in a transect of over 1000 km in southeast China for *Wiebesia pumilae*, the pollinator of *Ficus pumila* in the southern part of its range (Liu *et al.* 2015), and in a transect of 350 km throughout Taiwan for a pollinator of *Ficus septica*. It could well be that in many fig-pollinating wasps, species are constituted by a single, very large, population as suggested by Kobmoo *et al.* (2010). While very limited geographic differentiation is expected for pollinators of monoecious figs which disperse by drifting above the forest canopy, more limited long-distance gene flow was expected for pollinators of dioecious figs, such as *F. hirta* and *F. pumila*, as pollinators of dioecious figs seem to usually disperse more locally (Harrison & Rasplus 2006). Nevertheless, a number of *Ficus* species, both dioecious and monoecious and including *F. racemosa* and *F. hirta* (H. Yu, unpublished data), are pollinated by different species of wasps in different parts of their range (Moe & Weiblen 2010; Darwell *et al.* 2014). These different species could present different adaptations to ecological conditions. Indeed, north of the range of *Wiebesia pumilae*, *F. pumila* is pollinated by closely related but different species of *Wiebesia* (Chen *et al.* 2012). An analysis south of the city of Shanghai showed that the two pollinator species co-occurring locally presented different timing of emergence from figs after winter, suggesting different limiting temperatures for the completion of metamorphosis and hence adaptation to different climatic conditions (Liu *et al.* 2014). Hence, we suggest that substantial gene flow in fig-pollinating wasps may often limit adaptation to local conditions, especially at the limit of their range where conditions are expected to vary. Such situations may favour the establishment of different species of pollinating wasps presenting different adaptations to environmental conditions, and substituting for each other geographically with limited contact zones following the general model developed by Case & Taper (2000): strong gene flow associated with the presence of several species, in the presence of environmental gradients, is predicted to lead to such a pattern.

Within this context, the genetic differentiation between the continent and Hainan Island, observed for both plant and insect, could suggest allopatric differentiation due to a rupture in gene flow between the continent and the island. However, the Qiongzhou Strait is only 30 km wide on average, and given the data on lack of genetic structuring on the continent, this should not constitute a major obstacle to pollinator migration between continent and island. In two other species of pollinating wasps associated with dioecious figs, it has been shown that 30 km of open sea is not sufficient to lead to population differentiation (Zavodna *et al.* 2005). However, a decrease in gene flow could allow local adaptation on Hainan, a scenario which could limit the relative fitness of migrants and, as a consequence, reduce effective gene flow from the continent. Hence, isolation by barriers to dispersal could be supplemented by isolation by adaptation (Orsini *et al.* 2013). The level of differentiation observed here between continental and insular wasp populations (0.7%) is much lower than the level of differentiation observed in other studies on wasp species pollinating the same *Ficus* species, for example about 10% among clades of pollinators of *F. pumila* (Chen *et al.* 2012). The Hainan situation in our study system is thus suggestive of an ongoing process of genetic differentiation that could potentially lead to speciation.

Genetic differentiation between Hainan populations and adjacent populations in continental China has been reported for several other flying organisms such as the silver pheasant *Lophura nycthemera* (Dong *et al.* 2013), the horseshoe bat *Rhinolophus sinicus* (Mao *et al.* 2013) and the bee *Apis cerana* (Zhao *et al.* 2014). No genetic

discontinuity was detected between continental China and Hainan Island for *F. pumila*, the only other species of *Ficus* investigated (Liu *et al*. 2015). However, Hainan is at the limit of the range of the species and in Flora of China, it is not listed as native to the island (Zhou & Gilbert 2003). Therefore, it is difficult to draw conclusions. For another species, *Ficus hainanensis*, individuals from Hainan Island, present figs on short specialized branches at the base of the trunk (Merril & Chun 1935), while individuals from the continent present figs on elongate runners (Berg 2007; Wei *et al.* 2014), suggesting genetic differentiation between Hainan Island and the continent. We may suggest that isolation by adaptation is of frequent occurrence in Hainan in dispersive species, including *Ficus*. This could be due to 1) some reduction in gene flow due to the strait associated with 2) limited adaptation of mobile species to local conditions in adjacent continental populations. Indeed, Hainan belongs to the 'marginal tropical zone', a climatic zone of very limited spread in continental China (Dai *et al.* 2015). In high dispersal species like *Ficus*, geographic barriers may be of major importance in allowing some, limited, genetic differentiation among populations as suggested for *F. insipida* between Costa Rica, Panama and Peru (Heer *et al*. 2015). Such differentiation, if supplemented by some isolation by adaptation, could facilitate diversification.

Little is known about genetic co-structuring within species in obligate plant–insect nursery mutualisms. Investigations on the *Chiastocheta* flies–*Trollius europaeus* interaction suggested lack of congruent phylogeographic and demographic history (Espíndola *et al*. 2014). However, the study investigated cytoplasmic genes in the insects and nuclear genes in the plant. Our results show that even within species, biogeographic signal may differ between nuclear and cytoplasmic compartments. The other nursery pollination system for which data on genetic structuring within species are available is the yucca-yucca moth interaction. A study on cytoplasmic genes of the two most wide ranging pollinators of yuccas concluded that the sampled populations were in genetic and demographic equilibrium, although some populations were quite recent. Further, a clear signal of isolation by distance was detected. The authors concluded that this pattern was consistent with long distance dispersal capacity of the yucca moth (Leebens-Mack & Pellmyr 2004). Our results suggest an alternative explanation. In the fig–fig wasp system, we may suggest that population instability when exposed to extreme climatic events, associated with long-distance dispersal, will result in frequent genetic signatures of demographic expansion as documented now for two species, *V. javana* in southeast China (this study) and a pollinator of *Ficus septica* in Taiwan (Lin *et al*. 2008). In contrast, yucca moth populations may be exceedingly stable when confronted with extreme climatic events. Indeed, length of diapause varies among larvae within population allowing populations to survive to bad years and a record breaking thirty years of larval diapause is documented in one species of yucca moth (Powell 2001). In the geographically much more restricted *Yucca brevifolia* system with most distant populations separated by only 600 km, the observed genetic structuring was much closer to what is documented in *Ficus*. This self-contained system includes two species of host-specific pollinating yucca moths and two species of host-specific parasitic yucca moths. The four moth species presented no signature of geographic differentiation but clear signatures of population expansion, while the plant presented little or no signature of population expansion but a clear signature of geographic differentiation (Smith *et al*. 2011). This is similar to the results on *Ficus pumila* obtained on a 1200-km transect (Liu *et al*. 2015). Comparing genetic structuring in yucca and yucca moths, Althoff *et al*. (2012) proposed that geographic isolation trumps coevolution as a driver of yucca and yucca moth diversification. Our results suggest that in highly dispersive mutualistic systems, isolation-by-dispersal limitation across a geographic barrier could be supplemented by isolation by adaptation, and maybe by coevolution, allowing further genetic divergence.

Reciprocally, in low dispersal mutualistic plant–insect specific interaction systems, genetic co-structuring is expected with isolation by distance allowing local adaptation and/or co-adaptation. This has been observed in the ant-plant *Leonardoxa africana* and its specific ants *Petalomyrmex phylax* and *Cataulacus mckeyi* (Léotard *et al.* 2009). This can be opposed to the higher dispersal interaction between the ant-plant *Barteria fistulosa* and its mutualistic ant *Tetraponera aethiops* for which preliminary reports suggest genetic structuring in the host and more limited structuring in the ant, as in *Ficus pumila* and *Yucca brevifolia* (Blatrix *et al*. 2013).

A major conclusion of this study is that, for obligate mutualistic systems, predictions and explanations of genetic structuring, genetic co-structuring and their bearing on (co)evolutionary and (co)diversification processes are largely dependent on biological traits of the species. Testing ideas and processes, such as the role of frequent regional extinction of pollinators proposed here, will require continued comparative investigation of variation within model systems.

Acknowledgements

We sincerely thank Lanfen Wu for the laboratory work. We also thank associated professor Haifei Yan for data analysis and Dr. Min Liu for helpful discussion and comments on ear-

lier versions of this manuscript. This study was supported by the National Basic Research Program of China (973 Program) (2014CB954103), National Natural Science Foundation of China (31370409; 30600078), Natural Science Foundation of Guangdong Province (S2013020012814) and the Applied Fundamental Research Foundation of Yunnan Province (2014GA003).

Competing interests

The authors declared that they have no competing interest.

References

Althoff DM, Thompson JN (2001) Geographic structure in the searching behaviour of a specialist parasitoid: combining molecular and behavioural approaches. *Journal of Evolutionary Biology*, **14**, 406–417.

Althoff DM, Segraves KA, Smith CI, Leebens-Mack J, Pellmyr O. (2012) Geographic isolation trumps coevolution as a driver of yucca and yucca moth diversification. *Molecular Phylogenetics and Evolution*, **62**, 898–906.

Alvarez N, Kjellberg F, McKey D, Hossaert-Mckey M. (2010) Phylogeography and historical biogeography of obligate specific mutualisms. In: *The Biogeography of Host–Parasite Interactions*(eds Morand S., Krasnov B. R.), pp. 31–39. Oxford University Press, Oxford, UK.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, Massachusetts, USA.

Avise JC, Arnold J, Ball RM *et al.* (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489–522.

Bänfer G, Moog U, Fiala B, Mohamed M, Weising K, Blattner FR. (2006) A chloroplast genealogy of myrmecophytic *Macaranga* species (Euphorbiaceae) in Southeast Asia reveals hybridization, vicariance and long-distance dispersals. *Molecular Ecology*, **15**, 4409–4424.

Berg CC (2003) Flora Malesiana precursor for the treatment of Moraceae 3: *Ficus* subgenus *Ficus*. *Blumea-Biodiversity, Evolution and Biogeography of Plants*, **48**, 536–540.

Berg CC (2007) Precursory taxonomic studies on *Ficus* (Moraceae) for the Flora of Thailand. *Thai Forest Bulletin (Botany)*, **35**, 4–28.

Blatrix R, McKey D, Born C (2013) Consequences of past climate change for species engaged in obligatory interactions. *Comptes Rendus Geoscience*, **345**, 306–315.

Borer M, Arrigo N, Buerki S, Naisbit RE, Alvarez N. (2012) Climate oscillations and species interactions: large-scale congruence but regional differences in the phylogeographic structures of an alpine plant and its monophagous insect. *Journal of Biogeography*, **39**, 1487–1498.

Bowen BW, Grant WS (1997) Phylogeography of the sardines (*Sardinops* spp.): assessing biogeographic models and population histories in temperate upwelling zones. *Evolution*, **51**, 1601–1610.

Brandt M, Fischer-Blass B, Heinze J, Foitzik S. (2007) Population structure and the co-evolution between social parasites and their hosts. *Molecular Ecology*, **16**, 2063–2078.

Bronstein JL, Hossaert-McKey M (1995) Hurricane Andrew and a Florida fig population mutualism: resilience of an obligate interaction. *Biotropica*, **27**, 373–381.

Case TJ, Taper ML (2000) Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. *The American Naturalist*, **155**, 583–605.

Chen Y, Jiang ZX, Compton SG, Liu M, Chen XY. (2011) Genetic diversity and differentiation of the extremely dwarf *Ficus tikoua* in Southwestern China. *Biochemical Systematics and Ecology*, **39**, 441–448.

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

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Corlett RT (2006) Figs (*Ficus*, Moraceae) in urban Hong Kong, South China. *Biotropica*, **38**, 116–121.

Crandall KA, Templeton AR (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics*, **134**, 959–969.

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.

Dai S, Li H, Luo H, Zhao Y, Zhang K (2015) Changes of annual accumulated temperature over Southern China during 1960–2011. *Journal of Geographical Sciences*, **25**, 1155–1172.

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 Evolutionary Biology*, **14**, 189.

Dong L, Heckel G, Liang W, Zhang YY (2013) Phylogeography of Silver Pheasant (*Lophura nycthemera* L.) across China: aggregate effects of refugia, introgression and riverine barriers. *Molecular Ecology*, **22**, 3376–3390.

Espíndola A, Carstens BC, Alvarez N (2014) Comparative phylogeography of mutualists and the effect of the host on the genetic structure of its partners. *Biological Journal of the Linnean Society*, **113**, 1021–1035.

Excoffier L, Laval G, Schneider S (2005) Arlequin 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **23**, 47–50.

Excoffier L, Foll M, Petit RJ. (2009) Genetic consequences of range expansions. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 481–501.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Florence J (1997) *Flore de la Polynésie Francaise*Vol1. ORSTOM Edition, Paris. IRD Editions. Publications scientifiques du Muséum national d'Histoire naturelle, Paris.

Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. *Genetics*, **133**, 693–709.

Galil J, Eisikowtich D (1968) On the pollination of *Ficus sycomorus* in East Africa. *Ecology*, **49**, 259–269.

Garrick RC, Nason JD, Fernandez-Manjarres JF, Dyer RJ. (2013) Ecological coassociations influence species' responses to past climatic change: an example from a Sonoran Desert bark beetle. *Molecular Ecology*, **22**, 3345–3361.

Grison-Pigé L, Bessiére JM, Hossaert-McKey M (2002) Specific attraction of fig-pollinating wasps: role of volatile compounds released by tropical figs. *Journal of Chemical Ecology*, **28**, 283–295.

Haine ER, Cook JM (2005) Convergent incidences of *Wolbachia* infection in fig wasp communities from two continents. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **272**, 421–429.

Hamilton WD (1967) Extraordinary sex ratios. *Science*, **156**, 477–488.

Harpending H (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology*, **66**, 591–600.

Harpending HC, Sherry ST, Rogers AR, Stoneking M (1993) The genetic structure of ancient human populations. *Current Anthropology*, **34**, 483–496.

Harrison RD (2000) Repercussions of El Nino: drought causes extinction and the breakdown of mutualism in Borneo. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **267**, 911–915.

Harrison RD (2005) Figs and the diversity of tropical rainforests. *BioScience*, **55**, 1053–1064.

Harrison RD, Rasplus JY (2006) Dispersal of fig pollinators in Asian tropical rain forests. *Journal of Tropical Ecology*, **22**, 631–639.

Hazell SP, Neve BP, Groutides C, Douglas AE, Blackburn TM, Bale JS (2010) Hyperthermic aphids: insights into behaviour and mortality. *Journal of Insect Physiology*, **56**, 123–131.

Heer K, Kalko EKV, Albrecht L *et al.* (2015) Spatial scales of genetic structure in free-standing and strangler figs (*Ficus*, Moraceae) inhabiting Neotropical forests. *PLoS ONE*, **10**, 1–18.

Hembry DH, Okamoto T, Gillespie RG (2012) Repeated colonization of remote islands by specialized mutualists. *Biology Letters*, **8**, 258–261.

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **359**, 183–195.

Hossaert-Mckey M, Gibernau M, Frey JE (1994) Chemosensory attraction of fig wasps to substances produced by receptive figs. *Entomologia Experimentalis et Applicata*, **70**, 185–191.

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

Joseph KJ (1958) Recherches sur les chalcidiens *Blastophaga psenes* L. et *Philotrypesis caricae* L. du figuier (*Ficus carica* L.). *Annales des Sciences Naturelles, Zoologie*, **13**, 187–260.

Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes*, **5**, 187–189.

Kobmoo N, Hossaert-Mckey M, Rasplus JY, Kjellberg F (2010) *Ficus racemosa* is pollinated by a single population of a single agaonid wasp species in continental South-East Asia. *Molecular Ecology*, **19**, 2700–2712.

Kubisch A, Holt RD, Poethke HJ, Fronhofer EA (2014) Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. *Oikos*, **123**, 5–22.

Leebens-Mack J, Pellmyr O (2004) Patterns of genetic structure among populations of an oligophagous pollinating *Yucca* moth (*Tegeticula yuccasella*). *Journal of Heredity*, **95**, 127–135.

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

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Lin RC, Yeung CKL, Li SH (2008) Drastic post-LGM expansion and lack of historical genetic structure of a subtropical fig-pollinating wasp (*Ceratosolen* sp. 1) of *Ficus septica* in Taiwan. *Molecular Ecology*, **17**, 5008–5022.

Liu H, Xing Q, Ji ZK, Xu LH, Tian YH (2003) An outline of Quaternary development of Fagus forest in China: palynological and ecological perspectives. *Flora-Morphology, Distribution, Functional Ecology of Plants*, **198**, 249–259.

Liu M, Zhao R, Chen Y, Zhang J, Compton SG, Chen XY (2014) Competitive exclusion among fig wasps achieved via entrainment of host plant flowering phenology. *PLoS ONE*, **9**, e97783.

Liu M, Compton SG, Peng FE, Zhang J, Chen XY (2015) Movements of genes between populations: are pollinators more effective at transferring their own or plant genetic markers? *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **282**, 20150290.

Mantel N (1967) Detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Mao XG, He GM, Zhang JP, Rossiter SJ, Zhang SY (2013) Lineage divergence and historical gene flow in the Chinese horseshoe bat (*Rhinolophus sinicus*). *PLoS ONE*, **8**, e56786.

Maraldo K, Holmstrup M (2009) Enchytraeids in a changing climate: a mini-review. *Pedobiologia*, **53**, 161–167.

McCoy KD, Boulinier T, Tirard C (2005) Comparative hosts-parasite population structures: disentangling prospecting and dispersal in the black-egged kittiwake *Rissa tridactyla*. *Molecular Ecology*, **14**, 2825–2838.

McKechnie AE, Wolf BO (2010) Climate change increases the likelihood of catastrophic avian mortality events during extreme heat waves. *Biology Letters*, **6**, 253–256.

McLachlan JS, Clark JS, Manos PS (2005) Molecular indicators of tree migration capacity under rapid climate change. *Ecology*, **86**, 2088–2098.

Merril ED, Chun WY (1935) Additions to our knowledge of the Hainan flora II. *Sunyatsenia*, **2**, 203–332.

Moe AM, Weiblen GD (2010) Molecular divergence in allopatric *Ceratosolen* (Agaonidae) pollinators of geographically widespread *Ficus* (Moraceae) species. *Annals of the Entomological Society of America*, **103**, 1025–1037.

Morrison C, Hero JM (2003) Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology*, **72**, 270–279.

Murtskhvaladze M, Gavashelishvili A, Tarkhnishvili D (2010) Geographic and genetic boundaries of brown bear (Ursus arctos) population in the Caucasus. *Molecular Ecology*, **19**, 1829–1841.

Nason JD, Hamrick JL, Fleming TH (2002) Historical vicariance and postglacial colonization effects on the evolution of genetic structure in *Lophocereus*, a Sonoran Desert columnar cactus. *Evolution*, **56**, 2214–2226.

Nieberding C, Morand S, Libois R, Michaux JR (2004) A parasite reveals cryptic phylogeographic history of its host. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **271**, 2559–2568.

Orsini L, Vanoverbeke J, Swillen I, Mergeay J, De Meester L (2013) Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Molecular Ecology*, **22**, 5983–5999.

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

Powell JA (2001) Longest insect dormancy: Yucca moth larvae (Lepidoptera: Prodoxidae) metamorphose after 20, 25, and 30 years in diapause. *Annals of the Entomological Society of America*, **94**, 677–680.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multi-locus genotype data. *Genetics*, **155**, 945–959.

Ramanantoanina A, Hui C, Ouhinou A (2011) Effects of density-dependent dispersal behaviours on the speed and spatial patterns of range expansion in predator-prey metapopulations. *Ecological Modelling*, **222**, 3524–3530.

Raymond M, Rousset F (1995) Genepop (version 1.2): population genetics software for exact tests and ecumenicism. *The Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.

Simonsen KL, Churchill GA, Aquadro CF (1995) Properties of statistical tests of neutrality for DNA polymorphism data. *Genetics*, **141**, 413–429.

Sinsch U, Leskovar C (2011) Does thermoregulatory behaviour of green toads (*Bufo viridis*) constrain geographical range in the west? A comparison with the performance of syntopic natterjacks (*Bufo calamita*). *Journal of Thermal Biology*, **36**, 346–354.

Slatkin M (1985) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53–65.

Slatkin M, Hudson RR (1991) Pair-wise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Smith CI, Godsoe WKW, Tank S, Yoder JB, Pellmyr O (2008) Distinguishing coevolution from covicariance in an obligate pollination mutualism: asynchronous divergence in joshua tree and its pollinators. *Evolution*, **62**, 2676–2687.

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.

Song H, Buhay JE, Whiting MF, Crandall KA (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 13486–13491.

Stone GN, Lohse K, Nicholls JA *et al.* (2012) Reconstructing community assembly in time and space reveals enemy escape in a Western Palearctic insect community. *Current Biology*, **22**, 532e537.

Svenning J, Gravel D, Holt RD *et al.* (2014) The influence of interspecific interactions on species range expansion rates. *Ecography*, **37**, 1198–1209.

Tajima F (1989) The effect of change in population size on DNA polymorphism. *Genetics*, **123**, 597–601.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Thompson JN (2005) *The Geographic Mosaic of Coevolution*. University of Chicago Press, Chicago, Illinois. 442 pp.

Tian EW, Yu H, Zhang DY, Nason JD (2011) Development of microsatellite loci for *Blastophaga javana* (Agaonidae), the pollinating wasp of *Ficus hirta* (Moraceae). *American Journal of Botany*, **98**, e41–e43.

Toju H, Sota T (2006) Phylogeography and the geographic cline in the armament of a seed-predatory weevil: effects of historical events vs. natural selection from the host plant. *Molecular Ecology*, **15**, 4161–4173.

Walther GR, Roques A, Hulme PE *et al.* (2009) Alien species in a warmer world: risks and opportunities. *Trends in Ecology and Evolution*, **24**, 686–693.

Wei ZD, Kobmoo N, Cruaud A, Kjellberg F (2014) Genetic structure and hybridization in the species group of *Ficus auriculata*: can closely related sympatric *Ficus* species retain their genetic identity while sharing pollinators? *Molecular Ecology*, **23**, 3538–3550.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wiebes JT (1994) *The Indo-Australian Agaoninae (pollinators of figs). Koninklijke Nederlandse Akademie van Wetenschappen Verhandelingen Afdeling Natuurkunde, Tweede Reeks, Deel 92*, p. 208. North-Holland, Amsterdam/Oxford/New York/Tokyo.

Wiens JJ, Donoghue MJ (2004) Historical biogeography, ecology and species richness. *Trends in Ecology and Evolution*, **19**, 639–644.

Yan HF, Zhang CY, Wang FY, Hu CM, Ge XJ, Hao G (2012) Population expanding with the phalanx model and lineages split by environmental heterogeneity: a case study of *Primula obconica* in subtropical China. *PLoS ONE*, **7**, e41315.

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 Phytologist*, **197**, 348–351.

Yu H, Zhao NX, Chen YZ, Deng Y, Yao JY, Ye HG (2006) Phenology and reproductive model of a common fig (*Ficus hirta* Vahl.) in Guangzhou. *Botanical Studies*, **47**, 435–441.

Yu H, Zhao NX, Chen YZ, Herre EA (2008) Male and female reproductive success in the dioecious fig, *Ficus hirta* Vahl. in Guangdong Province, China: implications for the relative stability of dioecy and monoecy. *Symbiosis*, **45**, 121–128.

Yu H, Nason JD, Ge XJ, Zeng JQ (2010) Slatkin's Paradox: when direct observation and realized gene flow disagree – a case study in *Ficus*. *Molecular Ecology*, **19**, 4441–4453.

Zavodna M, Arens P, Van Dijk PJ, Partomihardjo T, Vosman B, van Damme JMM (2005) Pollinating fig wasps: genetic consequences of island recolonization. *Journal of Evolutionary Biology*, **18**, 1234–1243.

Zhao WZ, Tan K, Zhou DY, Wang M *et al.* (2014) Phylogeographic analysis of *Apis cerana* populations on Hainan Island and southern mainland China, based on mitochondrial DNA sequences. *Apidologie*, **45**, 21–33.

Zhou ZK, Gilbert MG (2003) *Ficus*. In: *Flora of China (English edition) 5* (ed. Flora of China Editorial Committee), pp. 37–73. Science Press, Beijing, China & Missouri Botanical Garden Press, St. Louis.

E.W.T. collected samples, performed laboratory work and analysed data. J.D.N. and C.A.M. contributed to data analyses and wrote the manuscript. H.Y. designed research, collected samples, analysed data and wrote the manuscript. L.N.Z. analysed data and contributed to figures. F.K. analysed data and wrote manuscript. All authors contributed substantially to revisions.

Data accessibility

DNA sequence haplotypes: GenBank accessions KR873011-KR 873047. Microsatellite genotype data and mtDNA sequences for all individuals of *Valisia javana*: Dryad data repository at http://datadryad.org, doi: 10.5061/dryad.j59jf.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Decreasing microsatellite diversity in *Valisia javana* (red squares) but not in *Ficus hirta* (blue diamonds) with relative latitude including only continental populations. For *V. javana*, both allelic richness (*Ar*) and private allelic richness (*PAr*) decreased significantly with relative latitude. While for *F. hirta*, only private allelic richness (*PAr*) decreased marginally with relative latitude.

Fig. S2 Principal coordinate analysis on microsatellite data for *Valisia javana*, using a covariance matrix of Nei's genetic distances in the program GenAlEx, version 6.4.1. *Valisia javana* populations are separated into two main genetic groups, Hainan vs. continental populations on axis one which explains 54.75% of the variance. The second axis separates continental populations with no geographic consistency and explains only 14.83% of the variance.

Fig. S3 Mismatch distribution of *Valisia javana* including all individuals (A) and continental populations only (B) and for *Ficus hirta* (C) and (D). Dotted line indicates the observed frequency of pairwise differences among cytoplasmic DNA gene sequences (COI in *V. javana* and *trn*L-*trn*F and *trn*S-*trn*G in *F. hirta*), while the smooth line indicates the simulated distribution under a range expansion model for both the whole populations and with continental populations only (without Hainan).

Table S1 Sampling locations and sample sizes for *Ficus hirta*. Sampled populations with their abbreviations, geographical coordinates and rotated latitudinal distance (D_{RL}), the number ($n_{\text{nSSR and }}n_{\text{cpDNA}}$) of *Ficus hirta* samples used in microsatellite genotyping and chloroplast COI gene sequencing for each population.

Table S2 Hierarchical analysis of molecular variance (AMOVA) for nuclear microsatellite (nSSR) and mitochondrial DNA (mtDNA) sequences among and within populations of *V. javana*.

Data S1 Haplotypes of mtDNA COI gene within and among trees for the populations for which the data was recorded.