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Movements of genes between populations: are pollinators more effective at transferring their own or plant genetic markers?

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Abstract

The transfer of genes between populations is increasingly important in a world where pollinators are declining, plant and animal populations are increasingly fragmented and climate change is forcing shifts in distribution. The distances that pollen can be transported by small insects are impressive, as is the extensive gene flow between their own populations. We compared the relative ease by which small insects introduce genetic markers into their own and host-plant populations. Gene flow via seeds and pollen between populations of an Asian fig species were evaluated using cpDNA and nuclear DNA markers, and between-population gene flow of its pollinator fig wasp was determined using microsatellites. This insect is the tree's only pollinator locally, and only reproduces in its figs. The plant's pollen-to-seed dispersal ratio was 9.183–9.437, smaller than that recorded for other *Ficus*. The relative effectiveness of the pollinator at introducing markers into its own populations was higher than the rate it introduced markers into the plant's populations (ratio = 14:1), but given the demographic differences between plant and pollinator, pollen transfer effectiveness is remarkably high. Resource-availability affects the dispersal of fig wasps, and host-plant flowering phenology here and in other plant-pollinator systems may strongly influence relative gene flow rates.

Key words: Agaonidae, *Ficus*, gene flow, insect dispersal, pollination, population structure, seed dispersal, Slatkin's paradox

1. Introduction

Dispersal between populations plays a vital role in shaping the genetic structure of flowering plant populations. As a cohesive force that unites individual plant species into real evolutionary units [1], dispersal is of great interest amid rising concerns about the persistence of populations within increasingly fragmented landscapes. Gene flow is usually achieved via dispersal of seeds and pollen [2], but dispersal of pollen is almost always more significant than gene flow mediated by movements of seeds [3], except at small spatial scales, e.g. [4]. In addition to reducing overall among-population differentiation, dispersal of pollen between populations can also introduce new genes, and thereby rescue declining populations by reducing inbreeding depression and promoting offspring fitness [5]. Maintenance of inter-population pollen transfer should therefore be considered when drafting long-term management strategies for plants in fragmented habitats or facing declines in pollinators [6].

Insects are the sole pollen vectors of many flowering plants, especially in tropical and subtropical regions [7]. The foraging behavior of the insects that visit their flowers determines which species can act as pollinators, how much pollen they collect and how far the pollen can be transferred [6, 8]. Dispersal kernels of insects, and pollen flow mediated by them, have traditionally been expected to be left skewed, with most individuals dispersing over short distances and gene flow between populations being the result of rare long-distance dispersal events. Direct observations of insect movements are difficult, especially if they are small, and impractical for recording rare long distance dispersal [9], but molecular markers have made the detection of these rare events much easier. Average

64 pollination distances of hundreds of meters are reported [10], and are particularly long
65 among some tropical trees [11, 12], where paternity analysis has detected examples of
66 pollen flow between trees growing tens or even hundreds of kilometers apart [8, 13].

67 The distances that pollinators travel is only one aspect of inter-population pollen
68 transfer. The quantities of pollen that they collect and subsequently deposit on appropriate
69 flowers are equally important [14], and the latter may vary according to how far an insect
70 has dispersed. Insects generally acquire and deposit pollen passively during sequences of
71 visits to flowers. In general, longer times between floral visits, or more intervening floral
72 visits, will result in fewer pollen grains being deposited, due to grooming behavior and
73 abrasion [15]. Insects that have dispersed longer distances may also be weaker, less active
74 and less likely to deposit the pollen they carry. Consequently, insects that have travelled
75 further are likely to deposit less pollen than more locally-dispersing individuals.

76 Insect dispersal also contributes to gene flow between their own populations. Realized
77 gene flow among populations of small insects is often high, and in contradiction to the
78 apparently localized movements of individual insects [16]. This apparent contradiction
79 (Slatkin's paradox) may have been resolved because there is increasing evidence that small
80 flying insects can disperse over large distances [8, 9, 17, 18]. Much of this evidence is
81 based on analysis of the pollen that the insects are carrying, and in the same way as
82 transportation of pollen between populations does not necessarily ensure seed set, so the
83 fecundity of insects after they have dispersed long distances may be reduced [19]. In the
84 case of pollinating insects, any declines in their ability to reproduce after dispersal need not
85 necessarily be proportionate to changes in their ability to pollinate, so assessments of pollen

flow between plant populations do not necessarily reflect the extent of gene flow between populations of their pollinators.

Identification of plant offspring that result from between-population pollination events allows the extent and direction of gene flow between populations to be estimated using Bayesian approaches [e.g., 20], but the likelihood that pollen grains carried between populations will result in the addition of new genes into plant populations has not been estimated quantitatively. This is because we do not know how many insects entered focal populations, how much of the appropriate pollen they carried, and how much they deposited on appropriate stigmas. Also, most plants are pollinated by more than one insect species, each of which will have differing relative contributions to pollen transfer that are likely to vary in space and time.

Here, we combine information derived from between-population gene flow in a plant and in its host-specific unique pollinator to determine the relative effectiveness of gene flow in the two species. Our verbal definition of pollinator effectiveness for dispersing insects moving between populations is the ratio of genetic markers introduced and becoming established in a pollen vector's population compared with the markers that it introduces and that become established in host plant populations via the pollen it carried. Estimates of pollen-mediated gene flow between populations of fig trees can be obtained by comparing bi-parentally and uni-parentally inherited markers (reflecting pollen and seed inheritance respectively) [21], and gene flow among their pollinators can be estimated using bi-parentally inherited markers [22]. In combination, these allow the relative effectiveness of gene flow in fig trees and fig wasps to be estimated quantitatively. Because of their

strongly contrasting generation times, we hypothesise that pollinators disperse their own genes far more readily than plant genes, and that the relative effectiveness of gene flow should be much smaller than one.

To test the above hypothesis, we assessed pollinator effectiveness in a fig species (*Ficus*, Moraceae). Each fig species is exclusively pollinated by one or a small number of species of host plant-specific fig wasps (Agaonidae), that enter the trees' globular inflorescences (figs) in order to lay their eggs [23]. Pollinating fig wasps are short-lived, weak-flying insects, but paternity analyses and population structuring of their host populations suggest that whereas some species disperse locally [24], others disperse across much longer distances [8, 13, 25], initially using fast-flowing air to transport them passively in whichever direction it is moving [26, 27].

In this study, the focal plant species is an Asian fig, *F. pumila*. Firstly, we estimate pollen flow between populations by comparing its genetic structure based on cpDNA and nuclear DNA markers. Then we estimate gene flow of its pollinating fig wasp *Wiebesia pumilae* using nuclear microsatellites. Finally, we calculate the relative effectiveness of the pollinator at introducing genes into its own populations and those of its host plant.

2. Materials and methods

(a) Study system

Ficus pumila L. is a functionally dioecious creeping fig tree that grows on trees and walls. It is widely-distributed in subtropical China. The large, pear-shaped figs contain thousands of tiny female flowers. Figs of female individuals produce only seeds, whereas

figs on male plants support development of fig wasp offspring [28]. Foundress females of the pollinator fig wasp *Wiebesia pumilae* Hill enter the figs to lay their eggs, but cannot reproduce if they enter a female fig. Their wings are removed on entry into the figs and once they enter a fig they do not re-emerge. Usually several females enter each receptive fig. Female *F. pumila* produce one crop of figs each year, pollinated in Spring and early Summer. Male trees generally produce two crops a year with a Spring/early Summer maturing crop that releases the fig wasps that pollinate female trees, and a second crop that matures in Summer/Autumn [29]. The male figs that release adult fig wasps in late Spring contain large numbers of dehiscent male flowers that release pollen that covers the fig wasps before they emerge. Conversely, adult fig wasps released from their natal figs in late summer disperse at a time when there are no receptive female figs to enter, and their natal male figs produce no pollen. Using microsatellites, moderate levels of genetic diversity and low between-population differentiation have been recorded in *F. pumila* populations growing in fragmented landscapes, suggesting moderate to high gene flow among populations, including those located on different islands [30].

Ficus pumila supports three closely-related and largely allopatrically-distributed *Wiebesia* pollinators in China [28]. Unlike many fig wasps, *Wiebesia* species are passive pollinators that do not actively collect and disperse pollen. Based on the fine-scale spatial genetic structure of a *F. pumila* population, Wang et al. [31] inferred that its pollen is dispersed further than its seeds, and is routinely carried further than one kilometer. *W. pumilae* (*Wiebesia* sp. 2 of Chen et al. [28]) is the only pollinator of *F. pumila* in South China. A single *W. pumilae* female that enters a female fig of *F. pumila* results in the

152 production on average of 1000 seeds [32]. If she enters a male fig she can produce around
153 500 offspring [32], but most figs are entered by several foundresses (up to 10 or more), and
154 competition for oviposition sites together with interference between females reduces the
155 numbers of eggs that each female can lay.
156
157 (b) Collections of *Ficus pumila* and its pollinating wasps
158 Although its three associated fig wasps are mostly distributed allopatrically, there are
159 some areas of overlap, so we focused our study in South China, where only *W. pumilae* is
160 present [28]. A total of 17 populations, separated by up to 1100 km, were sampled (figure 1).
161 Between 7 and 27 plant individuals were sampled in each population, with each plant
162 separated by at least 30 m to avoid repeat-sampling of the same individuals. About five
163 healthy leaves were collected from each plant and dried using silica gel. Fig wasps were
164 collected from male trees by placing mature figs that did not have exit holes into netting
165 bags and letting the adult fig wasps emerge naturally. The fig wasps were stored in absolute
166 ethanol at 4 °C.
167
168 (c) Analyses of microsatellites and cpDNA sequencing in *Ficus pumila*
169 Total genomic DNA of *F. pumila* was extracted from about 30 mg of leaves dried in
170 silica gel, using a Plant Genomic DNA Kit (Tiangen, Beijing, China). Eight nuclear
171 microsatellite loci (FP9, FP38, FP102, FP134, FP213, FP540, FP556 and FP601) were
172 genotyped using fluorescently labeled PCR primers as described by Zhang *et al.* [33]. The
173 amplification products were mixed into two groups (group 1: FP9, FP134, FP213, FP556;

group 2: FP38, FP102, FP540, FP601), and each mixture was scanned on an ABI 3730 Automated DNA Sequencer (Applied Biosystems, Foster City, California, USA). Allele sizes were scored using PEAKSCANNER (Applied Biosystems).

For chloroplast DNA of *F. pumila*, three noncoding regions, trnS-trnG [34], atpF-atpH [35] and trnC–ycf6 [36] were amplified in a volume of 50 μL, which included approximately 60 ng of genomic DNA, 0.2 mM dNTPs, 0.2 uM of each primer, 1×PCR buffer, 2 mM Mg^{2+} and 0.4 U of DNA Taq polymerase (Sangon), under the following conditions: 5 min denaturation at 94°C; 35 cycles of 45 s at 94°C, 45 s at 58°C, 1 min at 72°C; and a final extension of 72°C for 8 min. We also amplified the three cpDNA fragments of *F. sarmentosa* var. *henryi* (the most closely-related species in the study region) and two outgroup species, *F. pubigera* and *F. erecta*. PCR products were cleaned and sequenced in both directions on an ABI 3730 DNA Sequence Analyzer.

(d) Microsatellite analyses of *Wiebesia pumilae*

Genomic DNA of the pollinating wasps was isolated from whole bodies of single females using the modified method of Sambrook *et al.* [37]. Genotyping was carried out using 10 microsatellite primers developed previously [38] with 5'-labeled with fluorescent dye on the forward primer. The PCR amplification was performed in a volume of 10μL. The amplification products were combined into three mixtures (mixture 1: WP447 (6-FAM), WP294 (ROX) and WP076 (6-FMA); mixture 2: WP403 (ROX), WP554 (TAMRA), WP399 (HEX) and WP231 (6-FAM); mixture 3: WP522 (6-FAM), WP439 (HEX) and WP004 (6-FAM)), and each mixture was scanned on an ABI 3730 Automated

196 DNA Sequencer. Allele sizes were scored using PEAKSCANNER.

197

198 (e) Analyses of genetic structure

199 For nSSRs of the plant and its pollinator, tests for deviation from Hardy-Weinberg
200 equilibrium (HWE) were performed with GENEPOP 4.0 [39] using exact tests followed by
201 sequential Bonferroni corrections [40]. Linkage disequilibrium (LD) among loci per
202 population was conducted using FSTAT v2.9.3 [41]. Genetic diversity was estimated using
203 the following parameters: mean number of alleles per locus (N_A), allelic richness per locus
204 (A_R, correcting for sample size to the minimal sample size), observed (H_O) and unbiased
205 expected heterozygosities (H_E). These analyses were performed using FSTAT and TFPGA
206 [42]. Population genetic differentiation $F_{ST(n)}$ [43] was evaluated based on all loci using
207 FSTAT. Isolation-by-distance patterns in *F. pumila* and its pollinator were tested by using
208 Mantel tests with the R package 'vegan' [44].

209 For cpDNA of *F. pumila*, sequences (Genbank accession numbers:
210 KJ576907-KJ576923) were aligned using Clustal *w*, implemented in MEGA 4.0 [45].
211 DnaSP [46] was used to count the number of haplotypes. Population differentiation was
212 estimated by calculating $F_{ST(c)}$ with 1000 permutations in Arlequin 3.11 [47]. The
213 phylogenetic tree was constructed by the maximum likelihood approach using PHYML 3.0
214 [48]. The appropriate nucleotide substitution model (TPMuf+I) was chosen by
215 JMODELTEST 2.1.5 [49] based on AIC criterion. Node support was estimated with 100
216 bootstrap replicates.

217 A Bayesian approach to infer population structure of *F. pumila* was performed in

STRUCTURE 2.3.1 [50]. We ran the admixture model with correlated frequencies, and 10 independent runs for each K (from 1 to 10) were performed with 100,000 MCMC repetitions and a burn-in of 10,000. We used LnP(D), the posterior probability of the data for a given K, to identify the most probable number of clusters using ΔK values [51]. After the best K was chosen, all individuals were assigned to the K populations probabilistically by using a burn-in of 300,000 and 1,000,000 MCMC repetitions.

The STRUCTURE analysis divides individuals into at least two clusters, even if all individuals belong to a single panmictic population. *F. pumila* populations showed latitudinal and longitudinal gradients in genetic composition, which might be the output of contact of two genetic clusters or caused by dispersal, given the neutral markers used in this study. To infer the potential cause and the most likely direction of dispersal [52], we tested the relationship between genetic and spatial distances to the most southern (population TC), most northern (population FS), most western (population LZ), and most eastern population (FQ) using a linear regression in R [53].

(f) Estimation of pollinator effectiveness

We defined pollinator effectiveness (*PE*) using the following equation:

$$PE = \frac{Nm_p}{Lg_p} / \frac{Nm_i}{Lg_i} \qquad \text{Eq. 1}$$

where Nm_p is pollen gene flow (number of pollen grains per generation) of the plant, Nm_i is gene flow (number of individuals per generation) of the pollinating insect, Lg_p is generation length (years) of the plant and Lg_i is the generation length (years) of the pollinating insect. Generation lengths (to reaching maturity) of *F. pumila* and *W. pumilae*

240 average about 10 and 0.5 years, respectively (unpublished observations). However, fig
241 wasps of the summer generation can themselves reproduce, but do not pollinate female figs.
242 That means that the insect spreads its genes twice a year, but only spreads the plant genes
243 once a year. Thus we applied a value of 1 per year instead of 0.5 years per generation in this
244 specific case.

245 To estimate pollinator effectiveness, we have to obtain gene flow of the pollinating
246 insect (Nm_i) and pollen-mediated gene flow (Nm_p). Under the assumptions of Wright's [22]
247 infinite island model of population structure, we can estimate Nm_i from the fixation of
248 alleles among populations of the pollinating wasp.

249 For parentally inherited markers, such as nuclear DNA allozymes or microsatellites,
250 fixation index and gene flow in plant species have the following relationship [22]:
251 $F_{ST(n)} = \frac{1}{4Nm+1} = \frac{1}{4Nm_s + 2Nm_p + 1}$, where Nm_s and Nm_p are seed and pollen gene flow,
252 respectively.

253 In most angiosperms, Nm_s can be estimated using maternally inherited markers, such
254 as cpDNA markers. For dioecious plants with a 1:1 breeding sex ratio, the relationship
255 between cpDNA genetic differentiation ($F_{ST(c)}$) and seed gene flow can be expressed as:
256 $F_{ST(c)} = \frac{1}{Nm_s + 1}$ [54]. Based on the above equations, pollen-mediated gene flow can then
257 be estimated using:

258 $$Nm_p = \frac{1}{2F_{ST(n)}} - \frac{2}{F_{ST(c)}} + 1.5$$

Eq. 2

259 Due to their extreme polymorphism, genetic differentiation estimates based on

260 microsatellites are generally underestimates [55], and produce overestimates of gene flow.
261 However, *F. pumila* and *W. pumilae* both have moderate genetic variation and display
262 similar F_{ST} values, so biases in estimations of gene flow should be low. The estimated gene
263 flow values were also slightly lower than those obtained using a private allele approach [56]
264 in Genepop, which again suggests that any biases were weak.

265 To check whether pollinator effectiveness *PE* was related to distance, we estimated
266 pair-wise *PE* based on pair-wise differentiation between populations, and tested its
267 relationship with spatial distance.

268 We also estimated the pollen-to-seed dispersal ratio in *F. pumila*. Assuming a low rate
269 of seed migration, for dioecious plants with a 1:1 sex ratio, the pollen to seed dispersal ratio
270 (r) can then be estimated by Ennos' [21] method:

271 $$r = \frac{m_p}{m_s} = \frac{(\frac{1}{F_{ST(n)}} - 1)(1 + F_{IS}) - 4(\frac{1}{F_{ST(c)}} - 1)}{2(\frac{1}{F_{ST(c)}} - 1)} \quad \text{Eq. 3}$$

272

273 **3. Results**

274 Diagnostic loci confirmed that all the fig wasps in the study populations were *W.*
275 *pumilae* (= *Wiebesia* sp. 2). In total, 331 *F. pumila* and 316 *W. pumilae* were genotyped
276 using microsatellite loci. In *F. pumila*, deviation from Hardy-Weinberg equilibrium (HWE)
277 was found at two loci (FP9 in populations RY and LC; FP134 in populations TC, CZ, MZ
278 and FS). No linkage disequilibrium (LD) was observed. In *W. pumilae*, four loci were found
279 to deviate from HWE (WP447 in XM; WP294 in FQ; WP076 in LZ; WP399 in DZ, RY, LC,
280 GJ). No LD was detected among *W. pumilae* populations.

281 The mean number of alleles (N_A) across all eight loci in populations of *F. pumila*
282 ranged from 3.6 to 7.0 with a mean of 5.4. Allelic richness (A) was lowest in population FS
283 (3.1) and highest in population JJ (5.2). Mean observed heterozygosity (H_O) ranged from
284 0.50 to 0.80, with an average of 0.63. The expected heterozogosity per population (H_E) was
285 between 0.55 and 0.72, with an average of 0.66 (Table 1). A total of 15 chloroplast
286 haplotypes were found in the 17 populations of *F. pumila*, with the Hong Kong population
287 having the most haplotypes (figure 1). The ML tree indicated that *F. pumila* haplotypes
288 were clustered together as a sister clade to *F. sarmentosa* var. *henryi* (Electronic
289 Supplementary Materials, figure S1), suggesting no cytoplasm transfer from other local
290 *Ficus* species.

291 In populations of *W. pumilae*, N_A was between 2.8 and 7.9 with an average of 6.4. H_O
292 and H_E ranged from 0.58 to 0.76 and 0.49 to 0.80, respectively. Allelic richness was lowest
293 in population GZ (2.8), and highest in LZ (5.9) (Table 1).

294 Mantel tests revealed a pattern of isolation-by-distance in populations of *F. pumila* (r =
295 0.527, $P < 0.001$) (figure 2), but not in its pollinator (r = 0.152, P = 0.149). The
296 STRUCTURE analysis indicated a gradient in genetic composition of *F. pumila* populations
297 (figure 3a). A significant positive relationship between genetic and spatial distances was
298 found to the most southern ($r^2 = 0.711$, $P < 0.001$) (figure 3b), northern ($r^2 = -0.371$, P =
299 0.007), western ($r^2 = -0.581$, $P < 0.001$) and eastern ($r^2 = 0.349$, P = 0.009) populations,
300 suggesting that dispersal other than secondary contact of two genetic clusters played a
301 critical role in shaping genetic structure of these populations of *F. pumila*. The coefficient
302 of determination for the relationship between genetic and spatial distances was highest to

303 the most southern population TC, and southern populations were located in the west of the
304 studied region, hinting that a most likely dispersal pattern was first from Hainan Island
305 (populations TC and DZ) to the mainland and then from the west to the east.

306 Based on nuclear variation, the populations of *F. pumila* were moderately
307 differentiated, with a fixation index ($F_{ST(n)}$) of 0.123 (95% CI: 0.099–0.151) and a
308 calculated gene flow (*Nm*) of 1.783 individuals per generation, which was smaller than that
309 estimate based on the frequencies of private alleles (3.282). Large differentiation in cpDNA
310 was observed among populations ($F_{ST(c)}$ = 0.750, P < 0.001). Based on differentiation
311 between cpDNA and nuclear DNA variation, we obtained values for the pollen-to-seed
312 dispersal ratio (r) of 9.183 and 9.437 when $F_{ST(n)}$ was estimated by F_{ST} and R_{ST},
313 respectively.

314 Low levels of genetic differentiation were found among populations of the pollinator
315 ($F_{ST(n)}$ = 0.059, 95% CI: 0.048–0.071). Gene flow between populations (Nm_i) was estimated
316 to be 3.987 individuals per generation. This value was slightly lower than that estimated
317 from private alleles (4.688).

318 Pollen-mediated gene flow (Nm_p) between populations was estimated at 2.898 pollen
319 grains per generation. From Eq. 1, inter-population pollinator effectiveness was calculated
320 to be 0.0727, meaning that for every 13.8 pollinating wasps from outside populations that
321 successfully introduced markers into its own populations, one marker was introduced into
322 populations of *F. pumila*, via the pollen that it carried. *PE* was 0.0959 and 0.0989 within the
323 eastern and western population clusters respectively, much larger than that between the two
324 clusters (0.0205). A slight but non-significant decline in *PE* was present as spatial distances

between populations increased (figure S2).

4. Discussion

(a) Dispersal in *Ficus pumila* and its pollinating wasps

Pollinating fig wasps play an important role in transferring their hosts' genes. However, the wasps are weak fliers and their long-distance dispersal depends on their ability to utilize the wind. Most dioecious fig trees are understory species and remain below the canopy, where wind speed is very slow [57]. Thus, strong genetic structure was expected in dioecious fig trees and their pollinating wasps [57], as has been found in another dioecious creeper in China [24]. However, *F. pumila* is a creeper that can approach the forest canopy, or cover rocks or abandoned walls. This will allow its pollinating wasps to more easily make use of the wind to disperse over long distances. Genetic differentiation is low among South Chinese *W. pumilae* populations separated by up to 1100 km, confirming that the wasps disperse widely between populations. Genetic differentiation of the host *F. pumila* was also not large over this wide range. Further north, *F. pumila* is pollinated by a different *Wiebesia* species, which displays similarly extensive dispersal between populations [30]. Clearly both of these pollinators disperse the pollen of *F. pumila* over wide areas.

Our result is consistent with those from monoecious figs, most of which are canopy trees or forest-canopy hemi-epiphytes. For example, the pollinator of monoecious *F. racemosa* showed limited genetic structure across a 1600 km expanse of continental South-East Asia [58]. A weaker dispersal ability has been inferred among the pollinating wasps associated with some dioecious figs, based on their rates of recovery after local

347 extinctions. In 1998, an El Nino event resulted in an absence of figs on the trees and the
348 consequent local extinction of pollinators of fig trees at Lambir Hills National Park,
349 Sarawak, Malaysia, Borneo. Several fig wasp species had recolonized within one year, but
350 recovery of pollinators associated with monoecious species was more rapid [59]. Elsewhere,
351 a relatively continuous distribution of high-density populations may be responsible for the
352 dioecious understory species *F. hirta* having extensive pollen dispersal across its range, as
353 shown by its populations' weak genetic differentiation [60].

354 Extreme events such as droughts, hurricanes and harsh winters can lead to the local
355 extinction of fig wasp populations, while at the same time leaving host plant populations
356 intact [14, 57, 59, 61]. Similar extreme events, especially if repeated, would disengage the
357 genetic structuring of the pollinator populations from those of their host plants. If the wasps
358 can disperse to long distances, such events reduce the genetic structuring of pollinator
359 populations, relative to those of their hosts. Alternatively, strong genetic structure will be
360 observed in the fig wasp populations due to bottlenecks or founder effects resulting from a
361 small number of colonizers. Dramatic environmental events are not infrequent in South
362 China and most years there are typhoons that could cause large fluctuations in the sizes of
363 *W. pumilae* populations. High inter-population dispersal of *W. pumilae* is evident because
364 its populations are less differentiated (F_{ST}=0.059) than those of its host ($F_{ST(n)}$=0.123).

365 Movements of pollinators, in combination with seed dispersal, determine gene flow
366 between the plants they visit. Microsatellites are often assumed to overestimate gene flow
367 [55], but our estimates based on genetic differentiation in *F. pumila* populations were lower
368 than estimates using private alleles, suggesting that they are not inflated. The fruit bats and

birds that eat ripe figs of *F. pumila* [62, 63] are capable of dispersing fig seeds over long distances [64]. Our estimates of the relative contribution of pollen and seeds to gene flow in *F. pumila* (9.183–9.437) is less than half of that recorded for another dioecious fig tree, *F. hirta* [17]. They are also lower than those recorded for most other plants, where a median value of 17 was reported by Petit et al. [3]. Nevertheless, the pollen-to-seed dispersal ratio shows that the nuclear genome is less structured than the cytoplasmic genomes, as was indicated previously by a study of the plant's fine-scale spatial genetic structure, which concluded that seed dispersal in an area elsewhere in the plant's range was mainly within a radius of 1 km [31].

(b) Pollinator effectiveness

The extensive dispersal displayed by *Wiebesia* species is achieved despite the limitations imposed by their short adult life spans and low flight speeds [9]. Long distance dispersal events may be a feature of many such small insects, not just fig wasps [18, 65, 66] and provide a likely explanation for 'Slatkin's paradox', that direct observations of insect dispersal underestimate their potential to generate gene flow [8, 17]. In the case of fig wasps, where they are the sole dispersers of their host's pollen, gene flow among the insect and plant populations is intimately linked.

Genetic studies of plant populations can provide estimates of the proportion of seeds or seedlings sired by pollen originating from outside focal populations, but give no indication of how many pollinators were responsible for moving the pollen. Partially consistent with our initial hypothesis, our comparison of the relative abilities of a fig wasp

391 to introduce markers that become established in its own and into its host plant's populations
392 showed that markers are introduced more readily into the insect's populations. For every 14
393 insects that dispersed between populations and successfully introduced genetic markers into
394 their own populations, one pollen grain successfully introduced markers into the plant's
395 populations. Pollen is haploid, whereas eggs that result in female offspring are diploid,
396 which should favor the introduction of pollinator markers. No significant relationship was
397 found between pair-wise pollinator effectiveness and spatial distance between populations
398 as a whole or within each of the two population clusters, indicating that inter-population
399 pollinator effectiveness was not influenced by the distances between populations. Fig wasps
400 can use fast-flowing winds for long-distance dispersal, and variation in wind speed and
401 direction may make variation in the distances the wasps are carried insignificant.

402 Although *W. pumilae* introduces markers into its own populations at a higher rate than
403 it transfers markers into populations of its host, its pollinator effectiveness can nonetheless
404 be seen as being remarkably high, given the differences in demography between the fig tree
405 and its pollinator. As in most plant species, the vast majority of seeds produced by *F. pumila*,
406 including those sired by pollen from other populations, must fail to become established
407 plants [67]. In contrast, female fig wasps that have successfully entered a male fig have a
408 much better chance of producing adult offspring that can themselves reproduce.

409 Factors that might be responsible for a lowered relative effectiveness of introducing
410 markers into the pollinator's own populations include a greater likelihood that those *W.*
411 *pumilae* that have dispersed long distances will enter female, rather than male figs.
412 Between-population pollen flow only takes place in late spring because there is only one

crop of female figs each year. Gene flow between its pollinator populations will be mainly in late summer, because very few receptive male figs are produced in spring. Any factors that favor more long distance dispersal in late summer rather than spring will therefore favor gene flow between plant populations. Wind speeds in the region do not differ consistently between these two seasons, so ease of dispersal is unlikely to be responsible. The 'selfish' fruiting phenology of *F. pumila* provides a more likely explanation, because it results in fig wasps that emerge from figs in spring having to leave their natal male trees and make themselves liable to undertake long distance dispersal. This is because those individuals that emerge from figs in spring find themselves on male trees where few if any receptive figs are present, so their only chance for reproduction is if they disperse in search of figs on other trees. Given that the reproductive success of the male plants depends on the fig wasps entering figs on female trees, this is clearly advantageous for the male plants. In contrast, pollinators that emerge from figs in autumn will often find receptive figs on their natal male trees and dispersal from these trees will be unnecessary. There are no female figs to pollinate at this time, so fig wasp populations are increased on their natal trees, ready to emerge the following spring, which is again to the tree's advantage, but reduces the likelihood that the fig wasps will undertake long distance flights. This effect may be further increased because those fig wasps that do disperse and successfully reach a fig on a non-natal male tree may be late-arrivals and face greater competition for oviposition sites from more locally-dispersed individuals. Those fig wasps that have dispersed long distances are also likely to be weaker than others, and capable of laying fewer eggs, even in figs where there is no competition for oviposition sites. Pollination is achieved when the

435 insects walk around the inside of a fig, whereas egg laying involves
436 energetically-demanding repeated probing down the styles of each flower where an egg is
437 laid. Consequently, the rigors of long distance flight are likely to impact more on
438 oviposition rates than pollination rates.

439 Slatkin's paradox reflects a surprising extent of gene flow among populations of small
440 insects, given their apparently poor dispersal abilities. Our results have generated a
441 somewhat contradictory paradox, namely that the extent of dispersal evident from a small
442 insect's movement of plant markers was not reflected to the expected extent in the dispersal
443 of its own genes. We have suggested that manipulation of the pollinators' dispersal behavior
444 by their host plant is largely responsible for this apparent anomaly in our study species, but
445 comparative studies of pollination effectiveness in other systems are required before any
446 general conclusions can be reached. Nonetheless, our study emphasizes that caution is
447 required when using plant population structure to infer the behavior of their pollen vectors.

448

449 **Data accessibility.** The data used in this paper can be accessed via Dryad:
450 doi:10.5061/dryad.g50b0.

451

452 **Author contributions.** X.-Y.C. designed the study. M.L., F.-E.P. and J.Z. conducted the
453 experiments. M.L., S.C. and X.-Y.C. performed analyses and wrote the manuscript. All
454 authors approved the manuscript.

455

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644

Table 1: Sampling information and genetic diversity of populations of *Ficus pumila* and its specific pollinating wasp *Wiebesia pumilae*. # loci: number of loci, *n*: sample size, N_A: number of alleles per locus, *A*: allelic richness, H_O: observed heterozygosity, H_E: expected heterozygosity, $F_{ST(n)}$: nuclear DNA microsatellite-based fixation index, # hap.: number of haplotypes, $F_{ST(c)}$: cpDNA haplotype-based fixation index, **: P<0.001. Numerals in parentheses are ranges of values except those of $F_{ST(n)}$. Means are presented ± SD.

		Ficus pumila	*Wiebesia pumilae*
nDNA SSRs	# loci	8	10
	n	19±6 (7-27)	19±8 (8-30)
	N_A	5.4±0.9 (3.6-7.0)	6.4±1.5 (2.8-7.9)
	A	4.3±0.5 (3.1-5.2)	5.2±0.8 (2.8-5.9)
	H_O	0.63±0.08 (0.50-0.79)	0.67±0.06 (0.58-0.76)
	H_E	0.66±0.04 (0.55-0.72)	0.72±0.07 (0.49-0.80)
	$F_{ST(n)}$	0.125 (95% CI: 0.099-0.151)	0.062 (95% CI: 0.048-0.071)
cpDNA	# hap.	15	/
	$F_{ST(c)}$	0.750 **	/

654 Figure captions

655

656 Figure 1. Locations of *Ficus pumila* sample sites in South China and the distribution of its
657 cpDNA haplotypes. Populations names are abbreviated to two letters, and
658 haplotypes are represented by different colours.

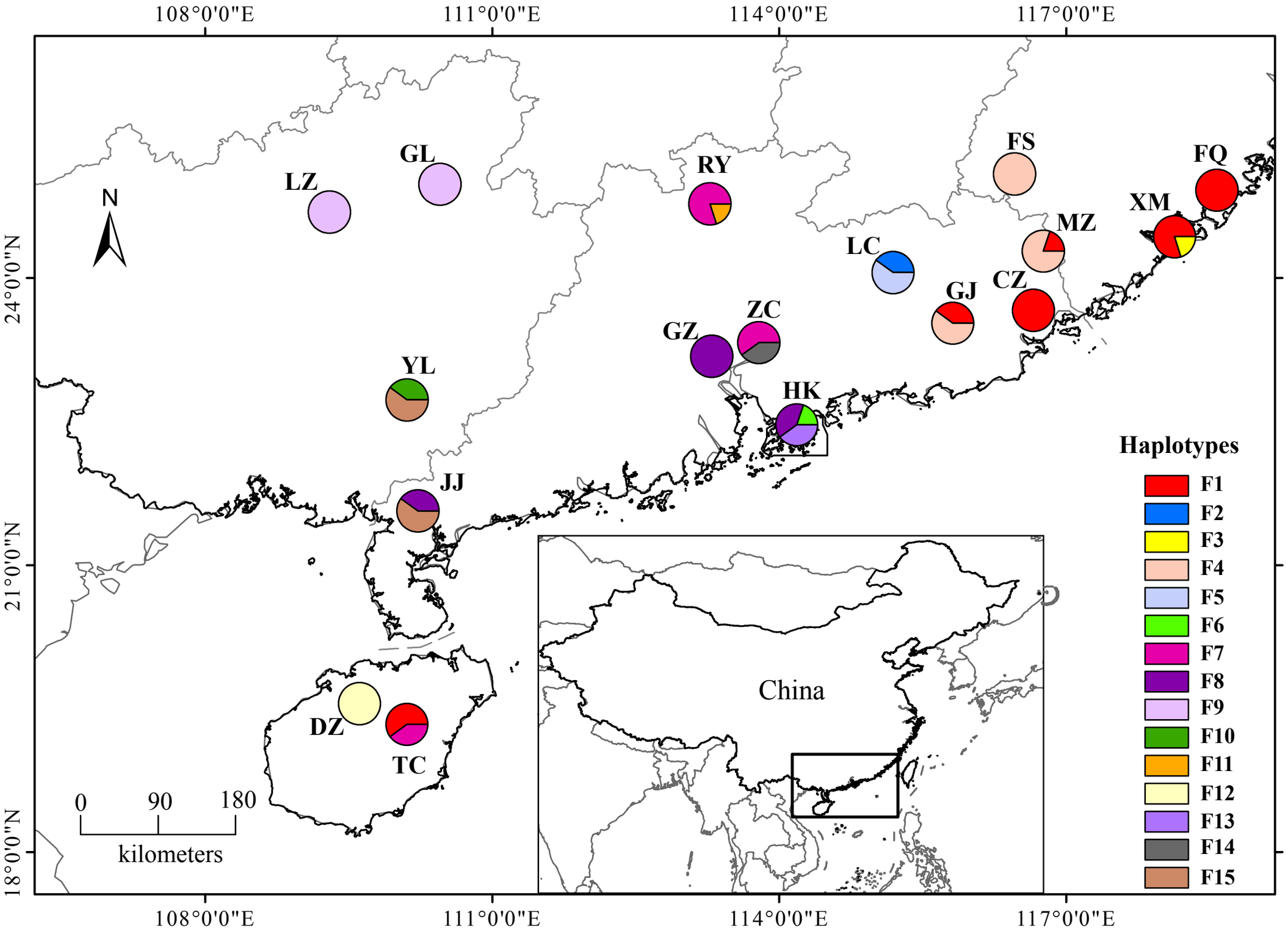
659

660 Figure 2. The relationships between genetic differentiation and geographical distance in
661 South China populations of *Ficus pumila* (left) and *Wiebesia pumilae* (right).

662

663 Figure 3. (a) Genetic clusters of individuals from 17 *Ficus pumila* populations assigned by
664 STRUCTURE. The red columns indicate the western group, and the green columns
665 the eastern group. The populations (left-right) are arranged from East to West. (b) A
666 linear regression between geographic distances from the most southern population
667 of *Ficus pumila* (TC) and the genetic differences of these populations from
668 population TC.

669


108°0'0"E
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114°0'0"E
117°0'0"E
24°0'0"N
21°0'0"N
18°0'0"N
N
LZ
GL
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FS
FQ
XM
MZ
LC
GJ
CZ
ZC
GZ
YL
HK
JJ
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0
90
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Haplotypes
F1
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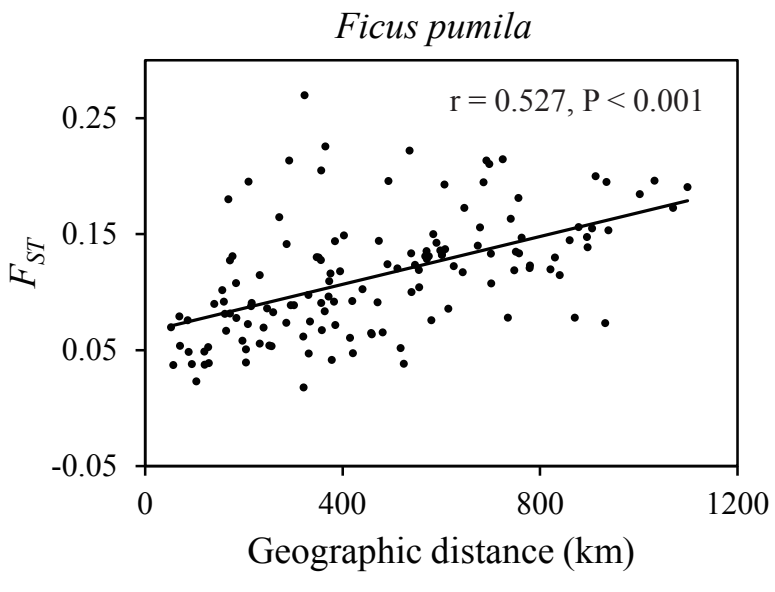
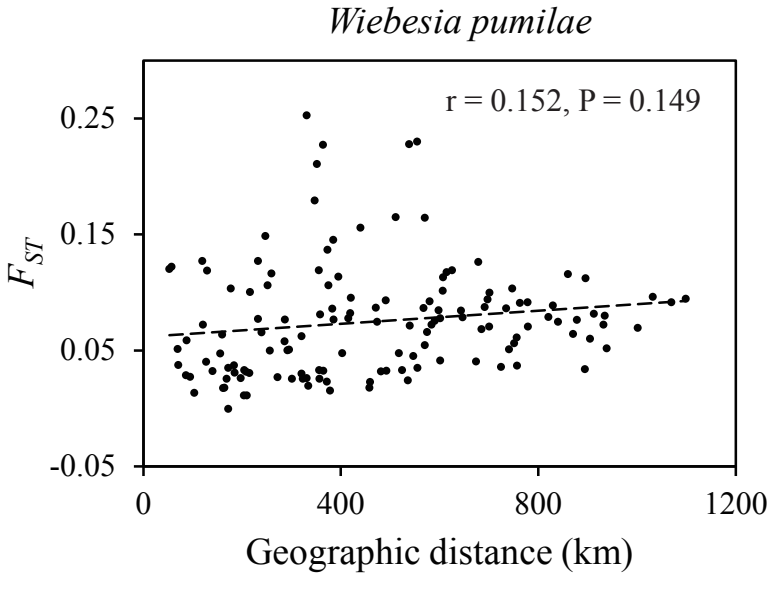
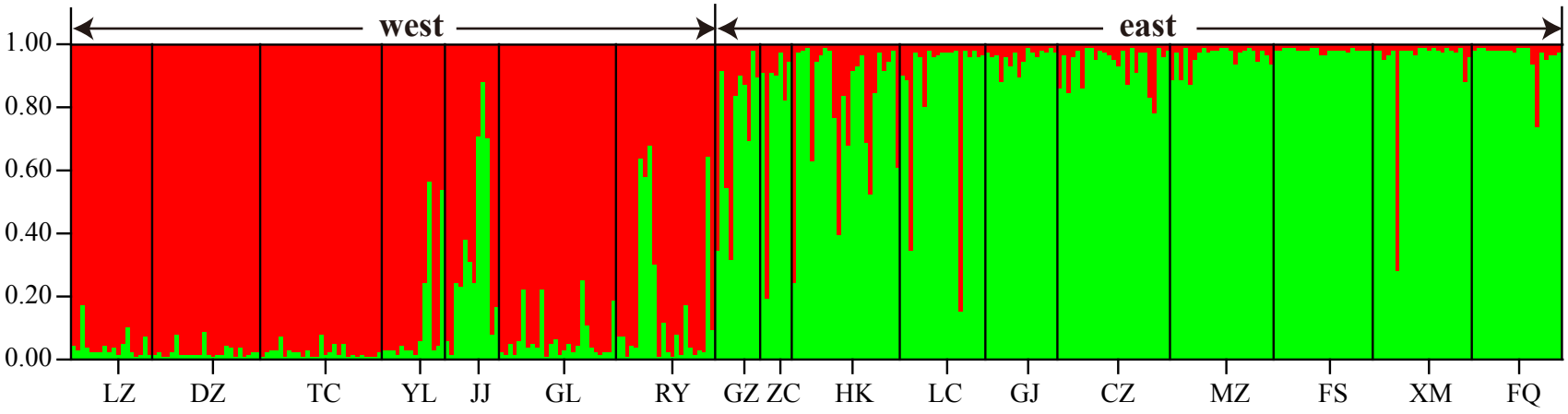
F15

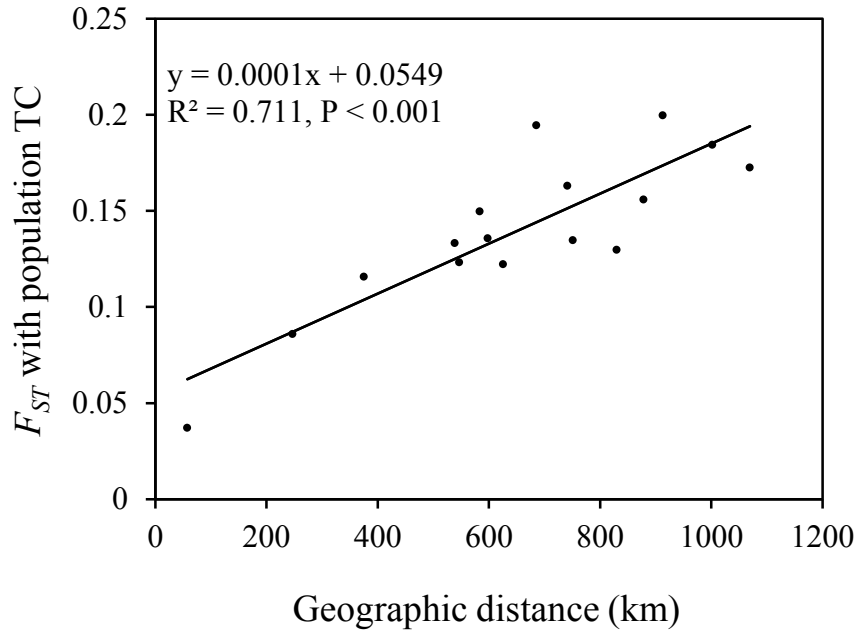

Ficus pumila
r = 0.527, P < 0.001
0.25
0.15
0.05
-0.05
F_{ST}
0
400
800
1200
Geographic distance (km)


Wiebesia pumilae
r = 0.152, P = 0.149
0.25
0.15
0.05
-0.05
F_{ST}
0
400
800
1200
Geographic distance (km)

(a)


west
east
1.00
0.80
0.60
0.40
0.20
0.00
LZ
DZ
TC
YL
JJ
GL
RY
GZ
ZC
HK
LC
GJ
CZ
MZ
FS
XM
FQ

(b)


y = 0.0001x + 0.0549
R² = 0.711, P < 0.001
F_{ST} with population TC
0.25
0.2
0.15
0.1
0.05
0
0
200
400
600
800
1000
1200
Geographic distance (km)