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Data for plants and associate

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Limited gene dispersal and spatial genetic structure as stabilizing factors in an ant-plant mutualism

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Abstract

Comparative studies of the population genetics of closely associated species are necessary to properly understand the evolution of these relationships because gene flow between populations affects the partners' evolutionary potential at the local scale. As a consequence (at least for antagonistic interactions), asymmetries in the strength of the genetic structures of the partner populations can result in one partner having a co-evolutionary advantage. Here, we assess the population genetic structure of partners engaged in a species-specific and obligatory mutualism: the Neotropical ant-plant, *Hirtella physophora*, and its ant associate, *Allomerus decemarticulatus*. Although the ant cannot complete its life cycle elsewhere than on *H. physophora* and the plant cannot live for long without the protection provided by *A. decemarticulatus*, these species also have antagonistic interactions: the ants have been shown to benefit from castrating their host plant and the plant is able to retaliate against too virulent ant colonies. We found similar short dispersal distances for both partners, resulting in the local transmission of the association and, thus, inbred populations in which too virulent castrating ants face the risk of local extinction due to the absence of *H. physophora* offspring. On the other hand, we show that the plant populations probably experienced greater gene flow than did the ant populations, thus enhancing the evolutionary potential of the plants. We conclude that such levels of spatial structure in the partners' populations can increase the stability of the mutualistic relationship. Indeed, the local transmission of the association enables partial alignments of the partners' interests, and population connectivity allows the plant retaliation mechanisms to be locally adapted to the castration behaviour of their symbionts.

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Introduction

Recent studies have shown that virulence is directly influenced by the spatial structuring of both host and parasite populations (Lion & Gandon, 2015). Parasites are thus thought to be 'prudent' in space as a result of kin selection. Spatial structuring leads to a local increase in relatedness between parasites, and the prudent exploitation of the local host supply can be

interpreted as an altruistic trait (Frank, 1996; van Baalen, 2002; Lion & van Baalen, 2008). This is especially true for sterilizing parasites: in spatially structured environments, host reproduction enhances the inclusive fitness of low virulence parasites by providing hosts that related neighbouring pathogens can colonize (O'Keefe & Antonovics, 2002). The intensity of spatial structuring can also select for different levels of virulence depending on migration patterns. Little migration decreases the ability of hosts and parasites to recolonize patches extinct due to a local 'tragedy of the commons', although high migration rates increase the risk of global extinction in case of high virulence (Kerr *et al.*, 2006). Finally, spatial structuring influences virulence by modulating the potential for co-evolution between parasite virulence and host immunity. On the one hand, a high rate of gene flow between parasite populations is thought to enhance local adaptation through the input of genetic novelty (Kaltz & Shykoff, 1998). Conversely, the local maladaptation of the parasite, or at least the absence of local adaptation, is thought to occur when host populations experience a higher rate of gene flow than the parasite (Gandon & Michalakis, 2002).

These predictions not only apply to host–parasite interactions but also to cheating and virulence in mutualistic relationships. Mutualisms are based on an exchange of services between partners, but they are also defined as reciprocal exploitation since they involve both benefits and costs for both partners (Herre *et al.*, 1999). As a consequence, mutualisms can give rise to conflicts in resource allocation and, ultimately, cheating, which occurs when one partner fails to provide a service or manipulates and overexploits the other (Douglas, 2010). The evolutionary trajectory of the relationship depends on the ability of the partners to adapt to one another. Such adaptation can result in a decrease/increase in the costs/benefits of being involved in the relationship or in the prevention of exploitation by the partner (De Mazancourt *et al.*, 2005). At the population scale, the magnitude of cheating and control mechanisms depends on the local genetic diversity of both partners. Moreover, the patterns of dispersion in both partners are one of the key factors enhancing the stability of horizontally acquired mutualisms (i.e. mutualisms implying the nonhereditary acquisition of the symbiont in the environment) as local dispersion potentially generates a genetic correlation between species (Wilkinson & Sherratt, 2001). However, the influence of spatial structuring on virulence in mutualisms remains largely overlooked (but see Szilágyi *et al.*, 2009) and only a few empirical studies have explored the population genetic structures of species engaged in species-specific mutualisms (e.g. Anderson *et al.*, 2004).

We focus here on a species-specific and obligate ant-plant mutualism between *Hirtella physophora* Mart. & Zucc. (Chrysobalanaceae) and *Allomerus decemarticulatus* Mayr (Hymenoptera: Myrmicinae). Like the vast majority of ant-plant mutualisms, this relationship is based on the provision of food and housing in exchange for protection. The ants are considered mutualists because they protect their host plant from phytophagous insects, ultimately resulting in an increase in leaf production (Grangier *et al.*, 2008; Orivel *et al.*, 2011). Moreover, the absence of ants negatively affects plant growth and ultimately survival because of a high level of resulting herbivory (Orivel *et al.*, 2011). However, the relationship comes at a cost for the plant since the ants destroy a part of its floral buds, which makes their host plant reallocate energy from reproduction to vegetative growth (Orivel *et al.*, 2011; Malé *et al.*, 2012). Retaliation mechanisms, however, prevent the ants from destroying all of the buds and the host plant is able to decrease its investment in a relationship with overexploiting ants (Malé *et al.*, 2014).

For there to be a low level of virulence in the ants, both *A. decemarticulatus* and *H. physophora* populations have to be spatially structured (Szilágyi *et al.*, 2009). Such structuring could stem from the patchy distribution of the associated species because the plants grow almost exclusively on hilltops in the study area (Solano *et al.*, 2003). The strong spatial population structure of both partners caused by the topology could result in a positive correlation between their reproductive success, thus lowering the advantage gained by the ants in castrating their host plant. On the other hand, gene flow could enhance the local capability of one partner to respond quickly to changes in the other partner (Wilkinson & Sherratt, 2001). Some degree of connection between populations is thus expected for the introduction of novel resistance/virulence types into these populations to be possible through migration.

To examine the putative role of population structure in the stability of the mutualism between *H. physophora* and *A. decemarticulatus*, we first used molecular and experimental approaches to assess the dispersal distance of both partners. We thus determined the prevailing mode of transmission of the association. Second, in the light of the constraints imposed by the dispersal capabilities of both species on the gene flow between populations, we characterized the spatial genetic structure of the species over a wide, regional level. Finally, we examined the potential for spatial genetic structuring to facilitate the stability of this association.

Materials and methods

Model system

Hirtella physophora is an understorey treelet that occurs strictly in pristine Amazonian rainforest. It has long-lived leaves that bear a pair of leaf pouches at the base of each lamina and extrafloral nectaries located on the abaxial surface of the lamina and inside the leaf

pouches (Leroy *et al.*, 2008). It is an obligate entomophilic outcrosser (Malé *et al.*, 2015) and although its dispersal mode is unknown, ornithochory is thought to be prevalent in the *Hirtella* genus. In the study area, plant individuals are mostly inhabited by *A. decemarticulatus* with a single mature colony per plant (Solano *et al.*, 2003), although another *Allomerus* species, *A. octoarticulatus*, can compete for the same host plant in a few locations. *Allomerus decemarticulatus* is a strictly monogynous ant species (Grangier *et al.*, 2009). Reproductive individuals are produced throughout the year, since there is no massive mating swarm (Grangier *et al.*, 2009). Founding queens disperse by flying from their mother colony to search for an available host plant.

Sampling and genotyping

We collected and successfully genotyped 394 *H. physophora* individuals and 377 *A. decemarticulatus* colonies from 14 different hilltops in French Guiana, hereafter referred to as 'sampling locations'. The sampling locations can be assigned to five broad geographical areas (see Table 1) and the distance between two sampling locations varied from 0.5 to 117 km (see Fig. 1). Between nine and 69 plants/colonies were sampled in each location. Four sampling locations were thoroughly censused to assess the dispersal distance of *H. physophora*. Three of them were located in the *Petit-Saut* area and the fourth in the *Nouragues* reserve (see Table 1). The censuses consisted of repeated extensive searches for *H. physophora* individuals over entire hilltops and the systematic collection of every plant individual and ant colony we found until we were confident that we had located the vast majority of the individuals. In contrast, in regular sampling locations, only the plants and colonies located along trails were sampled and no effort was made to sample the whole population. One leaf and several ant workers were collected for each plant individual and its respective ant colony. Plant tissues were vacuum-dried and ant workers were preserved in 96% ethanol. Each plant individual was geolocated using a Garmin GPSMAP® 60Cx GPS receiver (Garmin Ltd., Kansas).

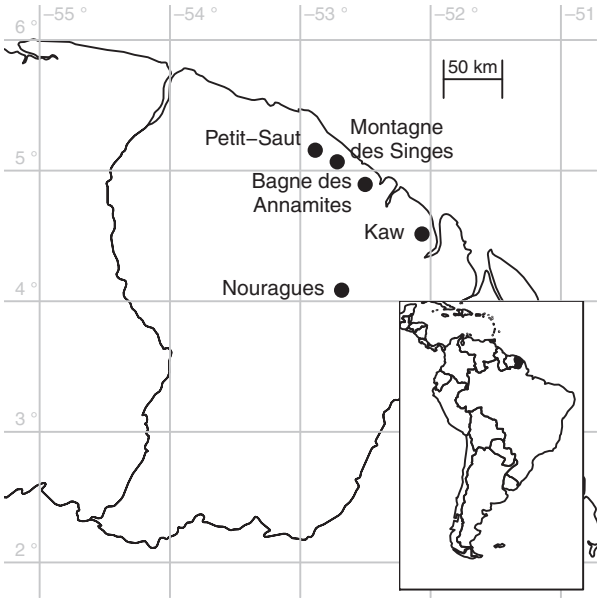

Fig. 1 Map of the five geographical areas sampled in French Guiana. One location was sampled in the *Kaw* area (FOU), one in the *Bagne de Annamites* area (BA), two locations were sampled in the *Nouragues* area (GP and PP) and in the *Montagne des Singes* area (SL and SS) and eight in the *Petit-Saut* area (Z1, Z2, Z6, Z7, Z9, Z11, ZP and PK24).

For each *H. physophora* sample, 10 mg of lamina tissue was frozen at −80 °C during 1 h and then immediately ground between two glass beads (8 and 4 mm in

Table 1 Minimum and maximum pairwise distances between individual plants/colonies (d_{min}/d_{max}), number of individuals (n) and inbreeding coefficient (F_{IS}) in the 14 locations sampled. Areas correspond to groups of sampled locations that are geographically close (see Fig. 1) and asterisks indicate locations that were exhaustively sampled.

			H. physophora		*A. decemarticulatus*	
Areas	Sampling locations	d_{min}/d_{max} (in m)	n	F_{IS}	n	F_{IS}
Kaw	FOU	0.9/139.2	21	0.227	20	0.004
Bagne des Annamites	BA	2.5/43.2	12	0.33	12	0.2
Nouragues	GP*	0.3/318.5	58	0.152	56	0.124
	PP	0.1/376.5	49	0.166	34	0.116
Montagne des Singes	SL	2.5/105.8	11	0.287	13	0.16
	SS	4.0/199.1	10	0.095	17	0.134
Petit-Saut	Z1*	0.1/362.3	45	0.173	44	0.008
	Z9*	1.4/324.5	41	0.125	42	−0.011
	Z6	4.1/441.9	17	0.209	16	0.179
	ZP	3.6/220	12	0.178	12	0.099
	Z11	4.1/61.9	9	0.165	9	−0.038
	Z7	11.0/223.3	20	0.214	21	0.073
	Z2	1.8/208.6	20	0.086	19	0.049
	PK24*	0.6/245.1	69	0.137	62	0.021

diameter) in a 2-mL microtube at 30 Hz for 5–10 min using a TissueLyser II (Qiagen, Courtaboeuf, France). These ground samples were then incubated overnight at 42 °C with 30 μL of proteinase K solution (Qiagen) and 400 μL of 2× CTAB solution. DNA was extracted from 200 μL of the lysate obtained using a BioSprint 15 DNA Plant Kit (Qiagen) according to the manufacturer's instructions. We subsequently amplified 14 species-specific microsatellite markers multiplexed in four different PCR sets using forward primers fluorophore-labelled on the 5′ end (see Molecular Ecology Resources Primer Development Consortium *et al.*, 2010 for details on the protocol). These 14 markers were Hphy-1165, Hphy-383, Hphy-901, Hphy-G108, Hphy-G43Q, Hphy-GGUE, Hphy-KG8A, Hphy-M4WK, Hphy-MFOM, Hphy-MVFC, Hphy-MYRJ, Hphy-NTVY, Hphy-O441 and Hphy-OINQ.

For each *A. decemarticulatus* sample, the total DNA was extracted from one ant worker per colony. Each ant worker was incubated overnight at 55 °C in 10 μL of proteinase K solution (Qiagen) and 150 μL of 10% Chelex solution (Bio-Rad, Marnes-la-Coquette, France). We used 2 μL of the solution obtained to amplify 10 microsatellite markers multiplexed in two different PCR sets using forward primers fluorophore-labelled on the 5′ end, as described in Malé *et al.* (2010). These markers were Adec-A21, Adec-A23, Adec-A41, Adec-A49, Adec-A60, Waur8Ω, Waur872, Waur813, Waur225 and Ad166.

All loci were genotyped using an ABI 3730 sequencer (Applied Biosystems, Courtaboeuf, France) coupled with the 500 LIZ™ GeneScanTM size standard and GENEMAPPER™ 4.0 software (Applied Biosystems).

Genetic assessment of the dispersal distance of *H. physophora*

We assessed the order of magnitude of the dispersal distance for *H. physophora* by estimating the mean parent–offspring distance within the four exhaustively censused sampling locations. The probability of identity (i.e. the probability of two plants having the same genotype by chance) was calculated based on allele frequencies in each sampling location using GENALEX v6.4 (Peakall & Smouse, 2006). Pedigree reconstruction was carried out using the likelihood-based parentage inference in the software CERVUS 3.0.3 (Kalinowski *et al.*, 2007). Since, in this plant species, the diameter at the base of the trunk is positively correlated with the age of the individual (Orivel *et al.*, 2011), we were able to limit the number of plausible parent–offspring relationships. Only plant individuals with a trunk diameter >7 mm were considered old enough to reproduce and were tested for a parent–offspring relationship against individuals with a trunk diameter that was at least 5 mm smaller than the older individual. We ran CERVUS assuming a 1% error rate and that 95% of the population had been sampled. Assignments were considered successful when the parent–offspring relationships were identified with an 80% confidence level.

Experimental assessment of the dispersal distance of *A. decemarticulatus*

We assessed the order of magnitude of the dispersal distance of *A. decemarticulatus* by experimentally triggering host plant colonization by founding queens and measuring the distance between the incipient colonies and the native colonies of both the founding queen and male. To accomplish this, seven *H. physophora* individuals from which the ant colonies had previously been removed were transferred to a new unmanipulated location situated on a *Petit-Saut* hilltop. Each plant was dug up and potted. The ant colonies were removed by brushing off patrolling ants and flushing out workers and brood from the domatia using water and a syringe. Particular attention was paid to the removal of queens. Uninhabited potted plants were then placed approximately 50 cm from a resident (i.e. naturally occurring) *H. physophora* to ensure that the micro-environmental conditions were favourable to the plants (see Figure S1). The sampling location was exhaustively censused, and workers from each of the 54 resident colonies recorded were collected. At least four workers per colony were genotyped as described above. After 8 weeks, each domatium of the transferred plants was opened and all founding queens were collected ($n = 46$, from one to 15 per plant). These queens were subsequently dissected to collect their spermatheca. Maternal DNA (queen) and paternal DNA (male) were extracted separately from heads and from spermathecae, respectively, and the microsatellites were then amplified as described above.

We used GENALEX v6.4 (Peakall & Smouse, 2006) to assess the probability of two ants from sibling colonies, that is colonies whose queens are sisters, having the same multilocus genotype. Each founding queen and male was then assigned to full-sib (i.e. of known paternal and maternal sibships) families constituted of workers from the resident ant colonies using the software COLONY v2.0.1.1 (Wang, 2004). The procedure consisted of four 'long runs' in which all workers coming from a given resident colony were considered full sibs, whereas no *a priori* assumption was made concerning founding queen and male sibships. The mating system was set as 'female monogamy' and 'male monogamy' with the 'inbreeding model'. The frequencies of genotyping errors were assessed for each marker by genotyping several ants twice. At the end of the procedure, the founding queen and male were considered assigned to their native colony when grouped in a full-sib family with workers from only one resident colony with an exclusion probability >0.99. Finally, we calculated the distance between the experimental plants hosting

incipient colonies and the resident colony that produced the founding sexuals.

Assessment of the fine-scale spatial genetic structure

The fine-scale genetic structures of both *H. physophora* and *A. decemarticulatus* were determined within sampling locations for each species separately. The isolation by distance between individuals within sampling locations was assessed by calculating the significance of the regression of the kinship coefficient described by Loiselle *et al.* (1995) as a function of the logarithm of geographical distances smaller than 0.5 km using Mantel's test in SPAGeDi v1.4c (Hardy & Vekemans, 2002). This distance was chosen because it is greater than the diameter of a patch and smaller than the distance between two patches. Since this kinship coefficient describes the genetic similarity between samples, significant isolation by distance should translate into a negative relationship between kinship coefficients and geographical distances. Note that this kinship coefficient is thought not to be biased by inbreeding.

The slope of this regression can be used to estimate the gene dispersal distance through an iterative approach provided that the effective density (D_e) is accurately known. We used SPAGeDi v1.4c to estimate σ^2, half the mean square of the parent–offspring distance, with a restricted distance range comprised between σ and 20σ. We assessed the density D in the four exhaustively censused sampling locations. Given that effective density is a fraction of census density because of variation between individuals in reproductive success and that overestimating D_e results in underestimating σ^2, we used $D_e = D/2$ in the SPAGeDi v1.4c computations. The σ^2 values were used to calculate the median of the dispersal distributions. Assuming that the distances travelled by dispersing individuals (d) follow a negative exponential distribution $P(d) = \lambda e^{-\lambda d}$, where $\lambda = 1/\sigma$ (see Broquet & Petit, 2009), the median of these distributions, calculated as $\ln(2)/\lambda$, represents the median dispersal distance (MDD), that is the distance travelled by 50% of dispersing individuals.

Assessment of the large-scale spatial genetic structure

The large-scale population structures of both *H. physophora* and *A. decemarticulatus* were determined at the regional level, that is between sampling locations. The analyses were performed for each species separately.

Weir & Cockerham's estimation of *F*-statistics and Nei's F_{ST} generalization for multiallelic loci G_{ST} were calculated over all 14 sampling locations, considered independent entities, using FSTAT v2.9.3.2 (Goudet, 1995, 2001). F_{IS} inbreeding coefficients were also calculated for each sampling location separately. Note that positive values for F_{IS} inbreeding coefficients can result from selfing and/or biparental inbreeding because such coefficients are based on deficiencies in heterozygosity (i.e. within-individual, within-locus correlations). Because *H. physophora* is an obligate outcrosser (Malé *et al.*, 2015) and *A. decemarticulatus* can obviously not self, it might just be possible to rule out selfing *a priori*.

The isolation by distance between sampling locations was assessed by computing pairwise F_{ST} indexes and then calculating the significance of the regression of $F_{ST}/(1\text{-}F_{ST})$ on the logarithm of the geographical distance between sampling locations using the function mantel.rtest in the *ade4* package in R v2.14.2 software (Dray & Dufour, 2007; R Development Core Team, 2012). Contrary to kinship coefficients, F_{ST} indexes do not describe genetic similarity but genetic dissimilarity between samples. As a consequence, significant isolation by distance should translate into a positive relationship between pairwise F_{ST} and geographical distances.

A discriminant analysis of principal components (DAPC) was used as a multivariate assessment of the differentiation between the five geographical areas and calculated with the dapc function in the *adegenet* package in R v2.14.2 software (Jombart, 2008). The genetic data were transformed, centred and scaled to create perfectly uncorrelated variables that summarize the total genetic variability in the principal component analysis. The variables were subsequently used as input in the discriminant analysis that maximizes the between-group variability. A DAPC was performed using predefined groups corresponding to the sampling locations (see Table 1). For both the plants and the ants, a total of 13 principal components were retained and used as input in the discriminant analyses.

We also performed Bayesian clustering analyses to infer the spatial structure of the genetic data. Because the use of different clustering methods with the same data set can lead to slightly different results (Chen *et al.*, 2007), we used two different approaches: (i) the most popular method which is implemented in STRUCTURE v2.3.3 (Pritchard *et al.*, 2000) and (ii) the spatially explicit method implemented in GENELAND v4.0.1 (Guillot *et al.*, 2012). These two approaches were performed hierarchically as described by Coulon *et al.* (2008). Briefly, we used the methods described above first on entire data sets, then on each of the defined clusters and so on until the number of genetic groups inferred or the number of sampling locations comprised in a group was equal to one.

The analyses performed using STRUCTURE were conducted on the freely available Bioportal server (Kumar *et al.*, 2009). Each round of STRUCTURE consisted of 20 runs for each of the K genetic groups tested, with K ranging from 1 to 15. All of the runs were conducted with the uncorrelated allele frequencies model, the

LOCPRIOR model and the admixture model for 100 000 iterations after a burn-in period of 10 000 iterations. This burn-in period was previously proven sufficient for the likelihood of it to be likely for the runs to stabilize. The number of genetic groups was determined using the ΔK method developed by Evanno *et al.* (2005) which consists in assessing the breakpoint in the slope of the distribution of $\ln P(D)$ as a function of K. This method was implemented using the online program STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Then, 100 runs were performed as described above, but with K fixed to the previously determined value. To determine the final assignment of individuals to clusters, we summarized the data on the outcomes of these 100 replicates using the CLUMPP program with the greedy algorithm (Jakobsson & Rosenberg, 2007).

The analyses performed using GENELAND were conducted with R v2.14.2 (R Development Core Team, 2012). Each round of GENELAND consisted of 100 runs with K ranging from 1 to 20. All of the runs were conducted with the uncorrelated allele frequencies model for 100 000 iterations. The number of genetic groups was defined as the modal group number of the 50 000 last iterations of the 20 runs with the highest posterior density. Then, 100 runs were performed as described above, but with K fixed to the previously determined value. To determine the final assignment of individuals to clusters, we assessed the features common to all or most of the genetic population structure defined by the GENELAND runs using the CONSANA function for R (Coulon *et al.*, 2008). We chose to assign individuals to the same group when they were grouped in at least 90% of the 85 replicates with the highest posterior density.

Results

Genetic assessment of the dispersal distance of *H. physophora*

The probability of two plant individuals sharing the same multilocus genotype was lower than 10^{-8}, demonstrating that, in our samples, one plant cannot be confused for another based on their genotypes. Of the 213 plants tested, 60 (i.e. 28%) and 146 (i.e. 69%) were successfully assigned to at least one parent with a 95% or an 80% confidence level, respectively. These proportions are very likely underestimated since the missing parents could be individuals from other sampling locations as well as individuals from the same location that were not sampled (either dead or not seen).

Experimental assessment of the dispersal distance of *A. decemarticulatus*

The probability of two ants from sibling colonies sharing the same multilocus genotype was 0.002, demonstrating that, in our samples, ants from one colony cannot be confused with ants from another based on their genotypes. The maximum allele number per marker per colony was three, thus confirming that *A. decemarticulatus* colonies are monogynous and monandrous. Of the 46 founding queens collected, 42 were successfully genotyped as were 43 males from the spermatheca contents. The assignment algorithm in COLONY v2.0.1.1 successfully assigned 17 founding queens and 27 males to a resident ant colony. Overall, these sexuals originated from 28 of the 54 resident colonies in the sampling locations. None of them originated from the neighbouring colony. This demonstrates that more than half of the colonization events occurred within the sampling locations, although this proportion is probably underestimated for the same reasons as for *H. physophora*.

Assessment of the fine-scale spatial genetic structure

The isolation by distance computed for individuals within sampling locations was highly significant for both *H. physophora* and *A. decemarticulatus*, indicating a significant fine-scale population genetic structure with closely related individuals being spatially close to each other. The coefficients of determination (r^2) were, however, surprisingly low, which reflects the great variability in the kinship coefficient values (Table 2).

The surface areas of the four exhaustively sampled locations were comprised between one and 10 hectares, and the census densities (D) were estimated at 255, 28, 70 and 124 plant individuals per hectare each with their associated ant colony. Based on these effective densities and on the resulting σ^2 values, MDDs were estimated to be very short, that is mostly <200 m. It should be noted, however, that σ^2 could not be computed for *A. decemarticulatus* in three of the four exhaustively sampled populations due to the lack of convergence of the algorithm (Table 2).

Assessment of the large-scale spatial genetic structure

Most of the genetic variation occurred at the individual level as shown by Weir & Cockerham's estimation of fixation indexes (Table 3). Global F_{ST} and G_{ST} values indicated a moderate to strong genetic differentiation between sampling locations. However, this result must be interpreted carefully because of the very short distances (i.e. mostly <50 km) between sampling locations. Positive F_{IS} values indicated a heterozygote deficiency, both at the regional level and at the scale of sampling location (Tables 1 and 3). This deficiency was more likely to result from inbreeding rather than from selfing since both species are obligate outcrossers or

Table 2 Estimates of σ^2 (i.e. half the mean square parent–offspring distance ± standard error) and the median dispersal distance (MDD; i.e. the distance travelled by 50% of dispersing individuals, in metres) for the four exhaustively sampled locations and isolation by distance parameters between individuals within sampling locations. Location names refer to those indicated in Table 1. '*D*' is the density of individual plants/colonies measured in the field (in individuals per hectare); 'IBD' is the statistic inherent to isolation by distance (i.e. regression of pairwise kinship coefficients (Loiselle *et al.*, 1995) on the logarithm of pairwise geographical distances between individuals within sampling locations). Dashes indicate that the estimation procedure did not succeed.

	Sampling locations				IBD	
	Z1 (*D* = 255)	Z9 (*D* = 28)	PK24 (*D* = 70)	GP (*D* = 124)	Slope	r^2
H. physophora						
σ^2	0.030 (± 0.009)	0.101 (± 0.069)	0.074 (± 0.039)	0.040 (± 0.006)	−0.016***	0.020
MDD	120	220	188	139		
A. decemarticulatus						
σ^2	0.027 (–)	–	–	–	−0.015***	0.012
MDD	114	na	na	na		

*denote statistical significance at the 0.1% level.

Table 3 Parameters of the global genetic differentiation for *H. physophora* and *A. decemarticulatus*. 'F_{IT}', 'F_{ST}', 'G_{ST}' and 'F_{IS}' are the fixation indexes; 'IBD' is the statistic inherent to isolation by distance (i.e. the regression of pairwise $F_{ST}/(1\text{-}F_{ST})$ on the logarithm of pairwise geographical distances).

					IBD	
Species	F_{IT}	F_{ST}	G_{ST}	F_{IS}	Slope	r^2
H. physophora	0.197	0.038	0.035	0.165	0.015***	0.284
A. decemarticulatus	0.205	0.148	0.135	0.067	0.058***	0.557

*denote statistical significance at the 0.1% level.

from the Wahlund effect, that is combining more than one genetically differentiated unit in the same sample since the units for which F_{IS} were computed were often smaller than the clusters found through Bayesian and discriminant analyses (see below).

The isolation by distance was highly significant for both *A. decemarticulatus* and *H. physophora* with the coefficients of determination r^2 remarkably high, indicating the strength of the genetic structure between sampling locations (Table 3). For *A. decemarticulatus*, five genetic groups corresponding to the five geographical areas were well differentiated according to the discriminant analysis (Fig. 2a). For *H. physophora*, the population structure appeared less marked, with roughly three genetic groups identified. The *Kaw* area was well differentiated from the others and the *Nouragues* and *Bagne des Annamites* areas appeared genetically different from the *Petit-Saut* and *La Montagne des Singes* areas (Fig. 2b). These results demonstrate the strength of the genetic population structure that exists in both species. The Bayesian clustering analyses confirmed these results as the clusters obtained from STRUCTURE v2.3.3 and GENELAND were almost exactly the same (Fig. 3 and Figure S2). Overall, between seven and eight genetic clusters were determined for *A. decemarticulatus* and only three for *H. physophora*.

Discussion

Altogether, our results show similar short dispersal distances for the two species engaged in the mutualism, resulting in the spatial structuring of the genetic information at both the local and the regional levels. This spatial structuring is, however, less marked for the plants than for the ants, probably due to a higher gene flow between plant populations. These two phenomena have the potential to enhance the stability of the mutualism by influencing the reciprocal local adaptation of the partners.

Our results cannot exclude the possibility of the local inheritance of the relationship. Although our experimental set-up was likely to favour colonization events from the immediately neighbouring colonies, such exceptionally short-distance dispersal events did not happen. However, pedigree reconstructions showed that, for both partners, probably much more than half of the dispersal events occurred within a sampling location. This experimental result was substantiated by genetic analyses at unmanipulated undisturbed locations. Isolation by distance between individuals was highly significant for both species, and inferred dispersal distances were very short. As a consequence, the F_{IS} values were positive, especially for *H. physophora*, reflecting biparental inbreeding resulting from short dispersal distances. Related ants thus interact mostly with related plants and the offspring plant population can be considered a 'public good' shared by related symbionts. The total castration of all of the plants could thus lead to a 'tragedy of the commons' (Rankin *et al.*, 2007) and the extinction of a too virulent ant population.

At the regional level, the ant sampling locations displayed a moderate to high degree of isolation between populations, whereas that degree of isolation was much less marked for the plants. The strict and obligate nature of the relationship associated with a patchy distribution and short dispersal distances probably

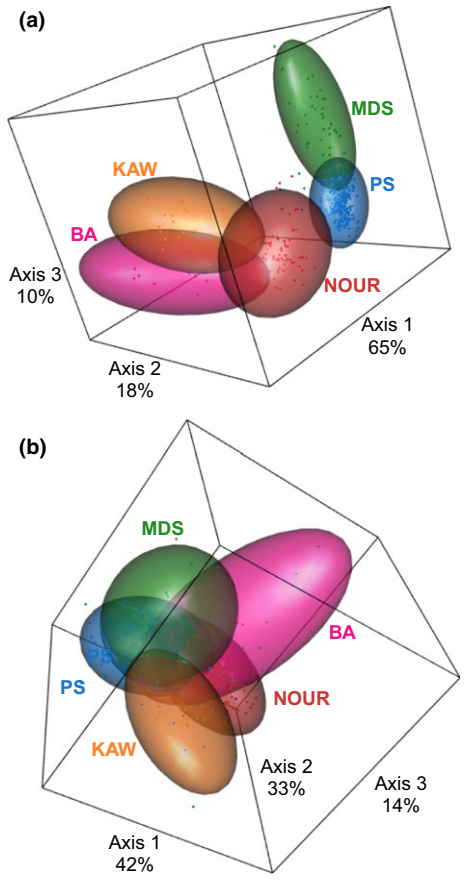

Fig. 2 Scatter plots of the discriminant analysis of principal components for *A. decemarticulatus* and *H. physophora*. The results obtained for the ants showing the first three axes of the discriminant analysis are displayed in the upper part of the graph (a), and the results obtained for the plants showing the first three axes of the discriminant analysis are displayed in the lower part of the graph (b). Dots representing individuals and groups (95% inertia ellipsoids) are shown in different colours according to the geographical area: blue, *Petit-Saut*; [PS] green, *La Montagne des Singes* [MDS]; red, *Nouragues* [NOUR]; pink, *Bagne des Annamites* [BA]; and orange, *Kaw* [KAW]. The percentages of variation explained by the axes are indicated on each axis.

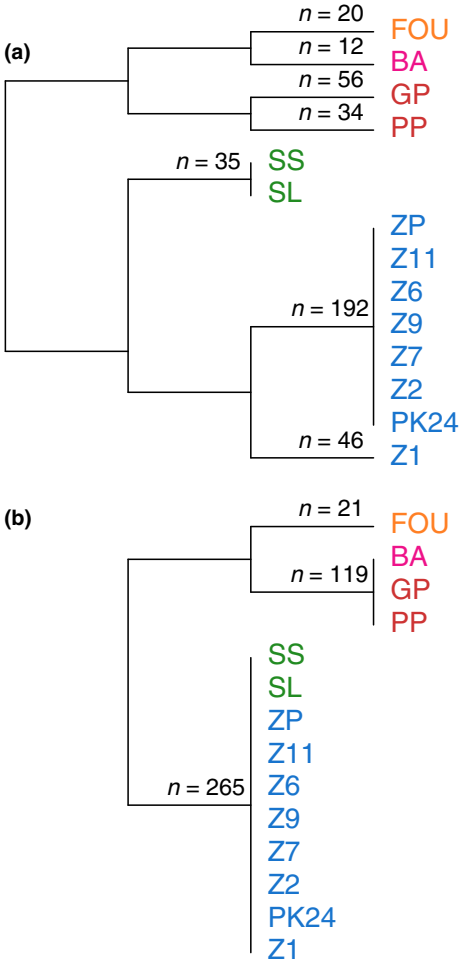

Fig. 3 Dendrogram of the sampling location clustering according to the results of Bayesian clustering Δ*K* hierarchical analyses with STRUCTURE. The results obtained for *A. decemarticulatus* are displayed in the upper part of the graph (a), and the results obtained for *H. physophora* are displayed in the lower part of the graph (b). Each node corresponds to the clustering of the sampling locations after one round of analysis. The number of individuals in each final cluster is indicated above the terminal branches, and sampling locations are indicated at the tip of the branches. The different colours represent different geographical areas (blue, *Petit-Saut*; green, *La Montagne des Singes*; red, *Nouragues*; pink, *Bagne des Annamites*; and orange, *Kaw*).

contributed to the partial congruence of the phylogeographical patterns (Alvarez *et al.*, 2010). Several factors can, however, account for the differences between fine-scale genetic structures. First, differences in generation times between the partners can result in an asymmetry in the differentiation rates. Although the maximum lifespan of an *A. decemarticulatus* colony is about 20 years, that of *H. physophora* can be more than 17 times longer (Orivel *et al.*, 2011). As a consequence of its longer generation time, *H. physophora* is expected to exhibit less genetic differentiation between populations than *A. decemarticulatus*. Moreover, because of this difference in lifespan, one cannot expect the strict codispersion of both partners. Second, the unequal biological dependence of the species can ultimately generate differences in their genetic structuring patterns (Alvarez *et al.*, 2010). Even if *A. decemarticulatus* and *H. physophora* are engaged in a strict and obligate mutualism, the plant can survive for a time without being inhabited by ants, whereas *A. decemarticulatus* colonies cannot complete their life cycle without having an *H. physophora* as their host. Finally, incomplete lineage sorting can result in patterns of local incongruence even though congruence exists when the distribution

ranges of the species are considered. Indeed, we observed a common, global pattern in both the ant and the plant population structures: eastern areas (i.e. *Petit-Saut* and *La Montagne des Singes*) vs. western areas (i.e. *Bagne des Annamites*, *Nouragues* and *Kaw*). These two genetically defined groups are congruent with the phylogeographical patterns observed for other plant species (Dutech *et al.*, 2003; Girod, 2010) and they probably result from paleoenvironmental events that affected the Guiana Shield during the late Quaternary (de Granville, 1982). Although comparative studies of population genetics have rarely been conducted on mutualists and commensals, partial congruence in the population structures between partners seems to be a common feature (Smith *et al.*, 2011; Widmer *et al.*, 2012; Andras *et al.*, 2013; Hurry *et al.*, 2014). Certain authors have suggested that external factors constraining population structures in the same way, such as historic glaciations, would result in comparable phylogeographical patterns, even in horizontally transmitted relationships.

The asymmetry we highlighted between the spatial population genetic structures at the regional level is likely to result in the greater capacity of *H. physophora* to finely adapt its retaliation mechanism to the cheating behaviour of the ants (Malé *et al.*, 2012, 2014). Indeed, asymmetry in gene flow is likely to provide the plant populations with more local genetic diversity than the ants because of trait remixing. This should induce the faster adaptation by the most genetically diverse partner to local changes in the traits of the other (Wilkinson & Sherratt, 2001). On the other hand, the local adaptation of the ants to the plants could be hampered because of the putative greater genetic diversity in the plants than in the ants. The asymmetry in gene flow could thus balance the evolutionary advantage experienced by the ants due to their shorter life cycles compared to their host plants (Gandon & Michalakis, 2002).

To conclude, although this amount of short-distance dispersal is unlikely to be high enough on its own to ensure the maintenance of the relationship (Szilágyi *et al.*, 2009), both the local and regional genetic patterns highlighted in our study have the potential to contribute to enhancing the stability of the mutualistic relationship between *H. physophora* and *A. decemarticulatus*. First, the local inheritance of the relationship penalizes too virulent, castrating ants. Second, the higher gene flow in *H. physophora* probably fosters the local adaptation of retaliation mechanisms much as the shorter generation time in *A. decemarticulatus* makes possible the local adaptation of cheating behaviour. These results highlight the necessity to consider the local genetic diversity of both partners when studying the outcome of selection on the evolutionary trajectory of mutualistic but conflicting relationships.

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Author contributions

PJGM designed the study, conducted part of the sampling, performed the molecular biology analyses and statistical analyses and wrote the manuscript. CL and AD conducted part of the sampling and participated in the writing of the manuscript. PH participated in the molecular biology analyses and the writing of the manuscript. AQ and JO supervised the study and participated in the writing of the manuscript.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Figure S1 Map of the hilltop where *A. decemarticulatus* dispersal distance was experimentally assessed.
Figure S2 Dendrogram of the sampling location clustering according to the results of Bayesian clustering ΔK hierarchical analyses with GENELAND.

Data deposited at Dryad: doi: 10.5061/dryad.20ck4

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