# Connectivity and selfing drives population genetic structure in a patchy landscape: a comparative approach of four co-occurring freshwater snail species

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**Abstract** (291 words)

The distribution of neutral genetic variation in subdivided populations is driven by the interplay between genetic drift, migration and extinction / colonization. The influence of environmental and demographic factors has also been increasingly examined in empirical studies, but generally focusing on a single species. An open question is whether these factors will similarly, or idiosyncratically, affect a guild of species occupying the same geographical space and exhibiting different traits, mating systems and histories. We addressed it by comparing the population genetic structure of the four most common species of hermaphroditic freshwater snails in Guadeloupe (Lesser Antilles), which occupy a network of patchily distributed sites experiencing temporal variation in water availability. We analyzed microsatellite variability in 21 to 43 populations per species, and built predictions on how several environmental and demographic variables, quantified from a long-term annual survey, as well as species traits, may affect the distribution of genetic variation. These species displayed similarities, such as fairly high levels of variation, but with marked differences among sites, as well strong genetic differentiation and limited isolation by distance, which can be explained by passive dispersal (strong role of site connectivity), extinction/colonization dynamics and variation in local population size. They also exhibit differences, largely due to the mating system with less genetic diversity and more genetic differentiation in the two selfing species when compared to the two outcrossing ones. These differences can also be attributed to interspecific interactions resulting from the ongoing invasion of Guadeloupe by one of the species studied, which affects the demography of other species, and, to a limited extent, to local environmental factors. Our comparative approach shows both differences and uniqueness in the way species occupy the same landscape, and provides a possible entry to interspecific interactions in community assembly.

## Introduction

The evolutionary dynamics of species occupying patchily distributed habitats has been the subject of a large body of both experimental and theoretical work (Hanski & Gaggiotti, 2004; Bell, 2008; Charlesworth & Charlesworth, 2010), highlighting the role of finite size of populations and migration, and of their spatial and temporal variation. Metapopulation approaches also consider the role of population extinction and colonization, which roles depend particularly from the origin of the propagule pool (Levins, 1969; Whitlock & Barton, 1997; Hanski & Gaggiotti, 2004). These demographic processes may also occur at the scale of whole metapopulations, for example in situations of biological invasions (e.g., Davis, 2008; Facon & David, 2006), with possible variation loss at migration front due to founder effects. Other factors, such as the mating system or environmental parameters (e.g., through local adaptation), should also be considered (e.g., Pannell, 2002; Hanski & Gaggiotti, 2004; Foll & Gaggiotti, 2006).

In this context, we will analyze here how the spatial distribution of neutral variation in a patchy landscape is affected by the following factors: (i) environmental factors, especially local ones, (ii) invasion dynamics, and (iii) traits, especially mating systems. (i) Variation in population size, as well as extinction / colonization dynamics, may affect the strength of both genetic drift and gene flow, and therefore genetic variation (Barton & Whitlock, 1997; Hanski & Gaggiotti, 2004). Both processes may depend on environmental variables and their temporal variation (Hanski & Gaggiotti, 2004; Lamy et al., 2013a). The role of local factors is increasingly accounted for in analyses of population genetic structure (e.g., Foll & Gaggiotti, 2006; Frichot, Mathieu, Trouillon, Bouchard, & François, 2014). An example of local factor is site size, which affects both population demography and migration (large sites can harbor larger populations and are larger targets for migrants). At larger scale, the connectivity of local sites to the whole network impacts gene flow and colonization. Connectivity can be evaluated based

on local features of accessibility, but also through some form of geographic distance, possibly taking into account landscape elements modulating dispersal (Barton & Whitlock, 1997; Rousset, 1997; Balkenhol, Cushman, Storfer, & Waits, 2015; Manel & Holderegger, 2013). (ii) Extinction and colonization may also occur at metapopulation scale in situations of regional invasion or extinction, both leaving traces in the distribution of genetic variability. For example, invasion is often associated with founder effect and variation loss at invasion front (Roman & Darling, 2007; Pannell, 2015), with increased frequencies of some alleles through genetic drift (Excoffier & Ray, 2008). (iii) Mating systems can vary widely among species, even closely related ones, or even within species, which affects the distribution of genetic variability, as they interact in a complex manner with both environmental factors and demography (see e.g. Charlesworth & Charlesworth, 2010). Self-fertilization is a striking example, on one hand decreasing the effective population size, and thus modulating the impact of demography on genetic diversity and population structure (see Charlesworth, 2003; Jarne, 1995). On the other hand, it may affect population demography itself (Abu Awad & Billiard, 2017), and increased selfing rates may be a way to cope with lack of partners / pollinators, protecting from local extinction and giving an advantage in colonizing situations (Jarne, 1995; Pannell, 2015). Other specific traits might be considered, such as the capacity to withstand bad conditions (e.g., escaping dryness through aestivation or seed banks), modulating the extinction dynamics (Lamy et al., 2013a; Fréville et al., 2013).

An important point in the perspective presented above is that the distribution of diversity is considered within species, and most studies of population focus on single species (see e.g. in Bell, 2008; Charlesworth & Charlesworth, 2010). We may wonder to what extent different species occupying the same landscape differ with respect to genetic diversity within and among populations, since they can both be differentially be affected by local and regional factors and exhibit different traits. This issue can be addressed through a comparative analysis of a species

set occupying the same landscape. The taxonomic range of the species set matters, since species belonging to very different groups (e.g., arthropods, gastropods and amphibians in freshwater habitats) are usually so different, for example with regard to migration, that even the delimitation of local populations strongly differs. The issue is much less trivial when considering closely related species (e.g., within arthropods, or gastropods, or amphibians). We report in Table S1 the few examples of multispecies studies analyzing genetic diversity at small geographic scale that we were able to find, which, incidentally, includes mostly studies conducted in freshwater organisms. These comparative analyses evaluated whether the environmental parameters mentioned above (e.g., affecting local population demography) are more important than species traits such as the mating system (e.g., monogamous *vs*. polygamous reproduction) in shaping population genetic structure. For example, connectivity and habitat patchiness play a role in structuring population structure in orthopterans from central Spain (Ortego, Garcia-Navas, Noguerales, & Cordero, 2015), while turtle species studied in the USA were sensitive to water availability (Reid, Mladenoff, & Peery, 2017; see Table S1). Note that a fraction only of these studies considers closely related species, and, even fewer, species sets occupying the same landscape.

We here conduct such a multispecies (comparative) analysis of population genetic structure in a guild of freshwater pulmonate snails (Hygrophila) occupying a patchy landscape in Guadeloupe (Lesser Antilles). Freshwater habitats, especially lentic ones (e.g., ponds), are appropriate systems for such a purpose, because the available habitats are clearly delimited from the inhospitable matrix and a wide variety of environmental parameters can be considered (see references in Table S1). In a pond network, snails disperse essentially passively through either more or less temporary waterways connecting sites, or aerial vectors such as birds (Jarne & Delay, 1991; Figuerola & Green, 2002; Kappes & Haase, 2012; Van Leeuwen et al., 2013). Importantly, our sampling covers the whole space occupied by freshwater snails from Grande-

Terre of Guadeloupe, meaning that migration takes place essentially at this scale, with limited 'natural' input from outside Grande-Terre, except through occasional introductions, essentially through aquarium trade or birds (see e.g. Duggan, 2010). Moreover, we focused on the four most common snail species in this landscape, with reasonably similar occupancy at metapopulation scale (50-70% of occupied sites), certainly because sampling is easier, but also because genetic and demographic parameters can more precisely be estimated than in rare species (Heino et al., 2015). The Guadeloupe system has been well characterized through a long-term survey of snail distribution on an island scale, as well as population genetics, demographic and ecological studies (Chapuis et al., 2017; Dubart et al., 2019; Lamy et al., 2012; Lamy et al., 2013a; Pantel et al., submitted).

We characterized microsatellite variation in 21 to 43 populations in these four species. They experience the same variation in environmental parameters, such as site size and connectivity, which may influence the distribution of genetic variation (Lamy et al., 2012). This variation may however be affected by differences among species (see Chapuis et al., 2017; Dubart et al., 2019; Lamy et al., 2012; Lamy et al., 2013a; Pantel et al., submitted). First, they exhibit demographic differences, for example in extinction and colonization rates, but also similarities (e.g., similar metapopulation occupancy, as mentioned above). Second, two of them are mainly outcrossing, while the remaining two are mainly self-fertilizing. They also differ in their aestivation capacity in dry sites. Third, one of them (*Physa acuta*) is an introduced species in the process of rapidly invading the whole Grande-Terre, while the other three are native and rather stable in terms of metapopulation occupancy. Our objective is to quantify genetic variation within and among populations within species in order to characterize the factors and processes to which these species react similarly vs. idiosyncratically (a comparative approach). We consider the role of mating systems and traits, population demography, and several environmental and landscape characteristics, building predictions on their influence (e.g., site

size should be associated with larger or more accessible populations and therefore more variation). Four species is certainly not a sufficient number to draw conclusions on the factors driving species differences in genetic structure in general. However, we suggest conducting more comparative studies, up to now rather rare, to evaluate the generality of these factors. We also discuss how such a comparative approach should help to progress towards a more integrated eco-evolutionary perspective on community building and species interaction (Hendry, 2017; Vellend, 2016).

## Materials and Methods

### *Long-term survey of snail communities*

The biological system, surveys and species studied have been described in papers spanning the last five decades (see Pointier & Combes, 1976; Pointier & David, 2004; Pointier, 2008; Chapuis et al., 2017; Dubart et al., 2019; Lamy et al., 2012; Lamy et al., 2013a; Pantel et al., submitted), and we provide here the relevant information for the current analysis. We initiated in 2001 a long-term yearly study of the guild of freshwater snails from the geographically close islands of Grande-Terre and Marie-Galante (Guadeloupe archipelago, Lesser Antilles). Grande-Terre is a plateau of about 800 $km^2$ harboring a network of ponds (*ca*. 2000) of various sizes, and a few small rivers (mostly temporary) and swamp grasslands connected to mangroves (back-mangroves). Marie-Galante is a smaller island (160 $km^2$) with a similar topography and pond network. Water availability varies widely in time with the highest water level at the end of the rainy season (August to November or December). A fraction of sites completely dry out either yearly, or more irregularly, and stay dry for up to several months.

This long-term survey includes almost 280 sites, 245 of which are in Grande-Terre (Lamy et al., 2012; Lamy et al., 2013a). Field sampling takes place in January-February when these communities peak in abundance. They include 24 mollusc species, which makes up the

major part of the macrobenthos. Each site was explored by three persons for at least 15 min at each visit (total searching time: 45 min). The presence and density of snails were estimated by foraging both the sediment and plant strata using a scoop, and rock surfaces or floating debris were visually surveyed. Snail density was estimated on a semi-log scale (no individuals, < 1 ind. / $m^2$, 1 to 5 ind. / $m^2$, 5 to 10 ind. / $m^2$ …). Several environmental parameters were characterized per site (detailed in Table S2) including size, aquatic vegetation cover, water connectivity to neighboring watersheds, hydrological regime, and density of favorable habitats. Site stability was defined based on the first four variables. From snail density and site size, we built a demographic parameter called 'long-term population size'.

*The species studied and population sampling*

Our analysis bears on two pairs of closely related species (Morgan et al., 2002; Wethington and Lydeard, 2007): *P. acuta* and *Aplexa marmorata* from the Physidae family, and *Drepanotrema depressissimum* and *Drepanotrema surinamense* from the Planorbidae family. In each pair, species are ecologically and morphologically similar, but do not hybridize. *Physa acuta* and *D. depressissimum* are outcrossers, while *A. marmorata* and *D. surinamense* are selfers (Escobar et al., 2011). These four species are the most common ones in the Guadeloupe metacommunity, occupying *ca*. 50-70% of sites on average, and co-occur in a large number of sites (Dubart et al., 2019; Pantel et al., submitted; Pointier & David, 2004). *Physa acuta* originates from the Eastern-central USA and has markedly increased its range over the last two centuries to now be cosmopolitan (Bousset et al., 2014; Ebbs, Loker, & Brant, 2018; Vinarsky, 2017). We observed it in two sites from Grande-Terre in 2001, but this figure increased to *ca*. 60% of sites in 2017. The three other species are neotropical and are considered native in the Lesser Antilles (Pointier 2008). The two Physid species have been shown to interact in Guadeloupe, most likely through direct reciprocal competition, with a depressing effect of *P. acuta* on the demography

**Figure 1.** Distribution of the sites studied in Guadeloupe (Grande Terre). Species name is indicated for each panel, and population acronyms are provided in Table S3. The four sites sampled in Basse Terre of Guadeloupe and Marie-Galante (Table S3) are not represented.

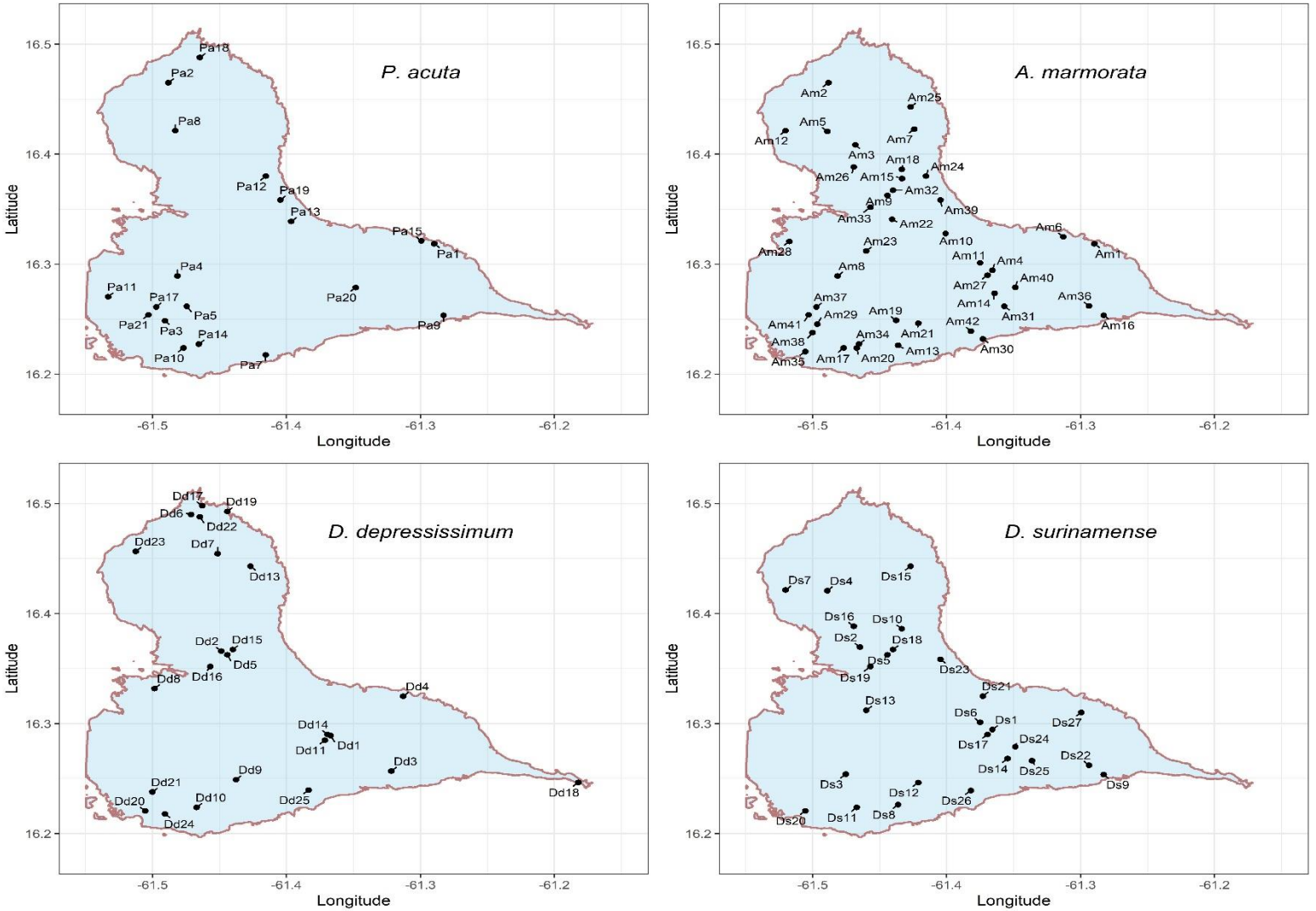


**Table 1.** Predictions on the influence of mating system, site (environmental) characteristics and demographic factors on population genetic diversity and structure in the four species studied. The rationale for these predictions is given in the main text. Although predictions for several characteristics are identical (e.g., size and connectivity), they are presented separately for the sake of clarity. The effects are given on genetic diversity within populations (Div) and differentiation among populations (Diff). Predicted effect: positive (+), negative (-), limited, and unclear (?).

| Characteristics | *Physa acuta* (outcrosser) | | *Aplexa marmorata* (selfer) | | *Drepanotrema depressissimum* (outcrosser) | | *Drepanotrema surinamense* (selfer) | |
|---|---|---|---|---|---|---|---|---|
| | Div | Diff | Div | Diff | Div | Diff | Div | Diff |
| Mating system | High | Weak | Low | Strong | High | Weak | Low | Strong |
| Effect of site characteristics | | | | | | | | |
| Size | + | - | + | - | + | - | + | - |
| Vegetation cover | - | + | + | - | + | - | + | - |
| Connectivity | + | - | + | - | + | - | + | - |
| Stability | + | - | + | - | Limited | Limited | Limited | Limited |
| Density of favourable habitats | + | - | + | - | + | - | + | - |
| Effect of demographic factors | | | | | | | | |
| Invasion age / years since *P. acuta* invasion | + | - | - | + | ? | ? | ? | ? |
| Long-term population size | + | - | + | - | + | - | + | - |

of *A. marmorata*, but a predicted stable co-existence (Dubart et al., 2019) and a character displacement (life-history traits) towards more 'r-strategy' in *A. marmorata* within sites, following the arrival of *P. acuta* (Chapuis et al., 2017). The two *Drepanotrema* species also resist better in dry sites than the two Physids, aestivating under rocks or vegetation (Pointier & Combes, 1976). This invasion allowed to introduce a second demographic variable in our analyses, the time in years since the first detection of the invasive *P. acuta* - referred to as 'invasion age' in this species and 'time since *P. acuta* invasion' in the other species (Table S2). We considered two classes, not or recently invaded by *P. acuta vs.* invaded for more than two years, as in Chapuis et al. (2017). In *A. marmorata* and *Drepanotrema* spp., populations of the first class are unlikely to show a detectable evolutionary response to the presence of *P. acuta* because of the limited number of generations (Chapuis et al., 2017).

The four species were sampled in Grande-Terre during the yearly field surveys in 74 sites in which one to three of the four species were co-occurring (Figure 1, Tables S3 and S4; 1.57 species per site). Four sites from Basse-Terre and Marie-Galante were also considered in the study of *P. acuta* (three sites) and *A. marmorata* (one site). *Physa acuta*, *A. marmorata*, *D. depressissimum* and *D. surinamense* were sampled in 21, 43, 25 and 27 sites respectively, from 2007 to 2010. Individuals were killed just after sampling in 80ºC water for 1 minute and preserved in 95º ethanol. The genetic analysis was conducted on at least 10 individuals per population for a total of 459 individuals in *P. acuta* (mean per population ± s.d.: 21.86 ± 5.51), 781 in *A. marmorata* (18.14 ± 6.42 s.d.), 749 in *D. depressissimum* (29.96 ± 2.73 s.d.), and 365 in *D. surinamense* (13.52 ± 6.3). Less than 10 individuals were available in three populations (Table S4).

Note that the population genetic data used in this paper have partly been published: a full analysis of population structure in *D. depressissimum* in Guadeloupe is reported in Lamy et al. (2012) and population genetic richness of both *D. depressissimum* and *A. marmorata* is

246 used in Lamy et al. (2013b). However, we will present all the genetic data for the sake of clarity,
247 and refer to previous work when required.
248
249 *Microsatellite characterization*
250 DNA extraction and allele characterization have been described in Sourrouille et al. (2003),
251 Bousset, Henry, Sourrouille, & Jarne (2004), and Escobar, Nicot, & David (2008) for *P. acuta*,
252 in Dubois, Nicot, Jarne, & David (2008) for *A. marmorata* and in Nicot, Jarne, & David (2009)
253 and Lamy et al. (2012) for *D. surinamense* and *D. depressissimum*. Briefly, DNA was extracted
254 using a Chelex method (Bio-Rad). Genotypes were obtained at polymorphic microsatellite loci
255 giving reproducible results, seven in *P. acuta* (Pac1, Pac2, Pac5, Sourrouille et al. 2003;
256 AF108762, AF108764, Monsutti & Perrin, 1999; Pasu1-11, Pasu1-2, Escobar et al., 2008),
257 eight in *A. marmorata* (Ama1, Ama4, Ama7, Ama9, Ama10, Ama12, Ama13, Ama14, Dubois
258 et al., 2008), 10 in *D. depressissimum* (DDE03, DDE04, DDE06, DDE09, DDE12, DDE14,
259 DDE16, DDE20, DDE22, DDE23, Lamy et al., 2012), and 12 in *D. surinamense* (DSU01,
260 DSU02, DSU04, DSU05, DSU07, DSU09, DSU11, DSU12, DSU13, DSU14, DSU15, DSU16,
261 Nicot et al., 2009). Allele reading was performed independently by two persons.
262
263 *Genetic diversity and differentiation*
264 The analyses were conducted in the same way in the four species, except when otherwise stated.
265 We computed the number of polymorphic loci, allelic richness (rarefied to the smallest sample
266 size per species ($R_A$, Petit, El Mousadik, & Pons, 1998; 8, 10, 14 and 7 diploid individuals in *P.*
267 *acuta*, *A. marmorata*, *D. surinamense* and *D. depressissimum* respectively) per population and
268 species using FSTAT 2.9.3.2 (Goudet, 2001). Gene diversity (unbiased $H_e$; Nei, 1987) and
269 genotypic disequilibrium for locus pairs (Weir, 1996) were computed using GENEPOP 4.4
270 (Rousset, 2008). In the two selfing species (*A. marmorata* and *D. surinamense*), we also

estimated the genotypic diversity ($R_G$; i.e. the number of genotypes) rarified to sample size of 10 and 7 respectively. We calculated the correlation between $R_A$ and $H_e$ in each species, as well as with $R_G$ in the selfing species, using Pearson's correlation coefficient.

Deviations from Hardy-Weinberg equilibrium (HWE) were tested at each locus using exact tests (Raymond & Rousset, 1995), and a global *P*-value for all loci was obtained using Fisher's exact test and Markov Chain algorithm with GENEPOP 4.4. A multilocus estimate of Wright's inbreeding coefficient ($F_{IS}$) was estimated following Weir and Cockerham (1984). The selfing rate (*S*) was calculated based on David, Pujol, Viard, Castella, & Goudet (2007)'s method, which is insensitive to null alleles, an acute issue in outcrossing species, and less of a problem in selfing species since null alleles are readily detected when occurring in the homozygous state (see Jarne & David, 2008). *S* was here derived from the $g_2$ method using RMES (David et al., 2007).

Genetic differentiation between population pairs and overall differentiation was assessed in each species based on exact tests using GENEPOP 4.4, and their magnitude was estimated based on the estimator $\theta$ of $F_{ST}$, which was calculated following Weir and Cockerham (1984). Hereafter, we refer to the estimates between populations pairs as pairwise $F_{ST}$. We also estimated the genetic differentiation between each local population and all other sampled populations, hereafter site-specific $F_{ST}$, using the Bayesian method implemented in GESTE 2.0 (Foll & Gaggiotti, 2006). We calculated Pearson's correlation coefficient between site-specific $F_{ST}$ and genetic diversity ($H_e$) in each species. The geographic distribution of genetic diversity was evaluated first by testing for isolation by distance in each species based on pairwise distance matrices (linear geographical distance and $F_{ST}/(1-F_{ST})$) using a Mantel test (1000 permutations) in *Vegan* R package (Oksanen et al., 2015; R Development Core Team, 2005 version 3.6.3). We also examined visually the pairwise $F_{ST}$ matrix after they have been restructured based on a seriation approach implemented with *seriation* in R (R Development

296 Core Team, 2005) – the occurrence of population clusters would be evidenced by population
297 blocks with lower intra-block $F_{ST}$ than with other populations. This also allows building
298 cladograms to better visualize population relationships using the "complete" method of *hclust*
299 (R Development Core Team, 2005). We also used a more formal clustering approach, sNMF,
300 proposed by Frichot et al. (2014). This method reconstitutes population clusters based on
301 ancestry coefficients. This method is not sensitive to strong inbreeding, and was previously
302 successfully used in the highly selfing snail *Biomphalaria pfeifferi* (Tian-Bi et al., 2019) – this
303 is the reason why we preferred it to popular approaches such as STRUCTURE (Pritchard et al.,
304 2000). sNMF was implemented using the R package *LEA* (Frichot & François, 2015 ; R
305 Development Core Team, 2005). We used a target number of clusters varying from 1 to 10, a
306 regularization parameter of 50, 50 runs were performed per analysis, and several analyses were
307 performed per species. Based on the optimal number of clusters, we drew barplots of average
308 ancestry coefficients per population, and reported these values as piecharts on the Grande-Terre
309 maps using the *gdal* R package (R Development Core Team, 2005).
310
311 *Environmental and demographic drivers of genetic diversity*
312 We assessed the influence of several site characteristics and demographic factors (described
313 above; see Table S2) on allelic richness ($R_A$) and on genetic differentiation (site-specific $F_{ST}$) in
314 the four species, but also on genotypic richness ($R_G$) in the selfing *A. marmorata* and *D.*
315 *surinamense*. The site characteristics were size, vegetation cover, connectivity, stability and
316 density of favorable habitats, and the demographic factors were invasion age and long-term
317 population size. Given that our first genetic sampling was performed in 2007, we used the value
318 per site of these variables over the 2001-2007 period (averaged for those variables for which
319 we got a value per year; Table S5). Because environmental data were missing, the following
320 populations were excluded from the analyses: Sainte Rose (Basse-Terre) for *P. acuta*, Ravine

321 Vinty (Basse-Terre) for *A. marmorata* and Pointe des Chateaux (Grande-Terre) for *D.*
322 *depressissimum*.

323 We built general predictions on genetic diversity and structure, based on (i) the mating
324 system, (ii) environmental parameters, and (iii) demographic parameters (Table 1). (i) We
325 expect less diversity and more divergence in selfing than in outcrossing populations, as detailed
326 in Introduction. (ii) The predictions depend on the model of population genetic structure
327 underlying species dynamics, especially on how the environmental variables are affected by the
328 demography and traits of species. However, we expect that site size, site connectivity, and
329 density of favorable habitats should contribute positively to intrapopulation variability and
330 negatively to differentiation among populations, because they positively affect effective
331 population size and gene flow. Stability might have the same effect than the first three
332 parameters in the two physids. The two *Drepanotrema* species are dwelling well in unstable
333 sites, because of their aestivating capacity (Pointier & Combes, 1976), which should limit
334 (perhaps annihilate) the effect. These predictions can be refined on the basis of previously
335 obtained estimates of extinction and colonization rates at metapopulation scale per species
336 (Dubart et al., 2019; Pantel et al., submitted). In most cases, these environmental parameters
337 did not affect these rates, and when they did, this was in the direction of the predictions above
338 (Table S6). For example, site size positively affect colonization in three species, which should
339 contribute positively to genetic diversity. Vegetation cover should also have the same effect in
340 *A. marmorata* and the two *Drepanotrema* species, but the reverse one in *P. acuta*, given its
341 incidence on extinction and colonization (Table S6). (iii) Invasion age is predicted to affect the
342 two *Physa* species. In the invasive *P. acuta*, young populations should be less diverse than old
343 ones reflecting temporary founder effects at the colonization front. Both demographic
344 population size and colonization rates are negatively affected in *A. marmorata* by the
345 competition with *P. acuta* (Chapuis et al., 2017; Dubart et al., 2019), which should depress

346 diversity in sites that have been invaded by the latter for a longer time. The expectation is less
347 clear in the two *Drepanotrema* species, since the arrival of *P. acuta* only slightly affects their
348 density ($P$ = 0.051 and 0.038 respectively *vs*. $1.2*10^{-4}$ in *A. marmorata*; see Chapuis et al., 2017
349 for details). Long-term population size should have the same effect as site size.

350 We compared size, vegetation cover, connectivity and stability over all sites studied
351 between the 2001-2007 and 2008-2015 periods using a paired *t*-test to check for temporal
352 homogeneity. The other variables were not considered, either because they do not vary in time
353 (density of favorable habitats), or because the comparison is irrelevant (e.g., invasion age). All
354 variables were compared between the populations studied for genetic variation and all other
355 populations from the long-term survey using linear models. We evaluated the effect of
356 environmental and site variables on genetic variability ($R_A$ in *P. acuta* and *D. depressissimum*
357 and both $R_A$ and $R_G$ in *A. marmorata* and *D. surinamense*) using linear models. We first built
358 models with the five site variables (size, vegetation cover, connectivity, stability and density of
359 favorable habitats), and then added invasion age and long-term population size in turn in order
360 to avoid considering too many variables compared to the number of data points. The most
361 parsimonious combination of explanatory variables was retained based on AIC (Akaike, 1974).
362 Explanatory and response variables were standardized in order to obtain comparable regression
363 coefficients in the linear models. All statistical analyses were performed using R (R
364 Development Core Team, 2005).
365
366 **Results**
367 *Genetic diversity within populations and species*
368 Summary results on genetic diversity in the four species are reported in Table 2, and full results
369 in Table S4. Much more variation was detected in the two outcrossing species (especially in *D.*
370 *depressissimum*, due to highly variable tetranucleotide loci), with all loci polymorphic in all

populations, than in the two selfing species. Some populations of these two species even showed no variation at all, i.e. exhibited a single, fully homozygous, multilocus genotype. The average allelic richness ($R_A$) per population was 3.287 (minimum 1.982 / maximum 4.426), 1.588 (1.000 / 2.555), 11.052 (6.260 / 14.160) and 2.039 (1.000 / 3.193) in *P. acuta*, *A. marmorata*, *D. depressissimum* and *D. surinamense* respectively. As a comparison, the mean number of alleles, averaged over loci, was 9.29, 6.00, 37.20, and 9.08 respectively. Genotypic diversity ranged from 1.000 to 8.816 in *A. marmorata*, and from 1.000 to 7.000 in *D. surinamense*. The average gene diversity ($H_e$) was 0.530 (minimum 0.311/ maximum 0.658) in *P. acuta*, 0.169 (0.0 / 0.504) in *A. marmorata*, 0.865 (0.736 / 0.921) in *D. depressissimum*, and 0.271 (0.00 / 0.471) in *D. surinamense*.

Significant deviations from HWE ($P < 0.05$) were observed in most populations of *P. acuta* (18 out of 21), and heterozygote deficiencies were low to moderate (mean $F_{IS} = 0.159$; Tables 2 and S4A). However, the selfing rates estimated with RMES were not significantly different from zero (mean $S(g_2) = 0.030$), suggesting that the significant heterozygote deficiencies are due to null alleles, as reported previously in this species (Bousset et al., 2004, 2014; Escobar et al., 2008). The selfing rates estimated with RMES were similarly very low in *D. depressissimum* (mean $S(g_2) = 0.012$), significantly departing from zero in a single population out of 25 (Table S4C). This corresponds to low $F_{IS}$ values (mean = 0.036) with only four populations in which Hardy-Weinberg proportions were rejected at $P < 10^{-3}$ (Lamy et al., 2012). The situation was very different in the other two species: most tests of departures from Hardy-Weinberg equilibrium were highly significant (Tables S4B and S4D). The estimates of $F_{IS}$ were accordingly very high (0.970 in *A. marmorata* and 0.922 in *D. surinamense*; Table 2), and the selfing rates as well (0.984 and 0.950 respectively). The selfing rates estimated with RMES were high and significantly different from zero in the subsets of populations of *A. marmorata* and *D. surinamense* in which it was possible to successfully run the analysis (Tables

S4B and S4D). Exceptions are one population for each of these two species in which a selfing rate estimated with $F_{IS}$ matched with an absence of selfing with the RMES approach.

*Population genetic differentiation*

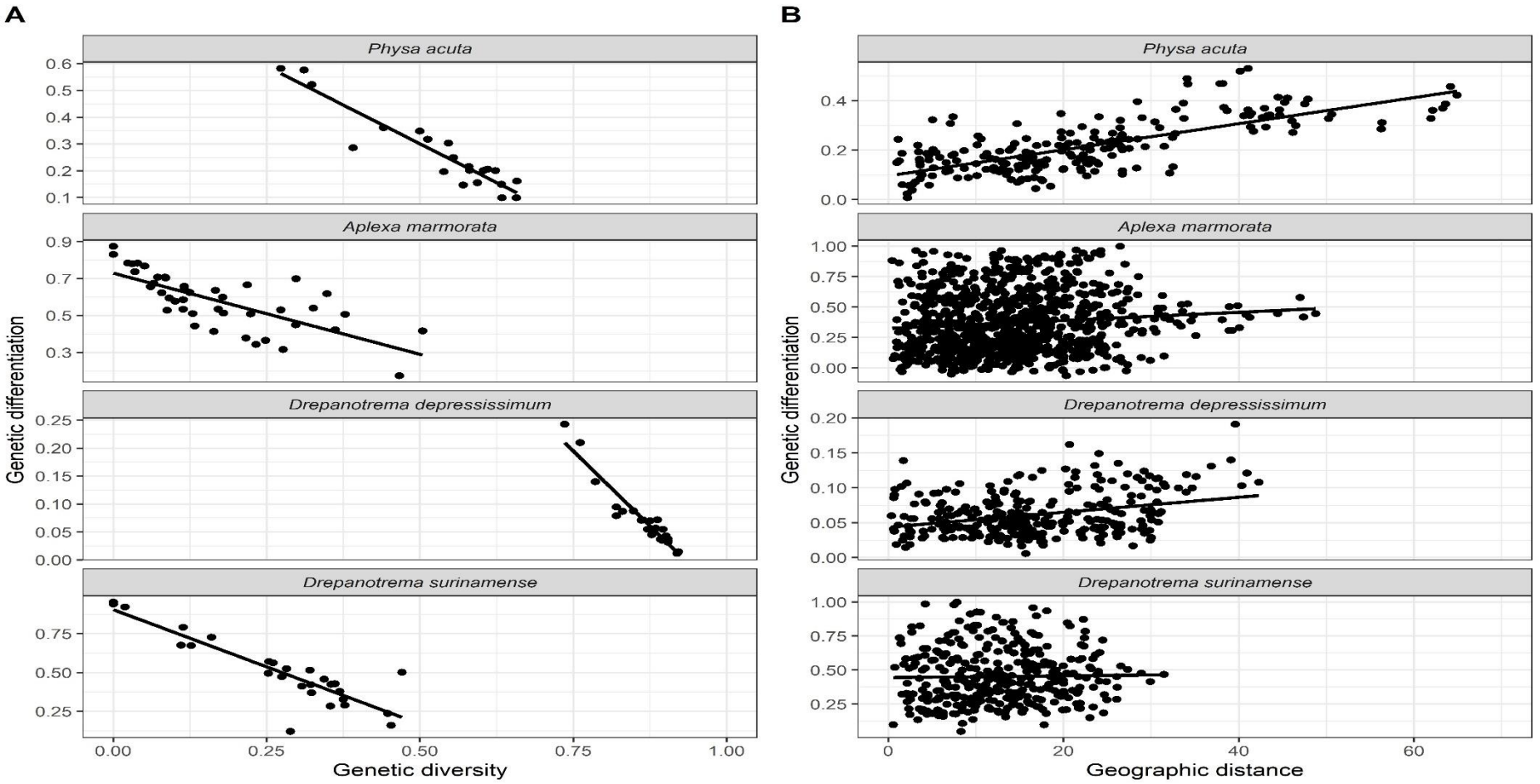
Highly significant genetic differentiation was detected between most population pairs in *P. acuta* and the two *Drepanotrema* species (Table S7). In both *P. acuta* and *D. surinamense*, a single *P*-value was higher than 0.01, and none in *D. depressissimum*. The situation is somewhat different in *A. marmorata* with 142 tests out of 841 with $P > 0.05$ (Table S7). Fourteen populations out of 43 were involved in more than 10 such tests (out of 42 per population), especially Favreau, Grand Maison, and Sainte Rose Est (18, 22, and 17 such results). Interestingly, eight of these fourteen populations are located in the Eastern part of Grande-Terre (Figure 1). The average estimate of pairwise genetic differentiation ($\theta$) between *P. acuta* populations was 0.213 (range: 0.007 / 0.533; Table 2). The two populations from Marie-Galante contributed significantly to this differentiation, since it went down to 0.170 when excluding them. The same estimates were 0.371 (0.000 / 1.000) in *A. marmorata*, 0.062 (0.006 / 0.191) in *D. depressissimum*, and 0.453 (0.049 / 1.000) in *D. surinamense*. Matrices of pairwise $F_{ST}$ estimates in the four species are reported in Table S7. The average site-specific $F_{ST}$ was 0.249 (range: 0.100 / 0.562) in *P. acuta*, 0.547 (0.215 / 0.872) in *A. marmorata*, 0.071 (0.012 / 0.243) in *D. depressissimum*, and 0.506 (0.121 / 0.954) in *D. surinamense*, and are not very different from the mean pairwise values. The average genetic diversity predicted quite accurately the site-specific differentiation, with more scattered data in *A. marmorata* than in the other species (Table 2, Figure 2).

Mantel test revealed isolation by distance in *P. acuta* populations ($r = 0.710$, $P = 0.001$; Figure 2), even when populations from Marie-Galante were excluded ($r = 0.301$, $P = 0.011$). Mantel test was significant in *D. depressissimum* ($r = 0.318$, $P = 0.020$), but neither in *A.*

**Table 2.** Mean values (range) of population genetic parameters in the four species studied. $N_{pop}$ and $N_{loc}$ are the number of populations and microsatellite loci studied. Other parameters are mean values across populations (mínimum / maximum): $n$ is the number of individuals, $R_A$ the allelic richness, $R_G$ the genotypic richness (in selfing species only), $H_e$ the gene diversity, $F_{IS}$ Wright's inbreeding coefficient, $S$ the estimate of the selfing rate based on $F_{IS}$, $S(g_2)$ the estimate of the selfing rate based on David et al. (2007)'s method, and the population-pairwise $F_{ST}$. Negative values of $F_{IS}$ were set to 0 when estimating the average $S$ value, and negative values of $F_{ST}$ were set to 0 when estimating the average $F_{ST}$. NC: not calculated because the values can be estimated in too few populations.

| Parameter | *Physa acuta* | *Aplexa marmorata* | *Drepanotrema depressissimum* | *Drepanotrema surinamense* |
|---|---|---|---|---|
| $N_{pop}$ | 21 | 43 | 25 | 27 |
| $N_{loc}$ | 7 | 8 | 10 | 12 |
| $n$ | 21.86 (8 / 32) | 18.14 (10 / 31) | 29.96 (22 / 32) | 13.52 (7 / 32) |
| $R_A$ | 3.287 (1.982 / 4.426) | 1.588 (1.000 / 2.555) | 11.052 (6.260 / 14.160) | 2.039 (1.000 / 3.193) |
| $R_G$ | - | 4.336 (1.000 / 8.815) | - | 4.662 (0.233 / 7.000) |
| $H_e$ | 0.530 (0.273 / 0.658) | 0.169 (0.000 / 0.504) | 0.865 (0.736 / 0.921) | 0.271 (0.000 / 0.470) |
| $F_{IS}$ | 0.159 (-0.050 / 0.253) | 0.970 (0.783 / 1.000) | 0.036 (-0.003 / 0.096) | 0.922 (0.483 / 1.000) |
| $S$ | 0.266 (0.000 / 0.404) | 0.984 (0.878 / 1.000) | 0.070 (0.000 / 0.075) | 0.955 (0.651 / 1.000) |
| $S(g_2)$ | 0.030 (0.000 / 0.206) | NC | 0.012 (0.000 / 0.168) | NC |
| $F_{ST}$ | 0.213 (0.007 / 0.533) | 0.371 (0.000 / 1.000) | 0.062 (0.006 / 0.191) | 0.453 (0.049 / 1.000) |

**Figure 2**. Genetic diversity, genetic differentiation, and spatial distance in the four species studied. A. Genetic differentiation (population-centered $F_{ST}$) as a function of genetic diversity ($H_e$). B. Genetic differentiation (pairwise-population $F_{ST}$) as a function of geographical (Euclidean) distance between population pairs. The black lines represent linear regression lines. Note that the y-scale differs among species.



*marmorata* ($r$ = 0.037, $P$ = 0.236; $r$ = 0.074, $P$ = 0.130 when excluding the single population outside Grande-Terre), nor in *D. surinamense* (r = -0.077, P = 0.857; Figure 2). The seriation and clustering approach did not allow detecting clear patterns of population differentiation (results not shown). The few indications are consistent with the results of the more quantitative sNMF approach, and we therefore rely on the latter, considering each species in turn. In *P. acuta*, the optimal number of ancestral populations is four (Figure S1A). The two populations from Marie-Galante belong to a single cluster (V2). One cluster (V1) is largely represented in the North of Grande-Terre, and one (V3) in the South-West, while V4 is widespread. A lower optimal number of clusters was detected in *A. marmorata* (K = 2; Figure S1B), with the smallest cluster rather distributed in the South-West of Grande-Terre, but not only. A single cluster was detected in *D. depressissimum*, suggestive, together with the limited isolation by distance, of an even distribution of genetic diversity at Grande-Terre scale. The pattern is much more complex in *D. surinamense* with eight clusters (Figure S1C), few populations (e.g., Belin or Poucet) belonging to a single cluster and no obvious population structure. Moreover, this analysis did not suggest common geographic patterns of clustering among species. Even when enforcing a fixed number of clusters for all species (e.g., K = 2, as in *A. marmorata*), no common pattern emerged (data not shown).

*Environmental and demographic drivers of genetic diversity*

The mean values of site characteristics (Table S4) for the 2001-2007 period did not differ significantly from those of the 2008-2015 period in the same set of sites (Table S8), suggesting temporal stability over our survey. It should be added that no temporal variation in cumulative rainfall over both the rainy season and the little rainy season has been detected over the same period (Pantel et al., submitted). We also compared values for these sites with those of all other sites in our survey over the 2001-2007 period, and detected no difference, except for stability:

the genotyped populations were in more stable sites than the others (Table S8), presumably because unstable sites are often dry by definition, limiting the possibility of sampling. With this exception, the sites in which genetic analyses were conducted therefore seem representative of all sites in our survey. We conducted the same two series of tests for each species separately. No differences were detected (all $P > 0.05$; results not shown), except when comparing the sites studied in *A. marmorata* and *D. depressissimum* and all other sites for vegetation cover ($t = -3.039$, $P = 0.004$ and $t = -4.009$, $P = 3*10^{-3}$ respectively): the sites sampled for genetic analyses showed less vegetation cover. We detected correlations between some of these variables when considering all genotyped populations (Table S9). Unsurprisingly, stability was positively correlated with size and vegetation cover, and connectivity was positively correlated with invasion age. It is less expected to find a negative correlation between size and connectivity and a positive one between stability and density of favorable habitats.

In both *P. acuta* and *D. depressissimum*, all descriptors of genetic diversity and differentiation ($R_A$, $H_E$ and site-specific $F_{ST}$) were highly correlated, $F_{ST}$ being negatively correlated to the other two descriptors ($R^2$ of linear regression of $F_{ST}$ on $H_E$ was 0.882 in *P. acuta*, and 0.909 in *D. depressissimum*, and the corresponding value for the regression of $F_{ST}$ on $R_A$ was 0.926 and 0.910; Figure 2 and Table S10). The same was true for $R_G$, $H_E$ and $F_{ST}$ in *A. marmorata* and *D. surinamense* ($R^2$ of $F_{ST}$ on $H_E$ was 0.529 in *A. marmorata*, and 0.784 in *D. surinamense*, and the corresponding value for the regression of $F_{ST}$ on $R_G$ was 0.539 and 0.530; Figure 2 and Table S10). When two species co-occurred in a large-enough number of populations, we tested for correlations between species pairs for these descriptors: the only significant correlation was found for *P. acuta* and *A. marmorata* ($R^2$ was between 0.300 and 0.326 for the three descriptors; $N = 11$ sites). We analyzed how genetic diversity and differentiation was affected by the environmental and population variables (Table S2) using linear models (results in Tables 3 and S11). The results were not qualitatively affected by

considering, or not, the populations outside Grande-Terre in *P. acuta* and *A. marmorata*. The most influential variable was connectivity with a positive effect on genetic diversity in three species (marginally significant for $R_A$ in *P. acuta*; Figure 3), except *D. surinamense*. Similarly, connectivity affected negatively population differentiation in *A. marmorata* and *D. depressissimum*. Vegetation cover had a significant positive effect on diversity, and a negative one, on differentiation in *D. depressissimum*. Long-term population size had the same effect in *D. depressissimum*, but that on differentiation was no more significant when vegetation cover and connectivity were included in the model. More surprisingly, this factor also had a positive effect on differentiation in *A. marmorata*. Allelic and genotypic diversity were higher in *A. marmorata* populations recently or never invaded by *P. acuta* than in those that have been invaded for longer. However, invasion age had no effect on *P. acuta* itself. These results can be compared to the expectations proposed in Table 1, and most were not fulfilled with a majority of non-significant effects. However, significant effects (15 situations) went in the expected direction, except that of long-term population size on differentiation in *A. marmorata*.

## Discussion

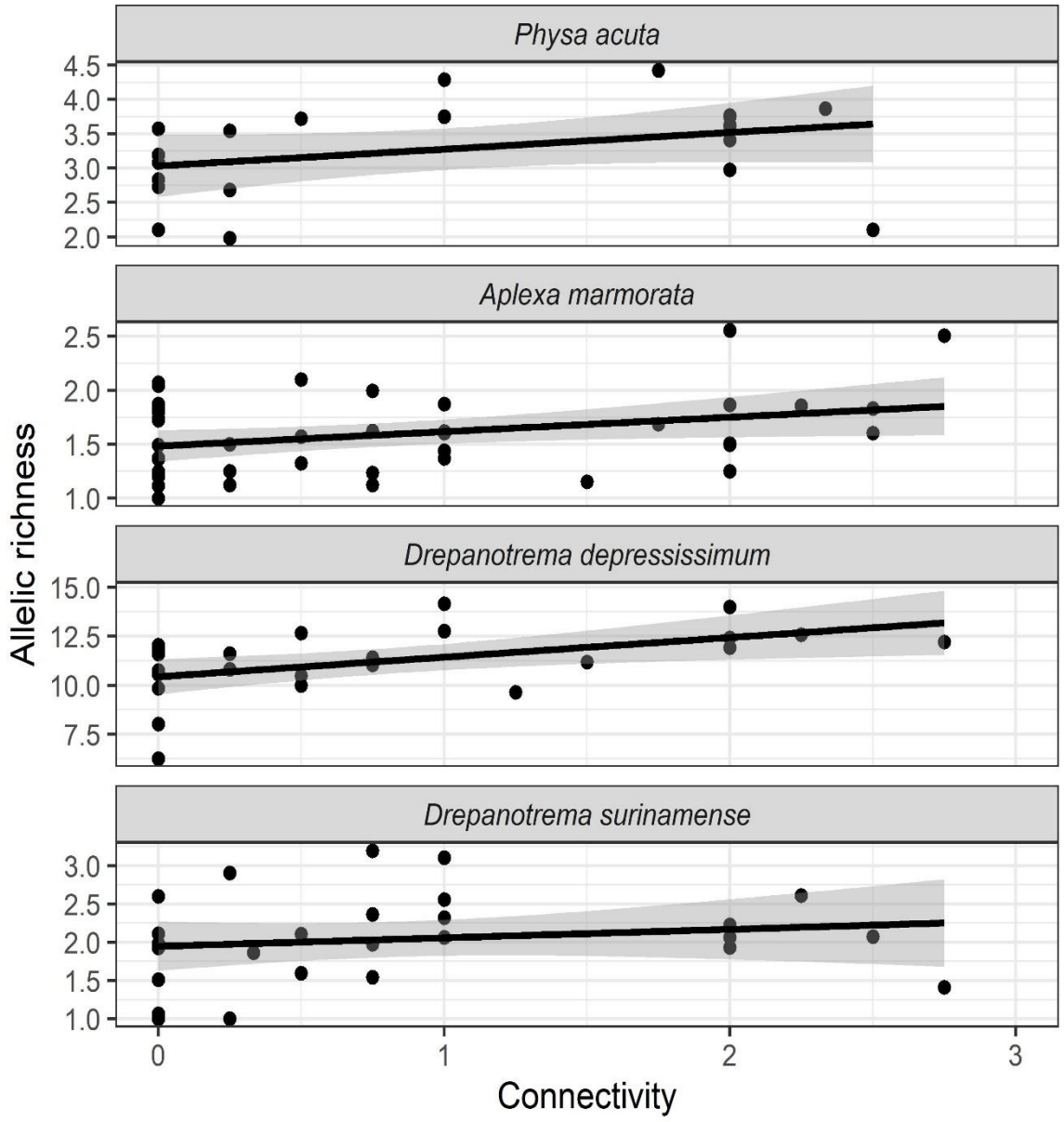
### *A comparative multispecies approach to population genetic structure*

The most classical approach for deciphering the neutral population genetic structure has been, and remains, to study several populations of a single species sampled at a given time, and to infer population processes based on signatures in molecular markers (Charlesworth & Charlesworth, 2010; Lewontin, 1974). These signatures have been in many cases related to traits, for example the mating system, sex or dispersal, but also to population demography, spatial distribution and environmental variables, using more and more sophisticated statistical approaches (Hanski & Gaggiotti, 2004; Foll & Gaggiotti, 2006; Balkenhol et al., 2015; Manel & Holderegger, 2013). We here analyzed genetic variation in several species at once, a

**Table 3**. Effect of environmental and population variables on genetic diversity and differentiation in the four species studied. Results are reported for $R_A$ and site-specific $F_{ST}$ in *P. acuta* and *D. depressissimum*, and for $R_A$, $R_G$ and $F_{ST}$ in *A. marmorata* and *D. surinamense*. Results are from linear models (see text for explanation), and the best models were evaluated based on Akaike Information Criterion. The significant (positive or negative) effects are indicated by + or - ($P < 0.05$), and by ++ or -- ($P < 0.01$). NS indicates no effect. The *P*-value for connectivity in *P. acuta* was indeed 0.08, and 0.07 for invasion age in *A. marmorata*. Full models and effect sizes are reported in Appendix Table S11. All significant effects were in the Direction predicted in Table 1, except the effect of invasion in *D. surinamense* for which we had no specific prediction.

| Variable | *Physa acuta* | *Aplexa marmorata* | *Drepanotrema depressissimum* | *Drepanotrema surinamense* |
|---|---|---|---|---|
| Size | NS/NS | NS/NS/NS | NS/NS | NS/NS/NS |
| Vegetation cover | NS/NS | NS/NS/NS | +/-- | NS/NS/NS |
| Connectivity | +/NS | ++/++/- | ++/- | NS/NS/NS |
| Stability | NS/NS | NS/NS/NS | NS/NS | NS/NS/NS |
| Density of favorable habitats | NS/NS | NS/NS/NS | NS/NS | NS/NS/NS |
| Invasion age | NS/NS | -/-/+ | NS/NS | NS/+/NS |
| Long-term population size | NS/NS | NS/NS/+ | +/- | NS/NS/NS |

**Figure 3.** Relationship between allelic richness and connectivity in the four species studied. Each point is a population. The black lines are simple linear regressions (with 95% SE as grey overlay). Connectivity is measured on an increasing scale (from 0 to 3).



comparative approach that remains relatively rare (Table S1). The four species studied display rather high levels of genetic variation within populations, but with strong variance among populations, suggesting a variable effect of genetic drift within sites, as already observed at similar spatial scale in *P. acuta* (Bousset et al., 2004; Escobar et al., 2008). The extremely high variation in *D. depressissimum* is largely due to highly variable (tetranucleotide) loci (Lamy et al. 2012). In the two remaining species, we do not know of previous studies to which our values can be compared. The marked differentiation among populations was associated with no to limited isolation by distance and an absence of clear geographic patterns. Moreover, most of genetic differentiation, especially in the two selfing species, is due to differences in within-population diversity, irrespective of geographic position, and therefore probably driven by population-specific factors. We can therefore suggest that the genetic structure of the four species at Guadeloupe scale corresponds more to an asymmetric island or a metapopulation model than to other models (e.g., a single panmictic population), as may be expected for freshwater snails occupying ponds (Jarne & Delay, 1991; Lamy et al., 2012). In the former models, we indeed expect marked variation in genetic diversity among populations and no marked geographic structure. Note, however, that cautiousness is required with *P. acuta* since quasi-equilibrium has probably not been reached because of its expanding dynamics. We previously show that the asymmetric island model should be favored over a metapopulation model in *D. depressissimum*, based on both spatial and temporal genetic data (Lamy et al. 2012). Temporal data are not available in the three other species, preventing further distinction in models of population structure – noting that the conditions under which the metapopulation model actually applies are rather narrow (Fronhoefer et al., 2012). In our study, we also observed large differences in genetic diversity and differentiation, lower and higher respectively in *A. marmorata* and *D. surinamense* than in *P. acuta* and *D. depressissimum*. These similarities and differences will be discussed below with regard to environmental parameters, demography

and the mating system.

*Connectivity is a major driver of the distribution of genetic variability*

We proposed in Table 1 how several environmental factors may affect genetic variation following the logic of Lamy et al. (2012). We found essentially no effect in *P. acuta* and *D. surinamense*, and two to three significant effects in the two other species. The most marked effect is that of connectivity, detected in three species, affecting both genetic diversity and population differentiation. Our definition of connectivity is based on local landscape structure, i.e. site accessibility linked to local topography, and therefore falls in the 'structural connectivity' category (see e.g., Storfer et al., 2007). In a comparative study of freshwater fish and invertebrates from Australia, structural connectivity corresponds to realized connectivity, estimated with genetic markers, in 50% of the 110 studies taken into account (Hughes, Huey, & Schmidt, 2013). It can therefore be considered as a rather good indicator of the distribution of genetic variation, as observed in our study. The central effect of connectivity on genetic variation was already highlighted by Lamy et al. (2012) in *D. depressissimum* in Guadeloupe, but also by Bousset et al. (2004) and Escobar et al. (2008) in southern France populations of *P. acuta*. Sites with high connectivity index are indeed connected during the rainy season, and the effective size of local populations is probably much enlarged by these connections. It is perhaps less obvious why *D. surinamense* is not affected by connectivity. This species might disperse less efficiently through flood or more actively through bird transportation. However, this should apply to *D. depressissimum*, since the two species have similar ecology and aptitude to colonize empty sites and an extinction/colonization dynamics driven by the same environmental factors (Pantel et al., submitted). Interestingly, colonization is not affected by connectivity, except in *P. acuta*, even if the colonization rate is higher in physids than in planorbids (Table S6; neither is extinction with no difference among species). This suggests that the effect of connectivity is

not mediated by the extinction/colonization dynamics – we already noticed that the latter has little influence on the distribution of genetic variability in *D. depressissimum* (Lamy et al., 2012). Moreover, there was no correlation in genetic diversity among species, except between the two physids. Overall, these results might be explained by the fact that dispersal is mostly passive (through water currents or birds), and leads to colonization or migration events that are both occasional and occurs at random with regard to both species and geographic scale.

*The impact of the mating system*

Our analysis confirms that the observed genetic patterns are consistent with an outcrossing mating system in *P. acuta* and *D. depressissimum* and a selfing one in *A. marmorata* and *D. surinamense*, as previously recognized (Escobar et al., 2011; Lamy et al., 2012). Genetic diversity is extremely similar in the two selfers, and comparable to what has been observed in other selfing snails (Charbonnel et al., 2002; Lounnas et al., 2017; Tian Bi et al., 2019). The results on population structure ($F_{ST}$) mirror those obtained with within-population variation, unsurprisingly given the strong correlations between within- and among-population variation in the four species, especially in *D. depressissimum*. We observed both lower diversity and higher differentiation in the two selfing than in the two outcrossing species. This was expected because of reduced effective population size and increased selective interference in selfers than in outcrossers (review in Jarne, 1995; Charlesworth, 2003; Ingvarsson, 2002). Interestingly, if we assume a neutral island model and an effective population size divided by two in selfers compared to outcrossers, based on the differentiation measured in the selfing species, $F_{ST}$ in the outcrossers should be that observed in *P. acuta* (see below the role of invasion in this species). Even if this reasoning is only approximate, the mating system alone might explain this difference. However, we observed strikingly large intrapopulation diversity and low differentiation value in *D. depressissimum*. Both could be due to a higher mutation rate,

consistent with the extremely large number of alleles detected, but also to a higher regional demographic size. This latter can be estimated as the product of the number of occupied sites multiplied by the number of individuals per site. The first does not differ markedly among the four species with 50-70% of occupied sites per species (Pantel et al., submitted). The second can be approximated through long-term population size (Table S2), which, however, was lower in *D. depressissimum* than in the other species. Demographic size therefore does not seem to be an explanation to the higher diversity / lower differentiation, except if the 'genetic size / demographic size' ratio is much higher in *D. depressissimum*. One cause might be aestivation since this species survives very well in dry sites (Lamy et al., 2013a), but this is also true of *D. surinamense* (Pantel et al., submitted). We also note that long-term population size did not affect genetic variation within species, suggesting that this estimator of demographic size does not capture adequately effective population size. As mentioned above, the four species differ in their colonization rate (not in extinction rates), but the difference is between the two families, not between selfers and outcrossers (Pantel et al., submitted). Since the difference is not between the two taxonomic families, it is likely that demographic extinction and colonization do not affect genetic variation strongly, as already shown in *D. depressissimum* (Lamy et al., 2012), but rather modulate the influence of the mating system. Overall, this latter seems to strongly influence genetic variation in the four species studied, due to its effects on effective population size.

*Which other factors affect genetic variation?*

We tested the influence of other factors than the mating system and connectivity with mostly identical expectations in the four species (Table 1). Most are not fulfilled (no effect), but when they are, it is generally in the right direction (Table 3). Vegetation cover shows the expected effect in *D. depressissimum* only. It should in principle favors large population size;

194 it indeed favors both population permanence in all species, except *P. acuta* (no effect; Pantel et
195 al., submitted), and colonization in *D. surinamense*. A possible explanation is that the
196 'vegetation cover' variable aggregates several plant and algae species (Pantel et al., submitted),
197 and that the four species react to different types of vegetation. It is also somewhat unexpected
198 that site size, site stability and the density of favorable habitats did not affect genetic variation,
199 all the more that stability is positively correlated with both site size and density of favorable
200 habitats. Site size might be a poor indicator of population size, as suggested by the fact that
201 long-term population size, which beyond size also includes population density, is connected to
202 genetic variation in two species. Stability is perhaps of limited impact, because the three native
203 species studied might have developed strategies allowing coping with drought (Pointier &
204 Combes, 1976; Lamy et al., 2013a). The absence of effect of density of favorable habitats is
205 suggestive of a limited role for passive dispersal through birds compared to passive dispersal
206 through water flow, given the strong effect of connectivity (the two variables are not correlated).
207 More generally, our work indicates that both common and idiosyncratic effects of the
208 environment, space and interspecific interactions on genetic variability can be detected, calling
209 for detailed studies. For example, Haag, Riek, Hottinger, Pajunen, & Ebert (2005) showed that
210 genotypic richness increases with population age in two *Daphnia* species, but that other factors
211 (e.g., presence of competitor species) did not. Other examples are mentioned in Introduction
212 and can be found in Table S1.

213 The distribution of genetic variation may also be affected by species invasion, especially
214 in the invasive species *P. acuta*. In terms of site occupancy at landscape scale, *P. acuta* has
215 been spreading since our first survey in 2000, and still shows an invasive dynamics in Grande-
216 Terre (Dubart et al., 2019; Pantel et al., submitted). This dynamics might be associated with
217 variation loss (Dlugosch & Parker 2008; Estoup et al., 2016; Wares, Hughes, & Grosberg,
218 2005;), but limited loss has been observed in invasive populations of this species at worldwide

scale (Bousset et al., 2014; Ebbs et al., 2018). Moreover, the average variability of Guadeloupe populations (e.g., $H_e$ = 0.530) is similar to that observed in the two studies conducted at similar scale (Bousset et al., 2004; Escobar et al., 2008). It therefore seems that the invasion of *P. acuta* in Guadeloupe is ancient enough – the species presence has been noted in some restricted areas since the early 1970's (JP Pointier, pers. obs.), and involved a large number of propagules as perhaps the result of several invasion origins. Theory also predicts a loss of variation at invasion front as a consequence of bottlenecks (Excoffier & Ray, 2008; Nei, 1975). However, we did not detect an effect of invasion age on genetic variability (except perhaps in the two populations of Marie-Galante, which are poorly variable), suggesting that site colonization involves large number of propagules.

A last point is whether this invasion may affect genetic variation in native species. The two physid species indeed showed competitive interactions through modified extinction and colonization when the other species is present (Dubart et al., 2019). Moreover, Chapuis et al. (2017) showed that, although the number of sites occupied by *A. marmorata* is stable, population density has strongly been affected by the invasion of *P. acuta*. Moreover, this study highlighted an evolution of life-history traits in *A. marmorata* in response to this invasion. This was mirrored in the present study by a loss of neutral genetic variation in *A. marmorata*. Such an effect was not detected in the two *Drepanotrema* species, although their population density is slightly affected by the invasion of *P. acuta*. This suggests that the effect might occur in *Drepanotrema* with some delay. Invasive species may have a negative effect on population densities of native species in introduction areas, which in turn may affect the dynamics of genes and induce rapid evolution (e.g., Strauss, Lau, & Carroll, 2006; Stuart et al., 2014). A rapid shift in life-history traits has previously been documented in *A. marmorata* as a result of the invasion of *P. acuta* (Chapuis et al., 2017). Whether the reduction in neutral genetic variation documented here is related to this shift remains an open question, but documenting such

244 decreases in other bioinvasions might be a first approach to analyzing their evolutionary impact.
245
246 **Conclusion and perspectives**
247 The comparative approach developed here leads towards a more general perspective on
248 population genetic structure than proposed in studies focusing on single species. Indeed, it
249 allows detecting common and idiosyncratic effects of the environment, space and interspecific
250 interactions on the distribution of genetic variation in sets of species occupying the same space
251 with limited migration from outside. The four species studied here displayed striking
252 similarities in genetic structure, with marked variation in genetic diversity among sites and
253 strong genetic differentiation, due to passive dispersal, extinction/colonization dynamics and
254 erratic variation in local population size. Differences are due to the mating system that amplifies
255 local drift in selfing populations, interspecific interactions, and to a very limited extent local
256 environmental factors. We also detected little geographic coherence in the distribution of
257 genetic variation among species. In studies conducted at the same (not too large) geographic
258 scale (Table S1), the effect of traits on genetic variation was detected in some cases (e.g.,
259 lifespan in Reid et al., 2017), but landscape structure (e.g., habitat fragmentation in Ortego et
260 al., 2015) and demographic factors (e.g., population age in Haag et al., 2006) also play a role.
261 However, none of these studies, with the possible exception of the last two studies cited, carry
262 out a comparative analysis to detect common genetic patterns and the role of idiosyncratic vs.
263 common influences among species. We suggest extending these comparative approaches,
264 moving towards comparative landscape genetics (see e.g. Burkhart et al., 2016; Ortego et al.,
265 2015).
266 We also suggest to include in such studies the impact of interspecific interactions, as we
267 did here and in previous genetic and demographic studies (Chapuis et al., 2017; Dubart et al.,
268 2019; Lamy et al., 2013a). Our approach constitutes a first entry into the processes shaping

these interactions, i.e. upscaling from population ecology to community ecology. The similarity between forces acting at species and community level has long been recognized (e.g. Bell, 2001; Hubbell, 2001), particularly when considering reasonably similar species occupying the same matrix (i.e. a metacommunity), and is at the heart of community genetics (Agrawal, 2003; Vellend, 2010; Vellend & Geber, 2005). Studying the relationships between species and genetic diversity and assessing how they are affected by environmental parameters and space is one way of highlighting these similarities (Lamy et al., 2017; Vellend & Geber, 2005). In the meta-community of Guadeloupe, this approach highlighted (again) the influence of connectivity (Lamy et al., 2013b; Lamy, Laroche, David, Massol, & Jarne, 2017). Taking into account eco-evolutionary dynamics would be an alternative path - at this stage they have essentially been studied at the intraspecific level (Hendry, 2017). The evolution of life-history traits and the demographic decline of *A. marmorata* following the invasion by *P. acuta* is an example of such eco-evolutionary dynamics (Chapuis et al., 2017). Integrative approaches, including long-term genetic, demographic and ecological studies, have been carried out in other species groups, such as guppies (Bassar, Simon, Roberts, Travis, & Reznick, 2017) or anoles (Pringle et al., 2019) in the Caribbean, to name just a few well-known examples, thus providing an insight into the links between population biology and community ecology. They should be continued and generalized to other model systems, including more complex communities.

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**Contributions**

PJ and PD framed the study and supervised all analyses. TL, EC, MD, JPP, PD and PJ did field sampling and were involved in the long-term monitoring in Guadeloupe. TL, EC, AS, and EC did the genetic analyses. PJ, ALdC, TL, and MD ran the statistical analyses. PJ wrote the manuscript with input from all other authors, especially PD, at all stages of writing.

**Data accessibility**

The Zenodo archive (https://zenodo.org/; 10.5281/zenodo.4534421) contains all information analyzed in this paper (microsatellite genotypes of the four species, environmental and demographic data).

**Conflict of interest**

The authors of this article declare that they have no financial conflict of interest with the content of this article.

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