Populations Genetic Study of the Corals *Acropora palmata* and *Acropora cervicornis* of Guadeloupe (French West Indies) in View of Their Preservation

Estudio de la Genética de Poblaciones de Corales *Acropora palmata* y *Acropora cervicornis* de Guadeloupe (Antillas Francesas) para su Conservación

Étude de Génétique des Populations des Coraux *Acropora palmata* et *Acropora cervicornis* de Guadeloupe (Petites Antilles) en vue de Leur Conservation

AURÉLIEN JAPAUD[1]*, CÉCILE FAUVELOT[2], and CLAUDE BOUCHON[1]

[1]*Universite des Antilles et de la Guyane, LABEX CORAIL DYNECAR EA, 926 BP 592 Pointe-à-Pitre Cedex, Guadeloupe 97159 Guadeloupe (FRANCE). *aurelien.japaud@univ-ag.fr.*

[2]*Centre IRD de Nouméa, UNIT 227 COREUS BPA 5 Nouméa Nouvelle-Calédonie 98848 France.*

ABSTRACT

In the Caribbean, *Acropora palmata* (Lamarck 1816) and *A. cervicornis* (Lamarck 1816) are major coral species for reef building. Since the 1980s, these species populations are decreasing and are now classified as critically endangered species by the International Union for Conservation of Nature (IUCN). To implement the development of an efficient and sustainable restoration method of the endangered populations, the genetic status of populations should be known. Recent studies, mainly conducted on the reefs of Florida and the Greater Antilles concerned the structure and the dynamics of *Acropora* populations, while the genetic status of the populations in the Lesser Antilles remains less studied. In this context, a genetic study of some populations of these two species, was realized on five reefs of Guadeloupe (French West Indies). This study suggests that the populations are genetically distinct and have a larval recruitment on a limited scale and need local conservation measures. In addition, results show that *A. cervicornis* populations are rare and genetically undiversified. Thus, in Guadeloupe, the survival of this species and its associated ecosystem services are threatened, which has never been shown before.

KEY WORDS: Caribbean corals, population genetics, *Acropora*

INTRODUCTION

For many decades now, Caribbean tropical marine ecosystems show signs of alteration (Jackson 2001, Knowlton 2001, Pandolfi et al. 2003, Wilkinson 2004, 2008). A reduction of 80% in the Caribbean coral reef cover has happened over the last 30 years (Gardner et al. 2003, Wilkinson 2004, 2008), including a dramatic decline in coral populations of Acroporidae. This observed massive decline is usually attributed to large-scale phenomena still difficult to predict (diseases, coral bleaching generated by high temperatures, physical damage due to hurricanes…) and a combination of many other additional natural and anthropogenic factors acting in synergy (Chadwick-Furman 1996, Precht et al. 2002, Wapnick et al. 2004, Donner et al. 2005, Williams and Miller 2005, 2012, Cramer et al. 2012).

Acropora palmata (Lamarck 1816) and *Acropora cervicornis* (Lamarck 1816) have an important role for the building and structuring of Caribbean reefs. Morphology, abundance and growth rates of *A. palmata* make this coral species one of the most important Caribbean reef builder. The branching morphology of *Acropora cervicornis*, and to a lesser extent, of *Acropora palmata*, spatially structures the coral reef ecosystem providing a high biodiversity (Gladfelter 1982, Bruckner 2002, Burke and Maidens 2004). Furthermore, coral reef structures have a significant physical role in inhibiting and dispersing wave energy and thus, protecting coral reefs, shorelines and human infrastructures against erosion. *Acropora palmata* and *A. cervicornis* are the only species of the genus *Acropora* in the Caribbean region in contrast to nearly 150 species of the same genus which exist in the Indo-Pacific reefs. These two Caribbean endemic coral species are classified as critically endangered since 2008 by the International Union for Conservation of Nature (IUCN). The current reduction of *A. palmata* and *A. cervicornis* abundance coupled with the small number of coral species in the Caribbean region seriously compromise coral reef ecosystem and the associated ecosystem services.

In this global context, to estimate the connectivity and the resilience of local Caribbean *Acropora* populations in order to better understand the health status and the functioning of these populations for conservation objectives is crucial. To assess genetic diversity at species or local population scale is the key to estimate the resilience capacity after a rapid environmental change. High genetic diversified populations resulting from sexual reproduction and genetic mixing, trigger new genotypes (genetic individuals) and are necessary to the species adaptation success against changes in environmental conditions (Miller and Ayre 2004, Yeoh and Dai 2009). Without genetic mixing, the asexual reproduction could maintain the adapted populations to an unchanged local environment. But in this case, these populations are potentially more vulnerable against changes in environmental conditions (Miller and Ayre 2004). For a geographically and genetically isolated population, genetic drift fixes some alleles, decreases genetic diversity and generates a differentiation between populations. By contrast, a weak differentiation is observed for genetically linked populations. Connectivity represents gene flows between populations. These gene flows could be estimated by studying genetic structure and genetic differentiation

between populations. For most marine species, gene flows between populations, and connectivity, are maintained through larval dispersion. Larval displacement in the water column is the result of passive transport (by currents) and active transport (swimming behavior of larvae, Ayata 2010). For corals, the recruitment process corresponds to the larvae fixation on the substratum. For an efficient recruitment process, larvae have to set up on reefs and to stay alive. Many biotic and abiotic factors may influence and limit larval settlement.

Since the beginning of the 2000s, the decline of *Acropora palmata* and *A. cervicornis* and their critically endangered status led the scientific community to be interested in the genetic structure and the dynamics of these populations. Population-based genetic studies have been mainly conducted on the reefs of Florida and the Greater Antilles while the genetic status of the populations in the Lesser Antilles remains less studied. In this context, the main objective of this study is to understand larval dispersion on Guadeloupe archipelagos reefs (Lesser Antilles). Especially, this work aims to answer these questions:

i) What are the spatial scales of larval dispersion?
ii) What is the connectivity level among populations?

It is not possible to follow larvae to know the spatial scales of larval dispersion; these scales have to be indirectly estimated with hyper-polymorph genetic markers to evaluate gene flows between populations. However, asexual reproduction (or clonal reproduction) by fragmentation is an important reproduction mode for branched corals with high growth rates, like *Acropora palmata* and *A. cervicornis* (Highsmith 1982). The asexual reproduction by fragmentation allows the installation of a new coral colony on the reef by the settlement of a coral fragment of a colony already set up on the same reef. The new colony and the original colony are genetically identical – they are clones. Mature clonal coral colonies would increase the sexual reproductive status of the cloned genetic individual increasing the quantity of its released gametes. Clonal reproduction would also help to secure high larval and juvenile mortality rates from sexual reproduction and to spread mortality risk for the cloned genotype. However, the consequences of a high clonal rate is a low genetic diversity and a potential increase of the associated dangers to stress events for which cloned genotype is not adapted (Reusch et al. 2005). Long terms effects of clonal reproduction depend on the balance between costs and benefits of this process (Lirman 2000).

For the present study concerning the genetic structure and the dynamics of *Acropora* coral populations of Guadeloupe, the first step was to determinate the number of genotypes among the sampled colonies and to estimate in a second step the diversity and the genetic structures of populations for each species and the associated connectivity.

MATERIALS AND METHODS

Study Sites and Field Samplings

Five sites were selected in Guadeloupe, Lesser Antilles (16°30'N; 61°30'W) to support the genetic study (Figure 1, Table 1). On those sites, individuals of both *Acropora* species were generally found, except for the site E where only *Acropora palmata* individuals were sampled.

On site A (*Caye à Dupont*) situated on the East Side of "Basse-Terre" island in the bay of *le Grand Cul-de-Sac Marin*, an *Acropora cervicornis* population and an *A. palmata* population were sampled on December 2011. The site B (*Îlet Fajou*) situated in the North Bay of Guadeloupe is about 22 km north from site A. The sampling area of the fore reef zone of site B was done between 0 and 8 m deep during the months of May, June and July 2011 and covered

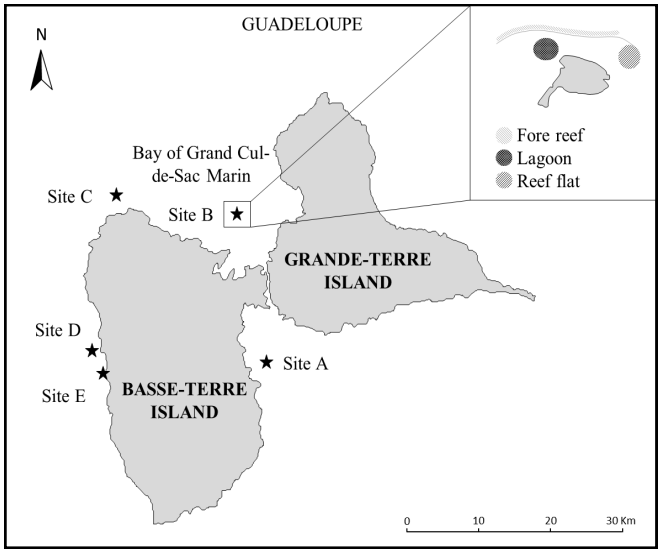

Figure 1. Locations of study sites in Guadeloupe.

Table 1. GPS locations of the study sites.

Site	Reef zone	Latitude	Longitude
Acropora palmata			
A		16°09'25.60"N	61°32'33.18"W
B	Reef flat	16°21'16.20"N	61°34'21.50"W
B	Fore reef	16°21'24"N	61°36'28"W
C		16°22'54.27"N	61°45'49.73"W
D		16°10'00"N	61°47'24"W
E		16°08'29.16"N	61°46'47.28"W
Acropora cervicornis			
A		16°09'27.07"N	61°32'42.36"W
B	Lagoon	16°21'24.19"N	61°35'35.12"W
B	Fore reef	16°21'24"N	61°36'28"W
C		16°22'54.27"N	61°45'49.73"W
D		16°10'00"N	61°47'24"W
Acropora prolifera			
B	Lagoon	16°21'24.19"N	61°35'35.12"W

a distance of 3.4 km (from 16°21'23.70"N; 61°36'27.5"W to 16°21'30.1"N; 61°34'36.7"W). Site B also included a reef flat zone and a lagoon zone sampled on January 2013. Only *Acropora palmata* individuals were found over the reef flat zone whereas only *A. cervicornis* individuals were found over the lagoon zone. Site C (*Tête à l'Anglais*) located on the North of "Basse-Terre" island about 20 km west from site B was sampled on March 2013. Site D (*Îlets de Pigeon*) located on the West of "Basse-Terre" island about 25 km South of the site C was sampled on September 2011. Site E (*Pointe à Lézard*) located about 3 km South of site D was sampled on October 2011.

In each site and for each colony, a fragment of a branch was sampled and put into a Falcon tube with seawater. When it was not possible to distinguish each colony because of their intermixing, the sampling was spaced in order not to sample the same colony twice. The fragments size (between 2 and 3 cm) was confined to what is necessary for the DNA extraction with the aim of disrupting as little as possible the corals biology (growth, sexual reproduction). Colonies that show large damaged surfaces (diseases, predation, and algae) or that were too small were not sampled. To preserve DNA, samples were reconditioned in the laboratory; seawater was replaced by 70% ethanol (analar NORMAPUR for analysis).

DNA Extraction

For each sample, about 20 polyps were recovered in a 1.5 ml Eppendorf tube and were leaved uncovered for a night for alcohol evaporation. Genomic DNA was isolated from these polyps with a DNA purification kit (Gentra Puregene; Qiagen, Germantown, USA) following the manufacturer's protocol. For some samples, the quantity of extracted DNA was controlled with a spectrophotometer.

Genotyping

Microsatellites sequences are non-coding regions from the genomic DNA which present small motifs of few nucleotides (3 or 4) tandemly repeated between 8 and n times, depending on the considered microsatellites. The length polymorphism of these amplified microsatellite fragments lead to genetically distinguish the genotypes, even if they are genetically close. Specific primers to the flanking regions of 10 microsatellite *loci* of *Acropora palmata* were used. Although these primers are not specific to the *A. cervicornis* species, they could be used for both species which are genetically very close (Baums et al. 2005a, 2009).

The polymerase chain reactions (PCR) were realized with a specific microsatellite PCR kit (Type-it® Microsatellite PCR Kit; Qiagen, Germantown, United States of America) on a GeneAmp®PCR System 2700 thermocycler (Applied Biosystems), following the manufacturer's protocol. Amplified fragments were sent to Genoscreen (Lille, France) where they were separated on an ABI 3730XL sequencer, with a GeneScan® LIZ-500 internal size standard (Applied Biosystems).

Acropora corals are diploid organisms. Amplified fragments represent the alleles of microsatellite regions. If an individual is homozygote, same size fragments are obtained. If an individual is heterozygote, the fragments have different sizes. A fluorescent primer (fluorochrome) with a color (blue, green, yellow or red) was linked to the 5' extremity of the forward primer of each microsatellite *locus*. With this technique, each fluorochrome was incorporated to each newly synthesized fragment during the amplification. Genotyping makes it possible to obtain fluorescence spectra which are analyzed with GeneMapper v5.0 software (Applied Biosystems) to determinate the alleles which are associated to the microsatellite *loci* for all samples. Thus, that enables to know the multiloci genotypes of the sampled coral colonies.

Data Analysis

Genotypic diversity — These species can reproduce by asexual reproduction; for each population, the presence of clones and the number of genotypes were determined with GenClone v2.0 software (Arnaud-Haond and Belkhir 2007). A diversity index, the genotypic richness index was also calculated for each population with this software. The values of the genotypic richness index (R) (Dorken and Eckert 2001) are between 0 and 1. The closer to 1 the values are, the higher the genotypic richness and the smaller the number of clones are. Genotypic richness was calculated as:

$$R = ((G - 1)) / ((N - 1))$$

where G is the number of genotypes and N is the number of sampled colonies.

Genetic differentiation — The genetic differentiation of the populations was evaluated with a principal component analysis realized with PCAGEN v1.2 software (Goudet 2005) using alleles found for all the different loci of each genotype found into the populations.

RESULTS

Number of Multi-locus Genotypes and Genotypic Diversity

A total of 448 colonies were sampled: 292 colonies of *Acropora palmata* and 143 colonies of *A. cervicornis*. The number of *A. palmata* sampled colonies varies between 15 on site D and 80 on site A. For *A. cervicornis*, the number of samples by site varies between 2 (site D) and 80 (site A).

In the lagoon zone of site B, a high number of colonies of the *Acropora prolifera* hybrid were morphologically identified *in situ* in January 2013 whereas these hybrid colonies were not found in 2011. Thirteen colonies of the hybrid were sampled.

The 448 samples did not lead to obtain complete genotypes with the 10 selected microsatellites markers. Only the 410 samples for which 5 loci or more were amplified, were used for the next analyses; *i.e.*, 255 colonies of *Acropora palmata*, 142 of *A. cervicornis* and 13 of *A. prolifera*.

Table 2 presents the number of samples (N), the number of genotypes (N_{MLG}), the ratio between the number of genotypes and the number of sampled colonies (N_{MLG} / N) and the genotypic richness index (R) of the sampled populations.

A total of 160 multiloci genotypes (MLG) were found from the 397 sampled colonies of *A. palmata* and *A. cervicornis*. For *A. palmata*, between 2 and 34 clones of a same genotype were found by site whereas for *A. cervicornis*, between 2 and 80 clones were identified by site. Clones were different from site to site. 143 MLGs were found from the 255 analyzed colonies of *Acropora palmata* and the associated genotypic richness (R) is 0.11.

For *Acropora palmata*, the number of MLGs varied from 2 (the reef flat zone of site B) to 46 (the fore reef zone of site B). The fore reef zone of site B and site E had the highest genotypic richness indices (R > 0.9). The reef flat zone of site B with 2 MLGs from 26 sampled colonies had the weakest genotypic richness index (R = 0.04), the majority of the sampled colonies of this site being clones of one genotype. For *A. cervicornis*, the number of MLGs varied from 1 (sites A and D) to 8 (the fore reef zone of site A). All sampled colonies of sites A and D were clones of a same MLG (different from site to site). For *A. cervicornis*, the fore reef zone of site B had the highest genotypic richness index (R = 0.54). The 13 samples of *A. prolifera* were clones; so, the genotypic richness index was zero.

Table 2. Number of sampled colonies (N), number of multilocus genotypes (NMLG), ratio between NMLG and N and genotypic richness index (R) for sampled *Acropora* populations.

Site	Reef zone	N	N_{MLG}	N_{MLG}/N	R
Acropora palmata		**255**	143	0,56	0,35
A		80	10	0,13	0,11
B	Reef flat	26	2	0,08	0,04
B	Fore reef	48	46	0,96	0,96
C		36	26	0,72	0,71
D		15	14	0,93	0,93
E		50	45	0,90	0,90
Acropora cervicornis		**142**	17	0,12	0,11
A		80	1	0,01	0,00
B	Lagoon	43	5	0,12	0,10
B	Fore reef	14	8	0,57	0,54
C		3	2	0,67	0,50
D		2	1	0,50	0,00
Acropora prolifera		**13**	1	0,08	0,00
B	Lagoon	13	1	0,08	0,00

Genetic Divergence in the Genus *Acropora*

In order to estimate the discriminating power of the microsatellite markers to the recognition of the two species and the hybrid taxon, a factorial correspondence analysis was realized with the Genetix v4.05.2 software (Belkhir et al. 2004).

The factorial correspondence analysis (Figure 2) with the alleles found at the different loci of the sampled colonies led to distinguish the MLGs found for *Acropora palmata, A. cervicornis* and *A. prolifera.* The second (vertical) axis split the *A. palmata* individuals (white dots) and the *A. cervicornis* ones (black dots). Some *A. palmata* MLGs are really close of some *A. cervicornis* MLGs. So, the two species seem to present a small genetic divergence. The *Acropora prolifera* genotype (grey dot) was situated in the overlapping area, which support the morphologically identification and the hybrid status of these colonies.

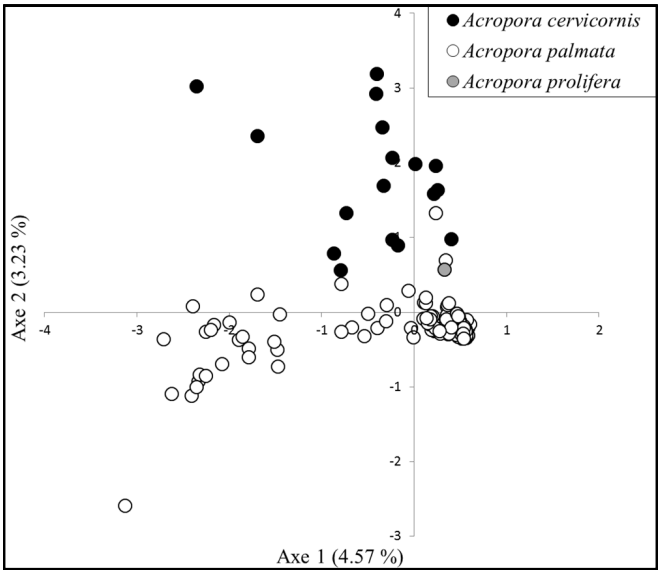

Figure 2. Results of the factorial correspondence analysis diagram realized with all genotypes of *Acropora palmata*, *A. cervicornis* and *A. prolifera*.

Genetic differentiation for *Acropora palmata*

The principal components analysis (PCA) shows the genetic differentiation between the *Acropora palmata* populations (Figure 3). Because of the low number of MLGs for the reef flat zone population of site B (N_{MLG} = 2), data could not be reliable to interpret the PCA. Therefore the PCA was realized without this site.

Axis 1 clearly split the population of site A from the other populations. Axis 2 clearly split the populations of the fore reef zone of site B and the site C from the others. In general, it would appear that the populations of sites D and E are genetically closed. The populations of site C and the fore reef zone of site B are genetically closed and the population of site A is significantly differentiated from all the others.

The *Acropora cervicornis* sampled sites have few MLGs (N_{MLG} < 10). Therefore, for this species, the interpretation of the PCA could not be reliable. The genetic differentiation between the *Acropora cervicornis* cannot be studied.

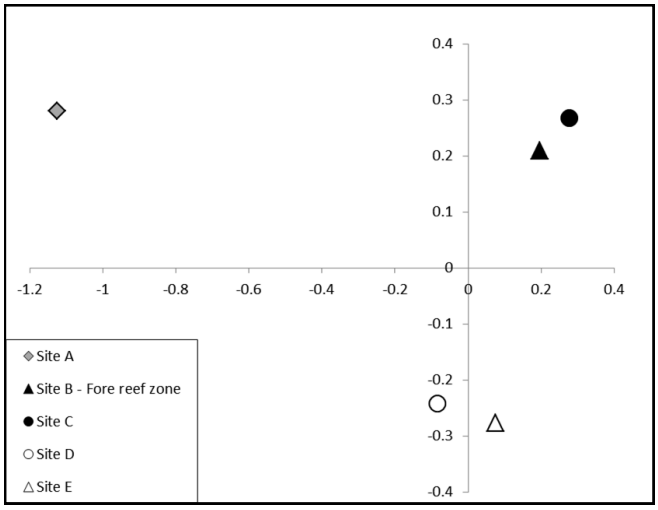

Figure 3. Results of the principal component analysis.

DISCUSSION

Hybridization and Genetic Divergence in *Acropora palmata* and *A. cervicornis* Species

Acropora palmata and *A. cervicornis* reproduce synchronously once or twice a year during a night of August or September which follows a full moon event (Szmant 1986, de Graaf et al. 1999). These species are simultaneous hermaphrodites and produce at the same time ovules and sperms, but some mechanisms prevent self-fertilization (Palumbi 1994). Nonetheless, *Acropora palmata* and *A. cervicornis* can form a viable F1 offspring; this hybrid form is called *Acropora prolifera*. The appearance of *Acropora prolifera* in the bay of *Grand Cul-de-Sac Marin* is recent because no individual was reported during surveys of 2011.

The colonies of the *A. prolifera* hybrid present morphological variations from *A. palmata*-like to *A. cervicornis*-like; that is why the morphological identification could be difficult (Vollmer and Palumbi 2002, *Acropora* Biological Review Team 2005). The factorial correspondence analysis (Figure 2) realized with the *A. palmata* and *A. cervicornis* genotypes show an overlapping area between the genotypes of the two species, which revealed a small genetic divergence between the species. This overlapping area includes the genotype associated to the *A. prolifera* hybrid form met in the lagoon zone of site B. Studies have recently described that ovules of *A. palmata* are less subject to hybridization than the *A. cervicornis* ones (Fogarty 2007, 2012, Palumbi et al. *2012*). The *A. prolifera* hybrid which makes gametogenesis could reproduce itself in some rare events with *A. cervicornis.*

Genotypic Diversity

For each population of *Acropora cervicornis*, the genotypic richness index was globally lower than *Acropora palmata* (Table 2). Because of the important number of clones in each population, *A. cervicornis* could spread more with asexual reproduction than *A. palmata* that could be explained by the branching structure which is much more fragile than the one of *A. palmata*. A weak genotypic diversity cans indicate an asexual reproduction strategy in order to increase the number of individuals of the species and maintain the genetic diversity during the population decline (Honnay and Bossuyt 2005). Genotypes are not sensible to the same environmental perturbations and populations with a weak genotypic richness like *A. cervicornis* populations could be threatened to disappear against extreme conditions without resistant genotypes. Many studies show that genotypic diversity of species which structure an ecosystem has a similar role to the species diversity by conferring resilience to the ecosystem towards perturbations of the environmental conditions (Willis et al. 2006). The low genotypic richness in *A. cervicornis* populations suggests that populations are declining and that the associated ecosystem services are threatened.

For *Acropora palmata*, previous studies realized on 26 reefs distributed into 8 Caribbean regions have shown a bio-oceanographic barrier situated at the Mona canal (between Puerto Rico and the Dominican Republic) which could limit the larval exchanges (Baums et al. 2005b and 2006). *Acropora palmata* westward populations are genetically distinct from the eastward populations because there were no recent genetic exchanges (or few). The population situated at the East of Mona canal have a higher genotypic richness ($N_{MLG}/N = 0.64 \pm 0.17$) than the populations of the western region ($N_{MLG}/N = 0.43 \pm 0.31$). Guadeloupe Island (Lesser Antilles) is situated in the eastern province. With all sampled *Acropora palmata* colonies, the genotypic richness is estimated to 0.56 (N_{MLG}/N). The richness and the genotypic diversity are negatively correlated to the colonies density. Zones with a high density of colonies have higher rates of asexual reproduction than populations with a low density of colonies (Baums et al. 2006). Only high density populations were sampled and that could explain a lower genotypic richness of Guadeloupean populations than the observed genotypic richness in the eastern province. To improve the sampling, it could be better to sample the other isolated colonies all around the island. Thus, the genotypic richness values could be increased and could approach the values found for the eastern province populations. *A. palmata* and *A. cervicornis* have the same reproduction mode and the reproduction period occurs at the same time. The barrier to the larval dispersion situated between Puerto Rico and the Dominican Republic could have the same impact on the *A. cervicornis* populations at the Caribbean scale.

Larval Dispersion and Genetic Differentiation of the Populations

For the two species, the individuals of site A were genetically distinct from individuals of the other populations (Figure 3). *Acropora* from site A is separated from sites D and E by "Basse-Terre" Island. The channel between "Basse-Terre" Island and "Grande-Terre" Island does not seem to authorize for the larval dispersion between the Northern and Southern bays of Guadeloupe. It could be explain by the poor water quality which could kill the larvae. This isolation could explain that the *Acropora palmata* population of site A significantly diverges than the other populations (Figure 3). For *A. palmata*, the populations of sites D and E on the east side of Basse-Terre Island and the populations in the bay of Grand Cul-de-Sac Marin seem genetically divergent. A genetic differentiation between *Acropora* populations is observed in Guadeloupe at a scale of a few tens of kilometers.

The larval pelagic phase of *Acropora* seems to be relatively short because larvae could settle after 3 to 5 days (Fogarty 2007, 2012). For a larval phase of 5 days, the potential of dispersion is estimated to few tens of kilometers (Baums et al. 2005b, Hemond and Vollmer 2010). However, this potential of larval dispersion depends on the speed and the direction of coastal currents. The zone chosen by the larvae to settle and to develop have to present some specific environmental conditions. For example, it seems that the presence of colonies of a species favorably affects the installation of other colonies of the same species (Carlon 2002, Baird et al. 2004, Vermeij et al. 2008). Because of these environmental constraints, the larval pelagic phase could be up to twenty days (Harrison and Wallace 1990, Hayashibara et al. 1993). Baums et al. (2005b) highlighted that the recovery of *A. cervicornis* populations mainly come from populations of local reefs and not from populations of distant reefs. In Guadeloupe, *Acropora* populations could be mainly self-recruiting. Thus, it is therefore crucial to protect the actual populations in decline at the local scale.

To conclude, the results showed that *Acropora palmata* populations are genetically differentiated for sites separated by tens of kilometers. In Guadeloupe, the larval recruitment seems to be limited to short distances. The insular context means that it is necessary to protect the actual populations in decline at the local scale. The results have proved that the *Acropora cervicornis* populations are in decline. However, the presence of the hybrid *A. prolifera* could give an evolutionary potential to the genus *Acropora*.

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