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Écologie moléculaire d'une relation hôte-parasite en contexte insulaire marin: crabes parasites des oursins spatangues en Mer des Caraïbes

Quentin JOSSART

Thèse présentée en vue de l'obtention du titre de Docteur en Sciences

Université Libre de Bruxelles (ULB)

Université de Bourgogne (uB)

Promoteurs : Chantal De Ridder (ULB), Bruno David (uB)

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INTRODUCTION

A. Le parasitisme

1. Définition et diversité

Le parasitisme est une symbiose où un organisme (le parasite) vit aux dépens d'un autre organisme (l'hôte), la relation étant dès lors bénéfique pour le parasite et préjudiciable pour l'hôte (Poulin 2007).

La définition du terme 'symbiose' varie selon les auteurs. Il s'agit toujours d'une association durable entre individus d'espèces différentes mais les modalités qui sous-tendent ces interactions peuvent être envisagées de manières différentes (Douglas 2010). Dans le cadre de ce travail, le terme 'symbiose' est utilisé selon la définition originale de De Bary (1879) basée sur l'étymologie du terme (du grec *sun* 'avec' et *bios* 'vivre', ou 'vivre ensemble'). Cette définition implique une intimité de contact entre les partenaires symbiotiques et la persistance de ce contact durant un temps 'relativement long'. Dans ce contexte, les interactions interspécifiques impliquant des contacts brefs entre les individus comme les interactions prédateurs-proies ou la majorité des interactions plantes-pollinisateurs, ne sont pas des symbioses. Outre le parasitisme, il existe deux autres catégories de symbioses : le mutualisme où les deux partenaires tirent profit de l'association et le commensalisme qui est sans effet pour l'un des partenaires et avantageux pour l'autre (Sapp 1994 ; Berthet 2006). Cependant, certains auteurs (voir Sapp 1994 et Douglas 2010), considèrent la symbiose comme étant exclusivement du mutualisme, et font du parasitisme et du commensalisme des associations non symbiotiques.

La diversité des parasites est remarquable. On estime que plus de 10% des espèces de métazoaires décrits sont des parasites (Poulin 2007). De nombreux phylums de métazoaires comptent des espèces parasites et certains le sont même exclusivement (ex : Acanthocephala, Pentastomida). On dénombre, par exemple, plus de 40.000 espèces de plathelminthes, 10.500 espèces de nématodes ou encore 30.000 espèces d'acariens (Tableau 1). De plus, le pourcentage d'espèces parasites atteint près de 50% des espèces actuelles décrites si l'on y inclut les microorganismes (de Meeûs & Renaud 2002).

La diversité des hôtes est, elle aussi, remarquable et il est fort probable que toutes les espèces libres soient parasitées (Thomas et al. 2007). Notons que même les virus peuvent être 'parasités' par des molécules simples (Combes 2001).

Taxa des parasites	Nb d'espèces parasites	Cycle vital	Habitat
Phylum Mesozoa	+80	S	M
Phylum Myxozoa	+1350	C	M, D
Phylum Cnidaria	1(?)	C(?)	D
Phylum Plathelminthes			
Classe Trematoda	+15000	C	M, D, T
Classe Monogenea	+20000	S	M, D
Classe Cestoidea	+5000	C	M, D, T
Phylum Nemertinea	+10	S	M
Phylum Acanthocephala	+1200	C	M, D, T
Phylum Nematomorpha	+350	S & C	D, T
Phylum Nematoda	+10500	S & C	M, D, T
Phylum Mollusca			
Classe Bivalvia	+600	S	D
Classe Gastropoda	+5000	S	M
Phylum Annelida			
Classe Hirudinea	+400	S	M, D
Classe Polychaeta	+20	S	M
Phylum Pentastomida	+100	C	D, T
Phylum Arthropoda			
Sous-Phylum Chelicerata			
Classe Arachnida			
Sous-Classe Ixodida	+800	S & C	T
Sous-Classe Acari	+30000	S	M, D, T
Sous-phylum Crustacea			
Classe Branchiura	+150	S	M, D
Classe Copepoda	+4000	S	M, D
Classe Cirripedia			
Sous-Classe Ascothoracida	+100	S	M
Sous-Classe Rhizocephala	+260	S	M
Classe Malacostraca			
Ordre Isopoda	+600	S	M
Ordre Amphipoda	+250	S	M
Sous-Phylum Uniramia			
Classe Insecta			
Ordre Diptera	+2300	S	T
Ordre Phthiraptera	+4000	S	T
Ordre Siphonaptera	+2500	S	T
Ordre Strepsiptera	+600	S	T

Tableau 1. Inventaire (non exhaustif) des parasites métazoaires. S = cycle vital simple ; C = cycle vital complexe. M = marin, D = dulcicole, T = terrestre (De Bruyn 2010 d'après Poulin 2007)

2. Importance dans les écosystèmes

Par leur diversité et leur impact sur les organismes impliqués, les parasites peuvent influencer de manière non négligeable la biodiversité et, par-là, les écosystèmes (Thomas et al. 2007). Ils peuvent moduler la compétition interspécifique (les parasites comme 'arbitres') ainsi que la disponibilité des niches (les parasites comme 'ingénieurs')

Arbitres dans la compétition interspécifique. Lors d'une compétition entre deux espèces, on assiste souvent à une inégalité devant le parasitisme c'est-à-dire que l'une des deux espèces sera plus affectée par la présence du parasite que l'autre. Le parasite va donc favoriser l'espèce la moins affectée, ayant ainsi une influence soit positive soit négative sur la biodiversité locale (Thomas et al. 2007).

Par exemple, si un parasite affecte préférentiellement une espèce dominante, il permettra le maintien des espèces moins compétitives ce qui se traduira par un maintien de la diversité locale. C'est ce type de situation qui a été observée par Ayling (1981) au sein de communautés subtidales en Nouvelle-Zélande. Il constate qu'une infection microbienne engendre une diminution de la couverture de certaines éponges et permet ainsi à plusieurs autres organismes benthiques de coloniser les zones mises à nu.

A l'inverse, la diversité spécifique d'un écosystème peut aussi diminuer suite à la présence d'un parasite, notamment dans le cas des relations prédateurs-proies. Par exemple, si un parasite virulent décime des populations d'un prédateur, on peut assister à une explosion démographique des proies ce qui peut avoir un impact négatif sur la biodiversité (Thomas et al. 2007). De même, la disparition d'un parasite peut déstabiliser un écosystème en affectant la densité de population de ses hôtes. C'est le cas de la peste bovine (virus) au sein du Parc Serengeti (Tanzanie). Durant les années 1950, le développement d'un vaccin éradiqua la maladie de la zone ce qui fut associé à une augmentation gigantesque de la taille des populations de gnous. Cette augmentation entraîna une croissance des populations de leurs prédateurs, une diminution des populations d'antilopes ainsi qu'une modification du couvert végétal. On remarque donc qu'un processus en cascade a eu lieu à partir de l'éradication du virus (Thomas et al. 2007).

Ingénieurs des écosystèmes. Les parasites peuvent être des ingénieurs des écosystèmes c'est à dire des organismes qui *« modulent directement ou indirectement la disponibilité des ressources pour les autres espèces en causant des changements physiques du matériel biotique ou abiotique »* (Jones et al. 1994). En modifiant son hôte (phénotype étendu), un parasite peut influencer la disponibilité des niches écologiques offertes par l'hôte. Il existe plusieurs exemples illustrant ces effets dont celui des sacculines. Ces cirripèdes parasites de crabes inhibent la mue de leurs hôtes dont la carapace reste alors accessible plus longtemps pour les épibiontes (Phillips & Cannon 1978 ; Thomas et al. 2007).

3. Implications évolutives

L'interaction parasitaire permet à deux espèces plus ou moins éloignées phylogénétiquement, de « partager » une partie de leur évolution ; ces espèces, l'hôte et le parasite, font donc 'un bout de chemin ensemble' en interagissant (Combes 2001) (Fig. 1).

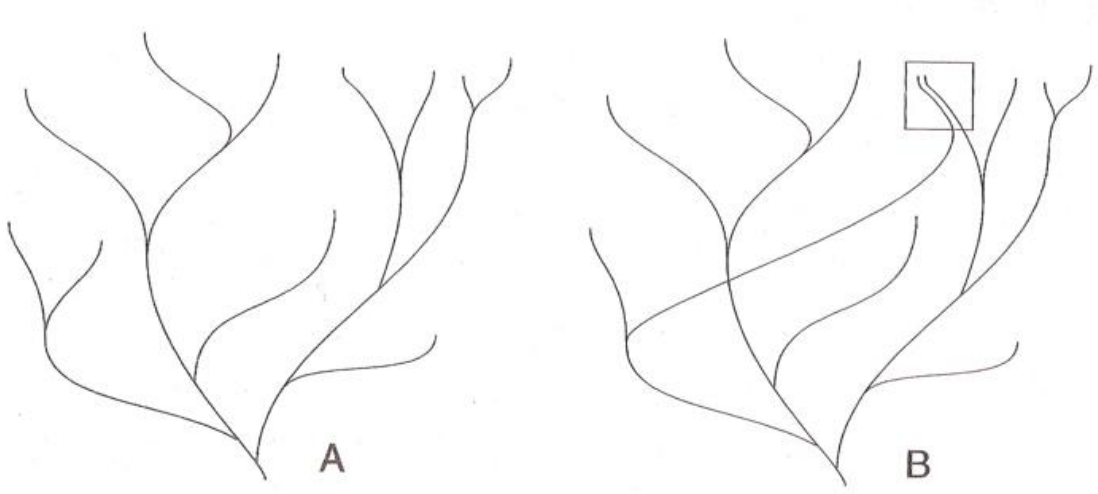


Fig. 1. Schéma d'arbres phylogénétiques fictifs. Dans l'arbre A, aucune association ne rapproche des branches séparées. Dans l'arbre B, suite à une association du vivant, une branche de la lignée de gauche partage un bout d'histoire évolutive avec une branche de la lignée de droite (d'après Combes 2001)

Si l'interaction persiste, hôtes et parasites vont exercer l'un sur l'autre des pressions sélectives réciproques. Ce processus, pouvant s'étendre sur de longues durées, est communément appelé « course aux armements » (Combes 2001). Le parasite développera des adaptations lui permettant de rencontrer son hôte puis de s'y maintenir. L'hôte présentera, en réponse, des adaptations limitant la rencontre avec le parasite et d'autres lui permettant de s'en débarrasser le cas échéant.

On notera une fréquente asymétrie au niveau de la relation hôte-parasite. En effet, le parasite est généralement associé à un nombre limité d'hôtes alors que ce dernier est souvent confronté à de nombreux et divers parasites (Combes 2001 ; Thomas et al. 2007). En général, les processus de défense développés par l'hôte devront donc agir sur un large spectre d''agresseurs', alors que les adaptations du parasite concerneront un set retreint d'hôtes.

Cette course aux armements peut être symbolisée par deux filtres (comparables à des diaphragmes): un filtre de rencontre et un de filtre compatibilité (Fig. 2) (Combes 2001). Via leurs adaptations respectives, les hôtes et les parasites tentent, respectivement, de fermer ou d'ouvrir ces filtres. Pour que le parasitisme s'installe, il faut que les deux filtres soient (et restent) ouverts.

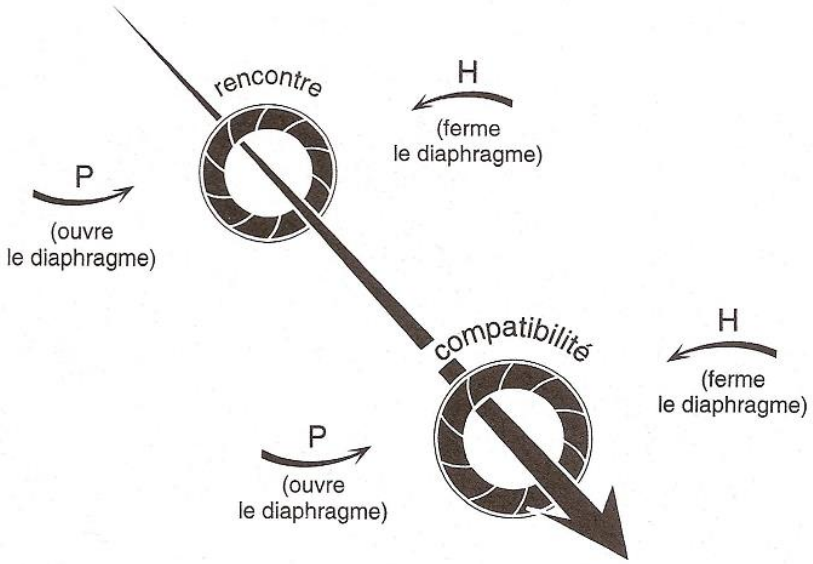


Fig. 2. Filtres (diaphragmes) de rencontre et de compatibilité dans un système hôte (H) – parasite (P) (Combes 2001)

Au fil des générations, on peut assister à une coévolution des deux partenaires symbiotiques durant laquelle ils acquièrent de nouvelles adaptations qui leur permettent de ne pas être distancés par l'autre (Combes 2001). Plus les adaptations du parasite portent préjudice à l'hôte, plus les pressions de sélection qu'il exerce sur celui-ci sont importantes. La sélection d'adaptations « antiparasites » va donc être favorisée chez l'hôte et elles-mêmes vont entraîner l'adaptation du parasite et ainsi de suite. Ce jeu des adaptations qui se répondent au cours de l'évolution permet le maintien de la symbiose et correspond au concept de la « Reine rouge » (« Red Queen hypothesis ») : les adaptations réciproques des espèces se succèdent (leur environnement sélectif

change donc constamment) ce qui entraîne le maintien de leur interaction/association (Van Valen 1973).

4. Traits généraux des populations

Une population de parasites peut être définie comme *« l'ensemble des parasites adultes associés à tous les individus d'une population d'hôte (ou aux populations d'espèces hôtes sympatriques) »* (Fig. 3). Deux caractéristiques importantes modulent les populations de parasites : les discontinuités spatiale et temporelle de leur habitat, c'est-à-dire de leurs hôtes (Poulin 2007). Les hôtes forment, en effet, des habitats fragmentés, comparables à des « îles au milieu d'un océan inhospitalier » (Combes 2001) (Fig. 3).

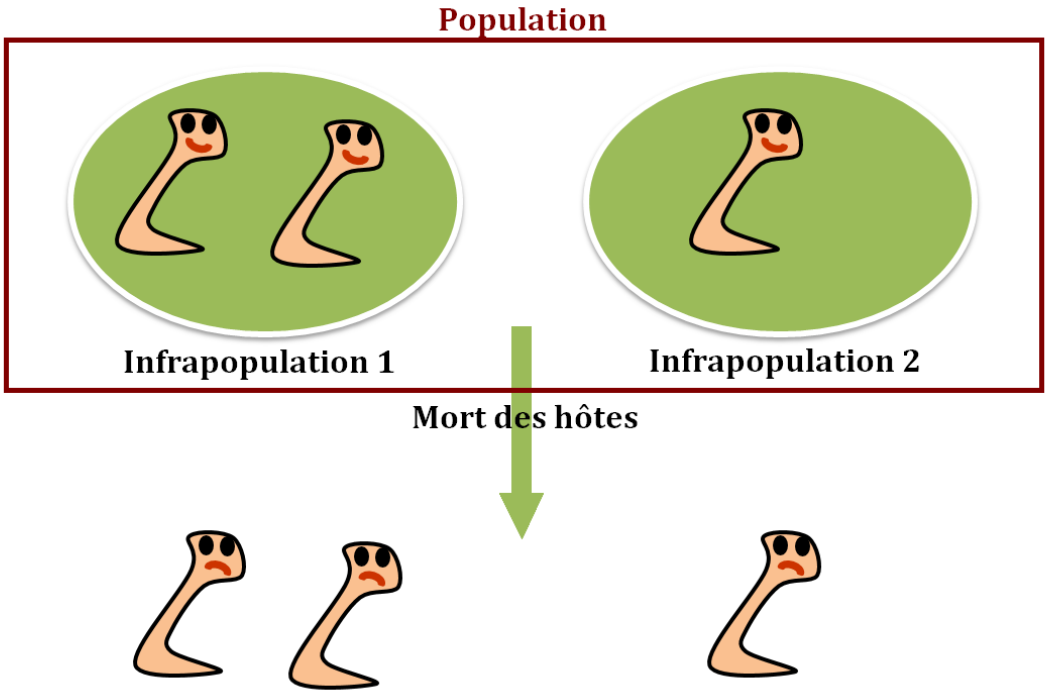


Fig. 3. Illustration de la fragmentation spatiale et temporelle de l'habitat d'un parasite. A la mort de l'hôte, chaque infrapopulation se retrouve sans habitat adéquat (sans hôte). Les hôtes sont figurés par les ellipses vertes, les parasites par les "vers" orangés

Une population de parasite est donc subdivisée en autant d'infrapopulations qu'il y a d'hôtes infestés. Les infrapopulations sont donc, elles-mêmes, éphémères et leur persistance ne dépasse pas la durée de vie de l'hôte (Fig. 3).

Les individus issus d'infrapopulations distinctes peuvent se mélanger et entraîner un brassage génétique au sein de la population (ex : déplacement de parasites adultes d'un hôte à l'autre, stades larvaires libres) (Poulin 2007).

Notons que la fécondité et la capacité de dispersion des parasites engendrent, selon les groupes, une grande variabilité de la différenciation génétique entre infrapopulations. Par exemple, cette différenciation semble improbable pour les taxons marins à larves pélagiques. Elle est par contre plus évidente dans le cas où la dispersion des parasites est intiment liée au déplacement de leurs hôtes. Ici, le comportement de l'hôte va jouer un rôle prépondérant dans la variabilité génétique observée (Poulin 2007). Paterson et ses collaborateurs (2000), en étudiant des nématodes parasites de rats, soulignent l'importance de la mobilité de ces hôtes dans l'homogénéisation génétique des infrapopulations de leurs parasites.

A une plus large échelle, les populations de parasites (et de leur hôtes) peuvent être plus ou moins connectées (Poulin 2007). Généralement, celles-ci ne constituent cependant pas des ensembles homogènes et continus sur leurs aires de distribution. Ces populations subiront donc des pressions évolutives différentes, ce qui peut entraîner de l'adaptation locale (Thomas et al. 2007). L'adaptation locale correspond à la situation où *« des génotypes locaux ont un plus grand succès reproducteur que des génotypes étrangers au sein d'un habitat local »* (Kawecki & Ebert 2004). Elle joue un rôle prépondérant dans la formation et le maintien des espèces (Via 2001) mais aussi dans la distribution, l'abondance et le rôle écologique des organismes. Enfin, elle aide à maintenir la variabilité génétique et peut servir à prédire la réponse des populations à une perturbation anthropique (Sotka 2005).

5. Flux de gènes et adaptation locale

Lorsqu'il y a interaction parasitaire, le potentiel évolutif (et donc la potentialité d'adaptation locale) de chaque symbiote dépend de trois paramètres principaux : (1) le taux de mutations, (2) le taux de migration et (3) la recombinaison génétique (Gandon & Michalakis 2002). Il est habituellement admis que les parasites ont un plus grand potentiel évolutif que leurs hôtes car ils possèdent généralement une plus grande taille efficace de population, un temps de génération plus court ainsi que des taux de mutations et de migration plus élevés (Gandon & Michalakis 2002). Cependant, certaines études (ex : Dufva 1996 ; Oppliger et al. 1999 ; Reichard et al. 2011) ont détecté une absence d'adaptation locale chez des parasites voire même de la «maladaptation» (parasites allopatriques mieux adaptés à un hôte local que les parasites sympatriques).

Ces études indiquent que ce n'est pas forcément le parasite qui est en avance dans la course aux armements (Gandon & Michalakis 2002).

L'importance du taux de migration (et donc du flux de gènes, Hellberg 2009) dans l'adaptation locale a fait l'objet de plusieurs travaux ces deux dernières décennies (Gandon et al. 1996 ; Gandon & Michalakis 2002 ; Kawecki & Ebert 2004 ; Sotka 2005 ; Greischar et Koskella 2007). Kawecki et Ebert (2004) puis Sotka (2005) soulignent que si le flux de gènes est faible (faible dispersion ou choix actif de l'habitat), l'adaptation locale sera favorisée. Par contre, si le flux de gènes est important, des génotypes à faible succès reproducteur vont recruter à chaque génération ; une sélection purificatrice (« élimination des allèles délétères ») est alors nécessaire pour permettre de l'adaptation locale (Sotka 2005).

Les modèles classiques indiquent que de forts flux de gènes vont homogénéiser les populations, contraignant ainsi l'adaptation locale (Storfer 1999). En effet, au-delà d'un certain seuil, il est impossible (par sélection naturelle) de prévenir un phénomène de « gene swamping » (envahissement de gènes), empêchant ainsi l'adaptation locale (Lenormand 2002; Blanquart et al. 2013). Cependant, un flux de gènes intermédiaire (au sein d'un modèle théorique) peut favoriser l'adaptation locale dans un milieu changeant ce qui est typiquement le cas des relations hôte-parasite (Gandon 2002; Blanquart et al. 2013). De plus, il a aussi été proposé que lorsque le flux de gènes du parasite est supérieur à celui de l'hôte, l'adaptation locale du parasite est plus importante. Ceci peut s'expliquer par une augmentation de la variabilité génétique dans les populations de parasites et donc d'une efficacité plus importante de la sélection (Gandon et al. 1996 ; Gandon & Michalakis 2002). Greischar et Koskella (2007) ont testé cette dernière prédiction dans une synthèse portant sur 54 études tant en milieu terrestre qu'aquatique. Les résultats confirment que les parasites ayant un flux de gènes plus important que celui de leurs hôtes montrent significativement plus d'adaptation locale. Les autres facteurs considérés (temps de génération, degré de virulence) n'ont que peu ou pas d'effet sur l'adaptation locale.

Pour la même raison que l'on associe un haut flux de gènes à une absence d'adaptation locale, on a longtemps cru que celle-ci était rare en milieu marin et que les organismes tendaient plutôt à présenter des phénotypes plastiques (Agrawal 2001 ; Sotka 2005). Cependant, plusieurs études ont montré que l'adaptation locale en milieu marin était

plus fréquente qu'attendue (Sotka 2005). De plus, elle a également été caractérisée chez des espèces possédant un stade larvaire pélagique (ex : le crabe *Pinnotheres novaezelandiae*, le ver polychète *Spirorbis borealis*) soutenant l'hypothèse qu'un fort flux de gènes n'empêche pas forcément l'apparition d'une adaptation locale. Ce flux de gènes va directement déterminer la structure génétique des populations du parasite et de celle de l'hôte. Caractériser et comparer ces structures génétiques est donc un prérequis indispensable afin de prédire une adaptation locale mais aussi l'échelle à laquelle l'interaction parasitaire peut évoluer (McCoy et al. 2005 ; Poulin 2007).

6. Comparaisons des structures génétiques hôtes-parasites

Comparer la structure génétique des populations de parasites avec celles de leurs hôtes est pertinente, notamment pour élucider les facteurs influençant la dispersion passée et présente (Criscione 2008). Les études menées sur ce sujet ont d'ailleurs montré tant de la congruence (ex : Mulvey et al. 1991 ; Criscione & Blouin 2007) que de l'incongruence (ex : Delmotte et al. 1999 ; McCoy et al. 2005).

Plusieurs paramètres vont théoriquement favoriser des structures génétiques communes. Il s'agit notamment d'une haute spécificité. En effet, la plupart des cas de congruence ont été observés dans des interactions 'hôte-parasite' hautement spécifiques, les parasites généralistes tendant à évoluer plus indépendamment de leurs hôtes (Froeschke & von der Heyden 2014).

Les autres paramètres favorisant une congruence sont : l'absence d'hôte intermédiaire, l'absence de stade libre, la transmission verticale, des facultés de dispersions proches, des tailles efficaces de populations comparables ou encore des taux de mutation voisins (Nieberding & Olivieri 2007). Cependant, on retiendra que ces paramètres influenceront différemment les marqueurs moléculaires utilisés. Le choix de ceux-ci est donc primordial pour cibler une échelle géographique et temporelle précise (Froeschke & von der Heyden 2014). Par exemple, si l'on désire mettre en évidence un cadre historique, on utilisera préférentiellement des marqueurs à séquences (souvent mitochondriaux) caractérisés par un taux de mutation faible à moyen. Au contraire, si on se concentre sur la structure génétique contemporaine, on utilisera plutôt des marqueurs à taux de mutations élevés comme les microsatellites (Hellberg 2009 ; Fratini et al. 2011). Des patrons ('patterns') peuvent être congruents à un moment donné mais incongruents à

un autre, si, par exemple, le parasite offre une subdivision plus fine que l'hôte (Nieberding & Olivieri 2007). Le nombre d'études sur la comparaison des structures génétiques hôte-parasite a considérablement augmenté durant les dernières décennies (Nieberding & Olivieri 2007 ; Criscione 2008), les recherches en milieu marin restant toutefois minoritaires (Kochzius et al. 2008 ; Froeschke & von der Heyden 2014).

Congruence des structures génétiques. Des 'patterns' congruents suggèrent que les mêmes facteurs ont façonné l'histoire des populations d'hôtes et de parasites (Criscione 2008 ; Hellberg 2009). Par exemple, des changements du niveau marin liés aux régressions et transgressions marines successives entraînent de la congruence lors de l'observation des structures phylogéographiques. C'est le cas de deux étoiles de mer (*Protoreaster nodosus*, *Linckia laevigata*) et de leur symbiotes (*Periclimenes soror*, *Thyca crystallina*) dont l'expansion géographique a été simultanée dans le Pacifique ('Triangle du corail') et liée aux fluctuations eustatiques lors du Pléistocène (Crandall et al. 2008).

Une congruence permet aussi un calibrage de l'horloge moléculaire pour les parasites. Ceci est particulièrement utile car les représentants fossiles des parasites sont rarement découverts (Criscione 2008).

Enfin, si un hôte est soumis à un programme de conservation, les données génétiques d'un parasite peuvent conforter les limites géographiques de conservation (ESU, « Evolutionarily Significant Unit ») préalablement établies. Ainsi, une congruence peut constituer un argument supplémentaire renforcant la validité biologique de ces limites (Criscione & Blouin 2007).

Incongruence des structures génétiques. La présence de 'patterns' contrastés est également informative. Elle peut être liée à des traits différents d'histoire de vie. Par exemple, une période de reproduction étendue, associée à une larve à haut potentiel de dispersion, expliqueraient l'incongruence entre le gastéropode parasite *Thyca crystallina* et l'étoile de mer hôte *Linckia laevigata* dans l'archipel indo-malais (Kochzius et al. 2009).

Ensuite, une incongruence peut aussi permettre d'utiliser le parasite comme proxy (« marqueur moléculaire ») pour l'hôte, ceci tant à fine échelle qu'à échelle phylogéographique. A une échelle locale, lorsque la dispersion du parasite est uniquement liée au mouvement de l'hôte, la structure génétique du parasite peut

informer sur l'activité de l'hôte durant une partie de son cycle vital. C'est le cas des tiques, où l'étude de la structure génétique (microsatellites) a révélé les comportements de recherche de colonies chez la mouette tridactyle (McCoy et al. 2005). Par ailleurs, une caractéristique phylogéographique non détectée chez l'hôte peut aussi être mise en évidence via l'étude de son « parasite-proxy » (Froeschke & von der Heyden, 2014). La variabilité génétique du parasite peut donc révéler l'histoire évolutive de l'hôte avant que l'ADN de celui-ci ne le puisse. Ainsi, si la structuration spatiale des populations d'un parasite est plus fine, on peut potentiellement utiliser ses génotypes pour assigner les hôtes à une population d'origine et ce, avec une plus grande précision que si on utilisait uniquement les génotypes de l'hôte (Froeschke & von der Heyden, 2014). Cependant, pour qu'un parasite serve de proxy phylogéographique, il doit au moins partager une partie de l'histoire généalogique de son hôte et lui être assez spécifique (Nieberding & Olivieri 2007).

Un très bel exemple d'utilisation d'un parasite comme proxy est celui d'un nématode du mulot en Europe de l'Ouest (Fig. 4) (Nieberding & Olivieri 2007). L'arbre phylogénétique de l'hôte (cytochrome b) montre deux régions géographiques distinctes. C'est aussi le cas du parasite mais celui-ci présente également des sous-groupes au sein de chaque grande lignée. Cette subdivison met en évidence certains évenements historiques comme une fragmentation ancienne des populations dans le nord de l'Italie (Fig. 4).

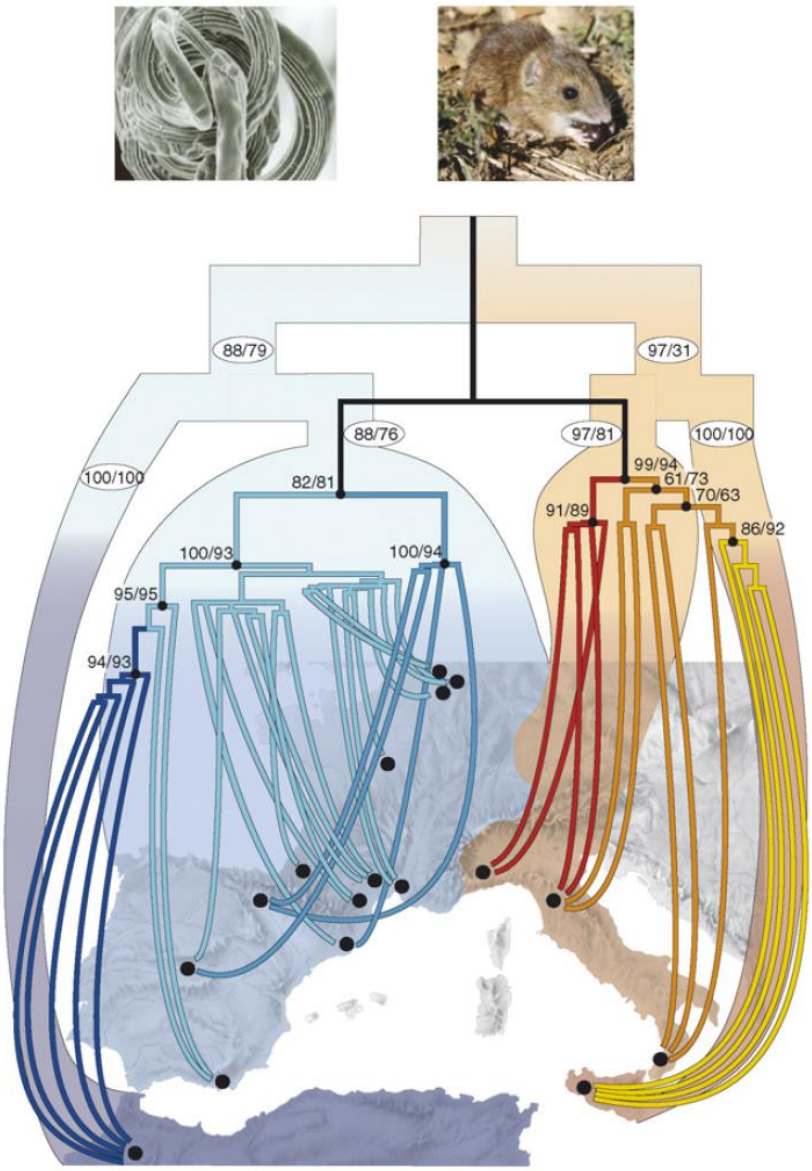


Fig. 4. Arbres phylogénétiques (cytochrome b) du mulot (branches larges) et du nématode parasite *Heligmosomoides polygyrus* (branches fines). L'arbre de l'hôte indique deux grandes lignées (Belgique-France-Espagne-Afrique du Nord vs Italie-Sicile), chacune subdivisée en deux sous-lignées, tandis que celui du parasite indique des subdivisions plus fines au sein de chacune d'entre elles, très nettes en France et dans le Nord de l'Italie (Nieberding & Olivieri 2007)

B. Le couple symbiotique *Meoma ventricosa – Dissodactylus primitivus*

1. L'échinide hôte *Meoma ventricosa*

Meoma ventricosa (Lamarck, 1816) est un échinide Euechinoidea, appartenant à l'ordre des Spatangoida (spatangoïdes ou spatangues) et à la famille des Brissidae (Fig. 5) (Kroh 2014). Le genre *Meoma* compte actuellement quatre espèces (ou sous-espèces) à savoir *M. ventricosa ventricosa* (Caraïbes et côtes américaines de l'Atlantique), *M. ventricosa grandis* (côtes américaines du Pacifique), *M. cadenati* (Afrique de l'Ouest) et *M. frangibilis* (eaux profondes des Caraïbes) (Schultz 2009).

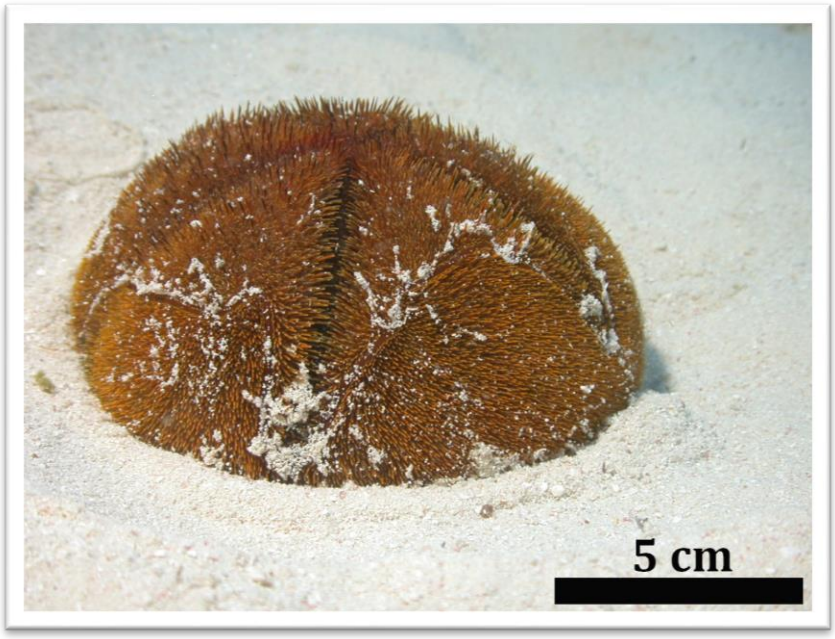


Fig. 5. Individu de *M. ventricosa* en cours d'enfouissement (Photo : P. Willenz)

Contrairement aux oursins « réguliers » à symétrie radiaire, les oursins irréguliers, comme les spatangues, ont acquis secondairement une symétrie bilatérale et un sens unidirectionnel de déplacement. Inféodés aux substrats meubles (sables, boues), ils sont fouisseurs ou laboureurs. Les spatangues sont psammivores et se nourrissent de la fraction organique associée aux sédiments ingérés (Fig. 6) (De Ridder 1986 ; De Ridder et al. 1985 ; De Bruyn 2010).

M. ventricosa est une espèce de grande dimension dont la longueur atteint 20 cm. Il est endémique des Caraïbes et des côtes américaines voisines (Fig. 7). Ses limites de distribution correspondent, au nord, aux côtes de la Caroline du Nord et, au sud, à celles du Brésil (Fig. 7). L'espèce est notamment présente dans les Petites Antilles, les Grandes

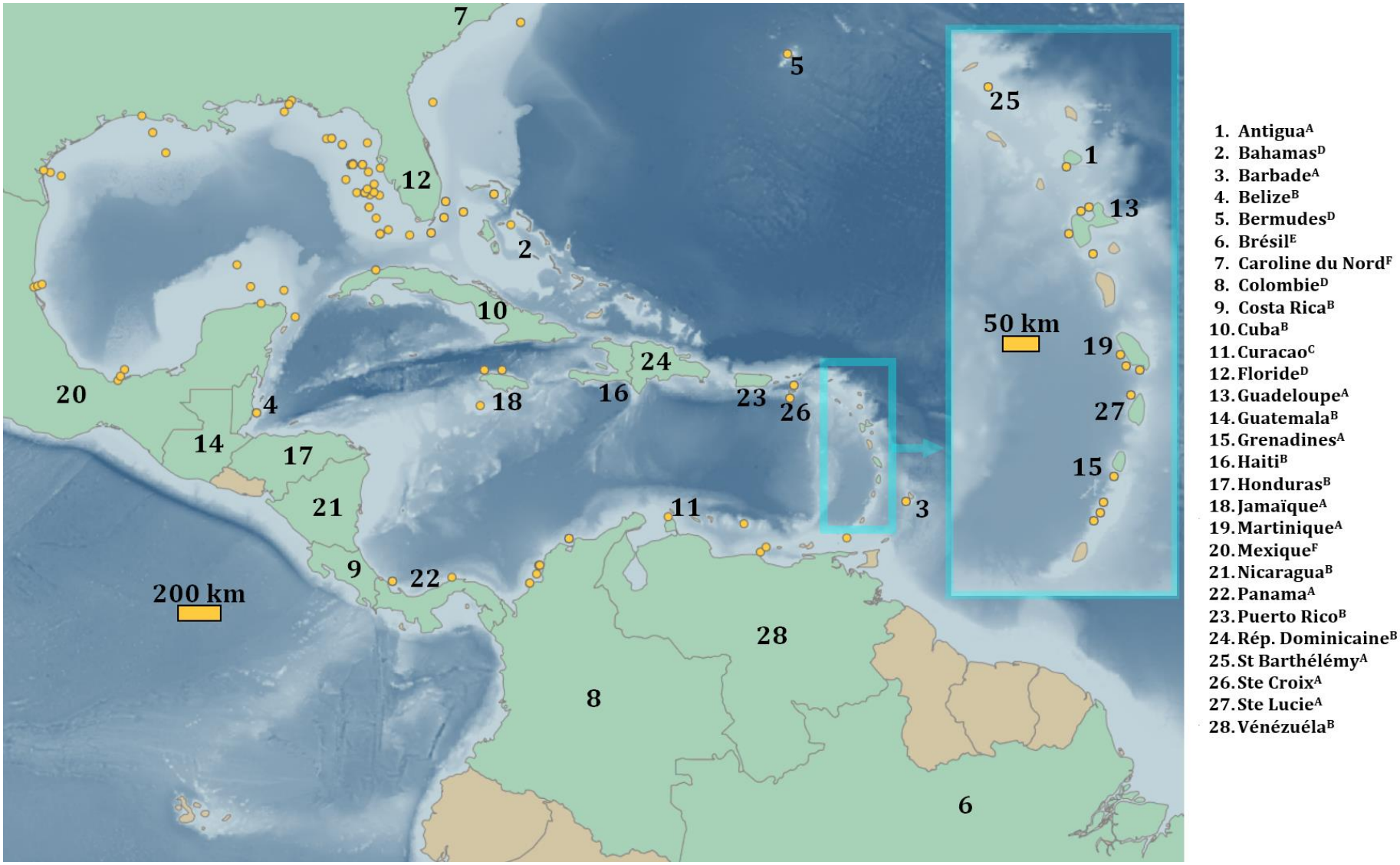


Fig. 7. Carte de distribution (OBIS, modifiée) de *Meoma ventricosa*. En vert, les états pour lesquels *M. ventricosa* a été répertorié le long des côtes. Les points jaunes indiquent des sites de recensement précis. La liste à droite reprend l'ensemble des états recensés sur la carte (A : observations personnelles ; B : Alvarado 2011 ; C : Nagelkerken et al. 1999 ; D : Chesher 1969 ; E : Wirtz et al. 2009 ; F : OBIS 2014)

Antilles, les Bahamas, le Golfe du Mexique, ainsi que dans les parties ouest et sud-ouest de la mer des Caraïbes (ex : Honduras, Panama, Colombie) (Fig. 7) (Chesher 1969 ; Hendler et al. 1995 ; Alvarado 2011 ; OBIS 2014).

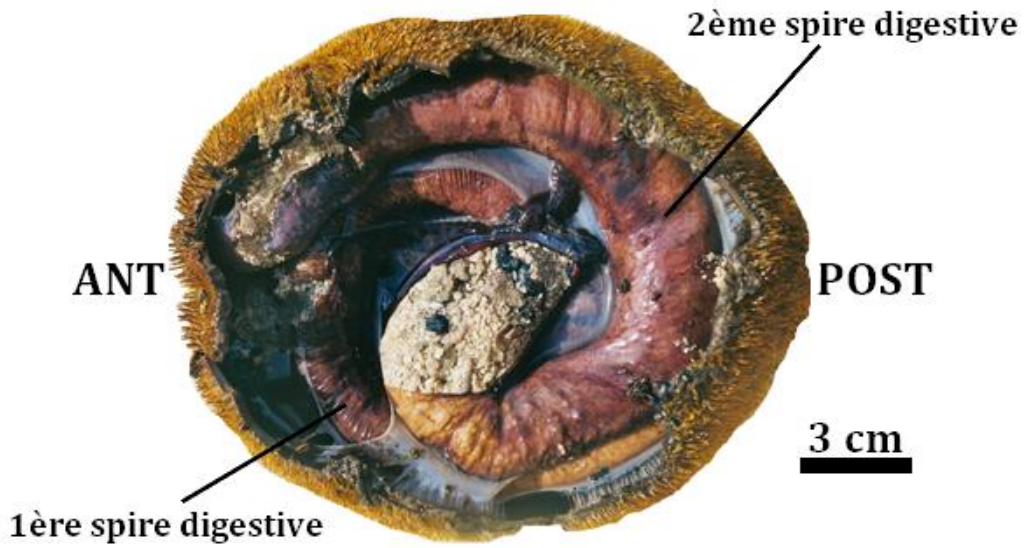


Fig. 6. *Meoma ventricosa*. Vue aborale du tube digestif. L'intestin postérieur est ouvert et les sédiments ingérés visibles (Photo : C. De Ridder)

M. ventricosa vit généralement dans des sédiments peu profonds, aux marges des récifs coralliens ou des herbiers, mais il peut être présent jusqu'à 200 mètres de profondeur (Hendler et al. 1995). Il colonise des sédiments de granulométrie variée allant de vases plus ou moins fines à des sables fins à grossiers (Chesher 1969). Cet oursin possède un cycle nycthéméral bien marqué: durant le jour, il est enfoui et relativement peu mobile ; au coucher du soleil et toute la nuit, il émerge des sédiments et se déplace en surface (Hammond 1982 ; Hendler et al. 1995). Chesher (1969) a estimé que *M. ventricosa* avait un rôle clé dans la perturbation du sédiment et donc sur la productivité des zones qu'il colonise. Au cours de son émergence nocturne, l'oursin se nourrit activement et ingère des sédiments superficiels riches en matière organique (Hammond 1982). Son enfouissement diurne lui permettrait d'éviter ses prédateurs parmi lesquels : la tortue caouanne, des raies, d'autres poissons ou encore l'étoile de mer *Oreaster reticulatus* (Chesher 1969 ; Hendler et al. 1995).

M. ventricosa est l'hôte de plusieurs espèces de symbiotes : le polychète *Ophiodromus obscurus* (Werding & Sanchez 1989), un bivalve du genre *Neaeromya* et le crabe

Dissodactylus primitivus. Des mortalités massives dues à une infection bactérienne ont été observées localement, notamment à Curaçao (Nagelkerken et al. 1999).

Comme la majorité des échinodermes (Jangoux & Lawrence 1982), *M. ventricosa* est gonochorique. Son développement est indirect : les œufs et le sperme sont émis dans la colonne d'eau où a lieu la fécondation (Ruppert et al. 2004). Le passage de la blastula au premier stade larvaire échinopluteus prend environ vingt heures chez la sous-espèce pacifique *Meoma ventricosa grandis* (Mortensen 1921). Cette information n'est pas connue pour la sous-espèce *M. ventricosa ventricosa*. On sait que cette larve est de type planctotrophe mais sa durée de vie n'a cependant jamais été évaluée (Emlet et al. 1997). La fécondité de l'espèce reste, elle aussi, inconnue (Chesher 1969).

2. Le crabe parasite *Dissodactylus primitivus*

Dissodactylus primitivus (Bouvier 1917) est un brachyoure appartenant à la famille des Pinnotheridae (Fig. 8).

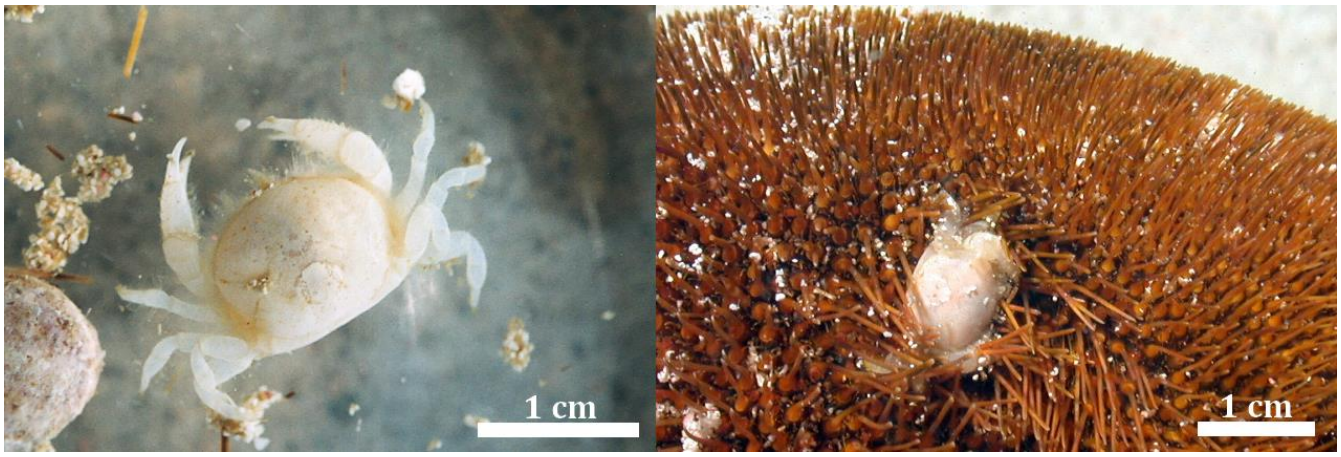


Fig. 8. Le crabe *Dissodactylus primitivus* isolé au laboratoire (à gauche) et sur son hôte (à droite) (Photos : C. De Ridder)

Les Brachyoures sont caractérisés par un céphalothorax bien développé dont la première paire de pattes marcheuses (péréiopodes) est transformée en pinces. L'abdomen est réduit et rabattu sous le céphalothorax (Tixier & Gaillard 1969). Les crabes Pinnotheridae sont caractérisés par un céphalothorax arrondi et de petite taille (<1cm), ce qui leur vaut le nom vernaculaire de 'crabes petits pois' (« pea-crab » en anglais) (Ruppert et al. 2004).

La famille des Pinnotheridae est exclusivement symbiotique et compterait de 287 à 316 espèces (Palacios-Theil et al. 2009). Les symbioses développées par ces crabes au cours de leur évolution sont diverses et correspondent aussi bien à des cas de mutualisme que de parasitisme (Takeda et al. 1997 ; De Bruyn et al. 2009). Le spectre d'hôtes associés est également varié (ex : mollusques, échinodermes, arthropodes, annélides) (De Bruyn 2010). C'est un groupe polyphylétique ayant une distribution cosmopolite, régions polaires exceptées (De Bruyn 2010). Sous nos latitudes, le membre le plus connu de cette famille est *Pinnotheres pisum* qui est associé à plusieurs espèces de bivalves dont la moule *Mytilus edulis* (Houghton 1963).

D. primitivus fait partie du complexe *Dissodactylus* qui regroupe 13 espèces réparties en deux genres (*Dissodactylus*, *Clypeasterophilus*) distribuées dans l'Atlantique Ouest et dans le Pacifique Est (Griffith 1987). Une étude phylogénique récente (Palacios-Theil et al. 2009) a démontré que ces deux genres étaient polyphylétiques. Tous les crabes du complexe sont associés à des échinides clypéastéroïdes de deux familles (Mellitidae et Clypeasteridae). Seul *D. primitivus* est associé à un autre ordre d'échinides, les spatangoïdes, et plus particulièrement à deux espèces de la famille des Brissidae, *Meoma ventricosa* et *Plagiobrissus grandis* (Griffith 1987). La distribution géographique de *D. primitivus* est probablement semblable à celle de ses hôtes (Fig. 7) (Griffith 1987, De Bruyn 2010).

La symbiose (*M. ventricosa* / *P. grandis* - *D. primitivus*) est considérée comme du parasitisme (ectoparasitisme) depuis l'étude de Telford (1982) constatant que le contenu digestif du crabe est composé à 60% des tissus de son hôte. Le crabe sectionne les piquants de son hôte et ingère ces piquants et le tégument. Des travaux plus récents (De Bruyn et al. 2009) ont entériné cette relation parasitaire en mesurant une diminution de l'index gonadique de *M. ventricosa* en fonction de l'intensité des lésions créées par le crabe (Fig. 9).

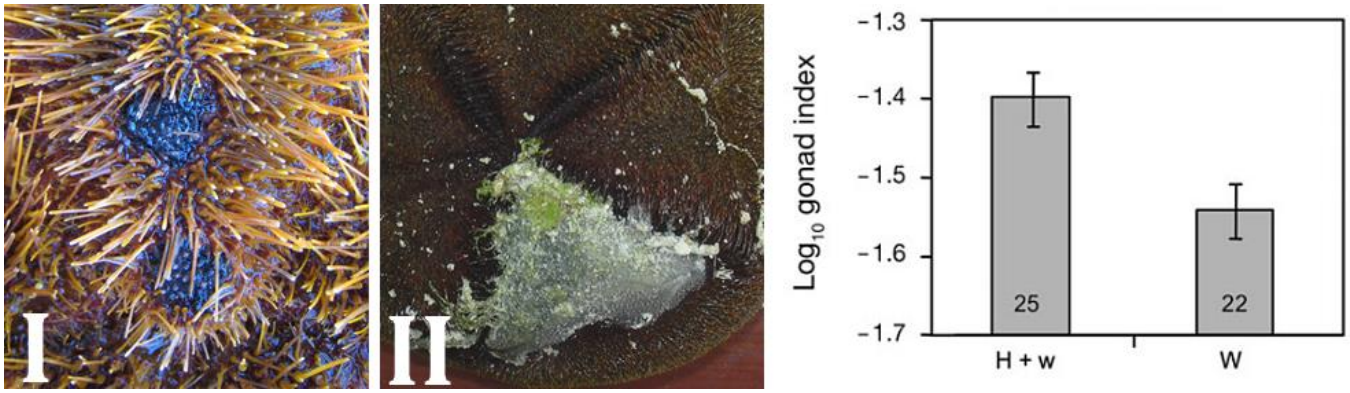


Fig. 9. Lésions sur *M. ventricosa* dues à *D. primitivus*. En (I) des piquants sont sectionnés tandis qu'en (II), le test est partiellement dénudé. Le graphique de droite indique le Log_{10} (Volume des gonades/Volume du corps) pour des oursins présentant pas (ou peu) de lésions (H+w ; cf. photo I) et ceux présentant des tests fortement lésés ou en cours de régénération (W ; cf. photo II) (De Bruyn et al. 2009)

La biologie de la reproduction de l'espèce a été examinée par plusieurs auteurs (Telford 1978 ; Pohle & Telford 1983 ; De Bruyn et al. 2009). Via des mesures démographiques et en aquariums, De Bruyn et al. (2009) ont suggéré que le système de reproduction de *D. primitivus* correspondrait à un des systèmes prédits par le modèle théorique de Baeza & Thiel (2007). Il s'agit de la « polygynandrie à femelles mobiles » qui se caractérise par un accouplement multiple des mâles et des femelles ainsi que d'un déplacement des deux sexes entre individus hôtes lors de la recherche de partenaires sexuels.

Une fois l'accouplement réalisé, la femelle porte les œufs sous son abdomen (moyenne de 274 ; Telford 1978). La fécondité des femelles gravides varie selon l'espèce d'hôte occupée : elle est plus importante sur *P. grandis* que sur *M. ventricosa* (De Bruyn et al. 2010). Le temps d'incubation des œufs est d'environ trois semaines (Pohle & Telford 1983) après quoi l'éclosion libère une larve pélagique planctotrophe de type zoé. Après le 3ème stade zoé, la larve se métamorphose en un autre type de larve pélagique (mégalope) qui effectuera le recrutement sur l'hôte (Fig. 10). La durée de vie larvaire totale (PLD, « pelagic larval duration ») a été évaluée à 15 jours (Pohle & Telford 1983). De Bruyn et al. (2010) ont montré que *D. primitivus* exploite ses hôtes de manière asymétrique. En effet, bien que la reproduction ait lieu sur les deux hôtes, il n'y a pas de recrutement sur *P. grandis* (De Bruyn et al. 2010) (Fig. 11).

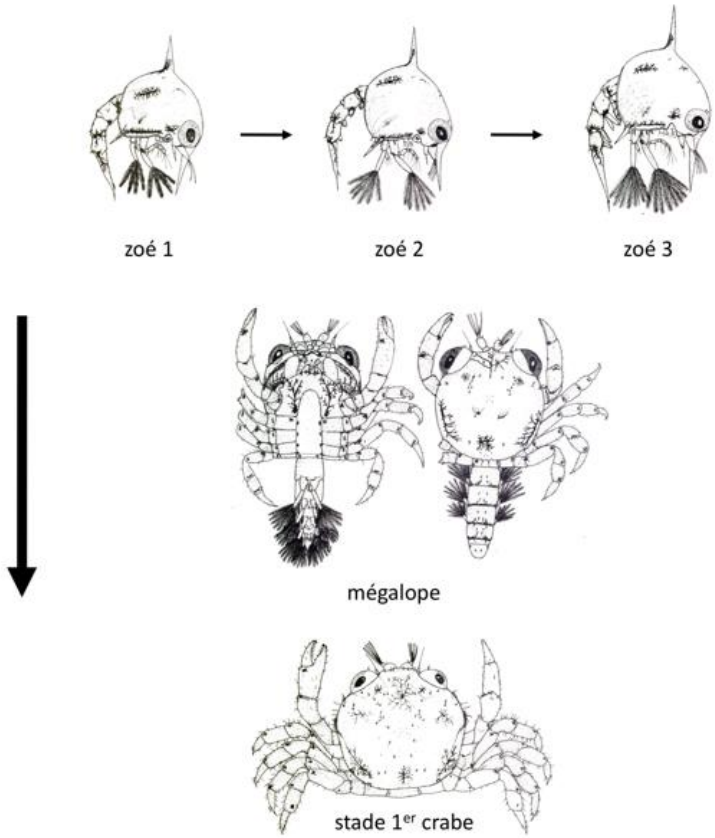


Fig. 10. Stades larvaires successifs de *D. primitivus* (De Bruyn 2010 d'après Pohle & Telford 1983)

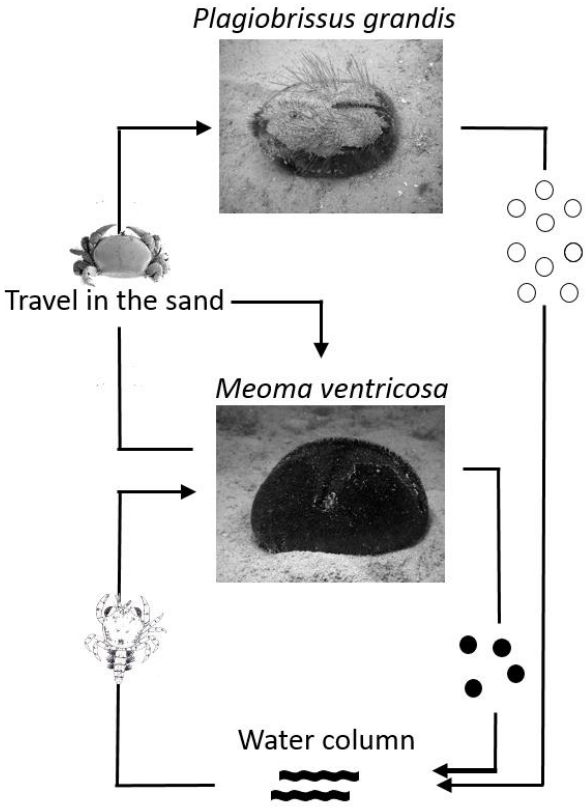


Fig. 11. Cycle vital de *D. primitivus* (De Bruyn et al. 2010). La reproduction a lieu sur les deux hôtes mais le recrutement larvaire n'aurait lieu que sur *M. ventricosa*

C. Les Caraïbes - contextes géologique et courantologique

Les Caraïbes sont une région comprenant la mer des Caraïbes, ses îles, ainsi que les côtes américaines l'encadrant et dont la superficie totale couvre environ 2,6 millions de km^2.

Au niveau tectonique, la Mer des Caraïbes se situe sur la plaque Caraïbes qui est limitée au nord et au sud par des zones de décrochement, et à l'ouest et à l'est par des zones de subduction (Germa 2008).

De manière synthétique, cinq événements géologiques majeurs ont rythmé l'émergence des différentes zones caribéennes depuis le Crétacé jusqu'au Pliocène (Germa 2008):

(I) **La formation de l'arc d'Amérique Centrale** (Costa Rica, Panama) s'est déroulée entre 85 Ma et 40 Ma suite à la subduction de la plaque tectonique Farallon (Pacifique) sous le bord occidental de la plaque Caraïbe en formation

(II) Sur l'autre côté de la plaque Caraïbe, se forment de manière concomitante **les Grandes Antilles** (ex : Cuba, Jamaïque, Haïti, Porto Rico) et **la ride Aves** (de 85 Ma jusqu'à environ 40 Ma). Ces deux entités sont intiment liées à une subduction orientée vers le Nord-Est.

(III) **L'édification des Petites Antilles** (Fig. 12) est liée à un événement de subduction plus récent (25-20 Ma), situé sur le bord oriental de la plaque Caraïbe, qui va conduire à la formation de l'arc externe des Petites Antilles (ex : Antigua, Grande Terre de Guadeloupe).

(IV) Plus récemment (moins de 6 Ma), suite à un saut de la subduction vers l'Ouest se forme l'arc interne (ex : Basse Terre de Guadeloupe, Martinique, Saba…).

(V) **La Barbade**, quant à elle, est une île correspondant à l'émersion du prisme d'accrétion lié aux arcs internes et externes des Petites Antilles. Celle-ci aurait émergé des eaux aux alentours du Pliocène (5,3Ma à 2,6 Ma). De plus, une grande partie de l'île s'est formée (roches sédimentaires) durant les derniers 700.000 ans ce qui en fait une île particulièrement récente (Donovan 2005).

Plus récemment, la région a également été influencée par les épisodes de glaciations du Quaternaire (Waelbroeck et al. 2002; Pillans & Gibbard 2012). Il a ainsi été estimé que, lors des périodes glaciaires les plus intenses, le niveau de la mer des Caraïbes était d'au moins 120 m inférieur au niveau actuel (Waelbroeck et al. 2002 ; Miller et al. 2005 ; Pillans & Gibbard 2012).

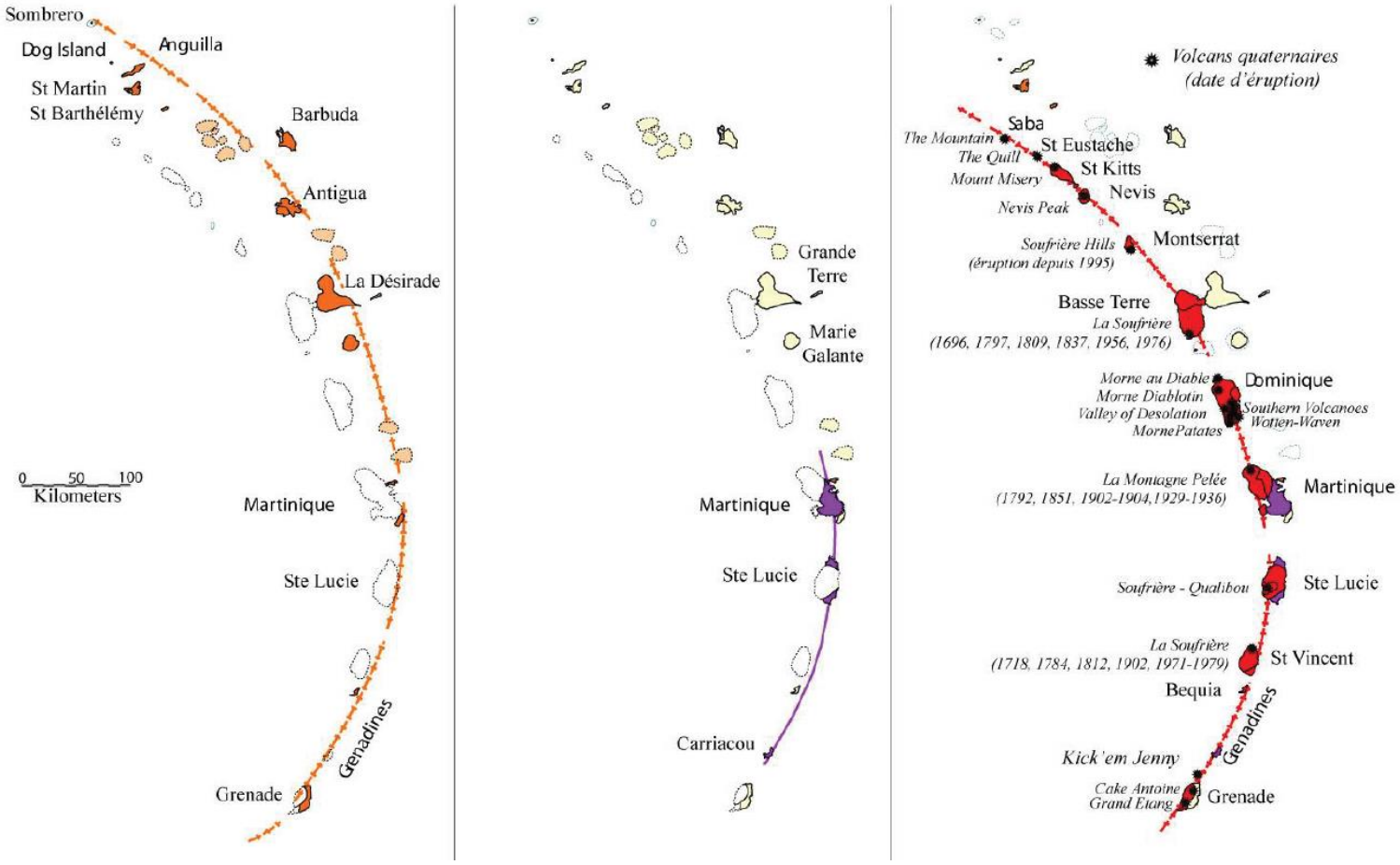


Fig. 12. Changement des Petites Antilles au fil des temps géologiques. >20 Ma (gauche), 20-6,5 Ma (centre), <6 Ma (droite) (Germa 2008)

Au niveau courantologique, les Caraïbes présentent un cas d'étude intéressant et abondamment caractérisé depuis les années 1960 (voir Molinari et al. 1981 et Gyory et al. 2013a, b). Plusieurs régimes de courants de surface sont à signaler (Fig. 13).

Le « Courant des Caraïbes » est le courant principal (Est-Ouest) de la mer des Caraïbes et débute aux Petites Antilles (Gyory et al. 2013a). Au niveau de l'Amérique centrale, ce courant rencontre une large gyre océanique allant du Panama jusqu'au Costa-Rica. Le courant des Caraïbes se poursuit jusqu'aux îles Caïmans puis rejoint le Golfe du Mexique (Lessios et al. 1984).

A ce niveau, se trouve le « Loop Current » qui est un affluant du courant de Floride (Gyory et al. 2013b). Enfin, plus à l'Est, se situe le « Courant des Antilles » qui se dirige vers le Nord-Ouest (jusqu'au Bahamas) depuis les Petites Antilles (Silberman et al. 1994) (Fig. 13).

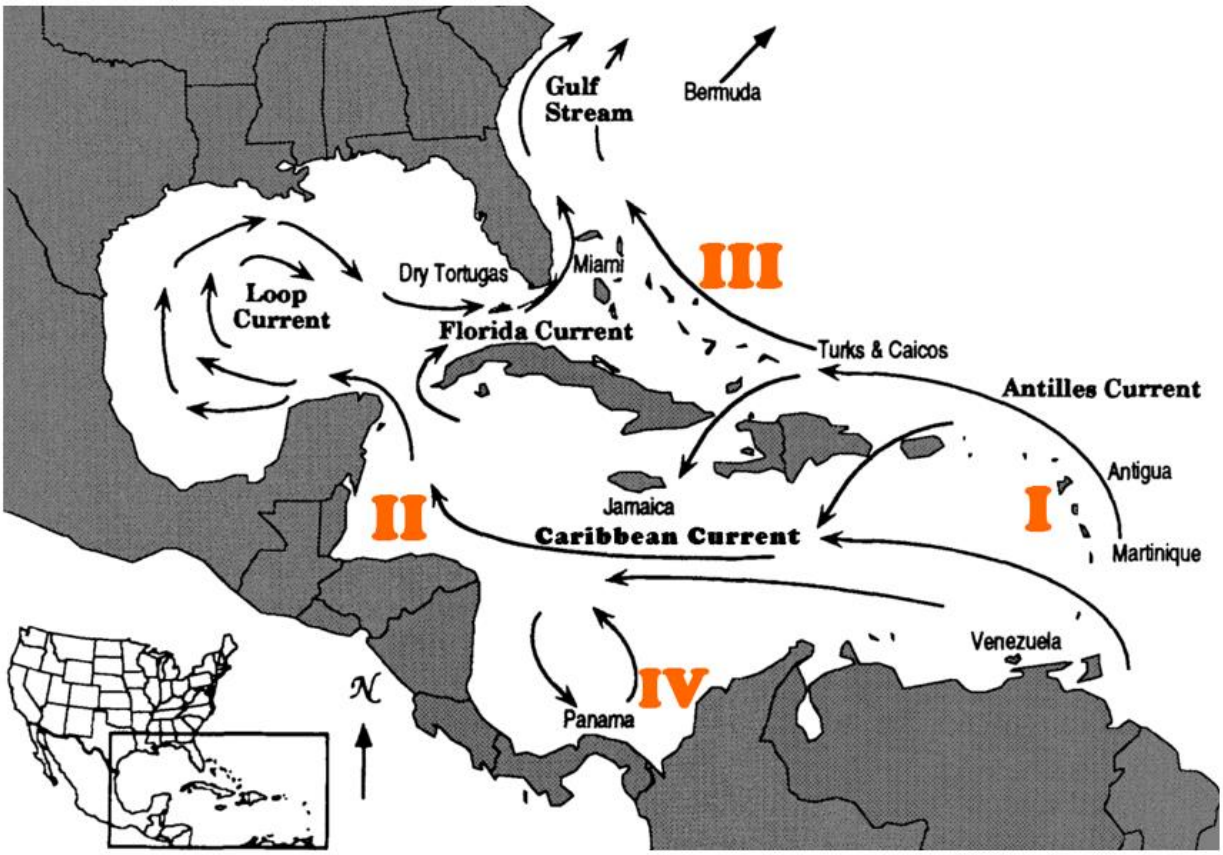


Fig. 13. Principaux courants de surface au sein des Caraïbes. Les chiffres en orange correspondent aux régions de connectivité définies par Cowen et al. (2006) (I : Est des Caraïbes ; II : Ouest des Caraïbes : III : Bahamas ; IV : Panama-Colombie ; d'après Silberman et al. 1994)

En utilisant ces courants au sein d'un modèle hydrodynamique complexe, Cowen et ses collaborateurs (2006) ont proposé quatre régions de connectivité qui correspondent à : l'Est des Caraïbes (I), l'Ouest des Caraïbes (II); les Bahamas (III) et la zone Panama–Colombie (IV) (Fig. 13).

Ce modèle prédit une dispersion importante au sein de chacune des régions mais une dispersion limitée voire absente entre celles-ci.

Plusieurs données génétiques (ex : poissons et coraux) ont conforté les régions de ce modèle. Cependant, le nombre de taxons testés reste limité et d'autres travaux ont récemment contredit les limites de certaines régions (Diáz-Ferguson et al. 2010 ; Johnston et al. 2012).

BUT DU TRAVAIL

BUT DU TRAVAIL

Les parasitoses en tant qu'associations intimes et durables entre organismes hétérospécifiques, fournissent des modèles biologiques particulièrement propices pour explorer les processus d'évolution. Les parasites sont en effet confrontés à différents environnements au cours de leur cycle vital. Ils vivent associés à un ou plusieurs hôtes et transitent éventuellement par le milieu ambiant (lorsque certains stades du cycle de vie sont libres, ou dans le cas des symbiotes facultatifs). Cette discontinuité des ressources a deux conséquences. Elle induit des stratégies d'accouplement et de reproduction qui peuvent être à la base de la structuration locale des populations, et la distribution spatiale hétérogène des hôtes peut se traduire par une différenciation entre populations de leurs parasites.

L'objectif de la thèse est de comprendre comment se structurent génétiquement les populations du couple symbiotique, le crabe ectoparasite *Dissodactylus primitivus* et son hôte principal, l'échinide *Meoma ventricosa*. Ces symbiotes font partie du biota caribéen qui couvre un archipel d'îles tropicales s'étendant sur près de trois millions de km^2 et compte des écosystèmes parmi les plus diversifiés de la planète. Ce biota a évolué dans un cadre géologique particulièrement complexe, complexité à laquelle s'ajoute celle du parcours actuel des courants marins qui délimitent quatre régions de connectivité. Les patrons observés étant le reflet d'événements tant historiques que récents, les discriminer n'est dès lors possible qu'avec l'usage de marqueurs moléculaires adaptés. Deux marqueurs moléculaires seront donc utilisés (à la fois pour l'hôte et le parasite). Des microsatellites qui se focalisent sur événements récents et un marqueur mitochondrial (COI, cytochrome oxydase I) qui offre une fourchette de temps plus large (événements présents et passés).

La différenciation génétique sera abordée hiérarchiquement ; d'abord à une large échelle géographique (Caraïbes) puis à une échelle locale (intra-île).

Le chapitre 1, 'Caractérisation de marqueurs génétiques', correspond à une mise au point technique. En effet, au départ de ce travail, des marqueurs microsatellites avaient déjà été développés pour *D. primitivus* mais aucun microsatellite n'existait pour *M. ventricosa*. Pour le COI, plusieurs paires d'amorces préalablement développées ont été testées sur les deux espèces.

Le chapitre 2, 'Comparaison des structures génétiques hôtes-parasites', est le corps principal de la thèse. Il a pour objectif de fournir un prérequis dans la prédiction d'une adaptation locale et de révéler les facteurs qui structurent (ont structuré) les populations de ces espèces au sein des Caraïbes. De plus, cette partie propose de tester le cas innovant d'une relation parasitaire au sein de deux régions de connectivité définies par le modèle hydrodynamique de Cowen et al. (2006). En effet, l'échantillonnage réalisé s'étend des Petites Antilles à l'Ouest des Caraïbes (Panama) en passant par une zone intermédiaire (Jamaïque). Enfin, cette zone explorée offre un cadre géologique complexe et abondamment décrit. De ce fait, la superposition potentielle des données génétiques avec des événements géologiques majeurs (émergence des îles, glaciations) sera également considérée.

Le chapitre 3, 'Différenciation locale en Jamaïque', porte sur la comparaison de populations de crabes issus de sites peu distants (1 km – 60km) localisés le long de la côte nord jamaïcaine. Les différenciations morphologique et génétique seront évaluées et comparées à celles observées entre crabes issus d'espèces hôtes différentes (d'un même site).

Enfin, **dans le chapitre 4**, **'Système de reproduction de *Dissodactylus primitivus*'**, l'analyse génétique des pontes et des spermathèques permettra de mieux cerner le système de reproduction et donc d'appréhender comment se structure la diversité génétique entre individus au sein d'une population. Ce chapitre est donc principalement axé sur la biologie de la reproduction du crabe parasite et il fournira aussi des informations sur le déplacement des crabes adultes d'un hôte à l'autre (cf. différenciation locale en Jamaïque).

Les différents chapitres de ce travail sont rédigés en anglais et précédés chacun par un résumé en français.

Une présentation générale des méthodes d'échantillonnage précède l'ensemble des chapitres et une discussion générale les suit. Ces deux parties sont rédigées en français.

STRATÉGIE D'ÉCHANTILLONNAGE

STRATÉGIE D'ÉCHANTILLONNAGE

Plusieurs échantillonnages ont été nécessaires afin de réaliser les analyses génétiques et morphométriques de ce travail. Ceux-ci ont été menés préalablement et durant ma thèse et ils s'étendent de 2005 à 2013 (Fig. 14 ; Tableau 2).

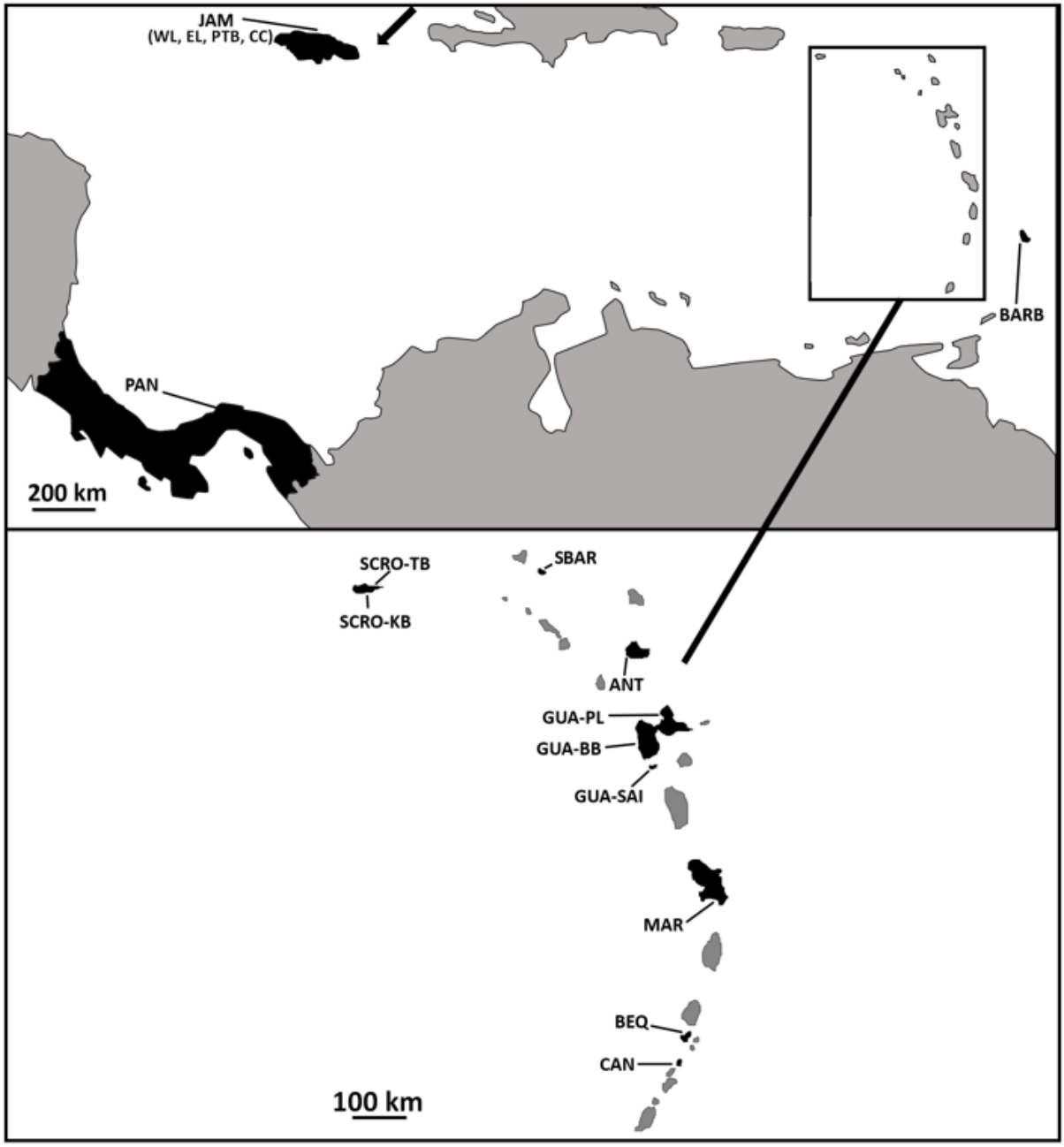


Fig. 14. Origine des échantillons utilisés dans les analyses génétiques et morphométriques. PAN : Panama ; JAM : Jamaïque ; BARB : Barbade ; SCRO : Ste Croix ; SBAR : St Barthélémy; ANT : Antigua ; GUA : Guadeloupe ; MAR : Martinique ; BEQ : Bequia ; CAN : Canouan.

Échantillonnages préalables. Ces échantillonnages correspondent à plusieurs missions jamaïcaines échellonées entre 2005 à 2009 ainsi qu'à une mission martiniquaise réalisée en 2010 (Tableau 2).

Échantillonnages durant la thèse. Une première mission d'échantillonnage s'est déroulée de mai à juin 2011 dans les Petites Antilles. Les prélèvements ont été réalisés sur six îles depuis la Martinique jusqu'aux îles Vierges américaines (limite nord) ainsi qu'aux Grenadines (limite sud) (Fig. 14 ; Tableau 2). Ceux-ci ont été effectués entre 1 et 17 mètres de profondeur, en plongée libre ou en scaphandre autonome. Les profondeurs de prélèvement propres à chaque site sont reprises dans le Tableau 2.

Les échantillonnages ont été réalisés en journée ; les individus de *M. ventricosa* étant alors repérables au dôme de sable qui les surplombe et/ou à la piste tracée dans le sable (Fig. 15).

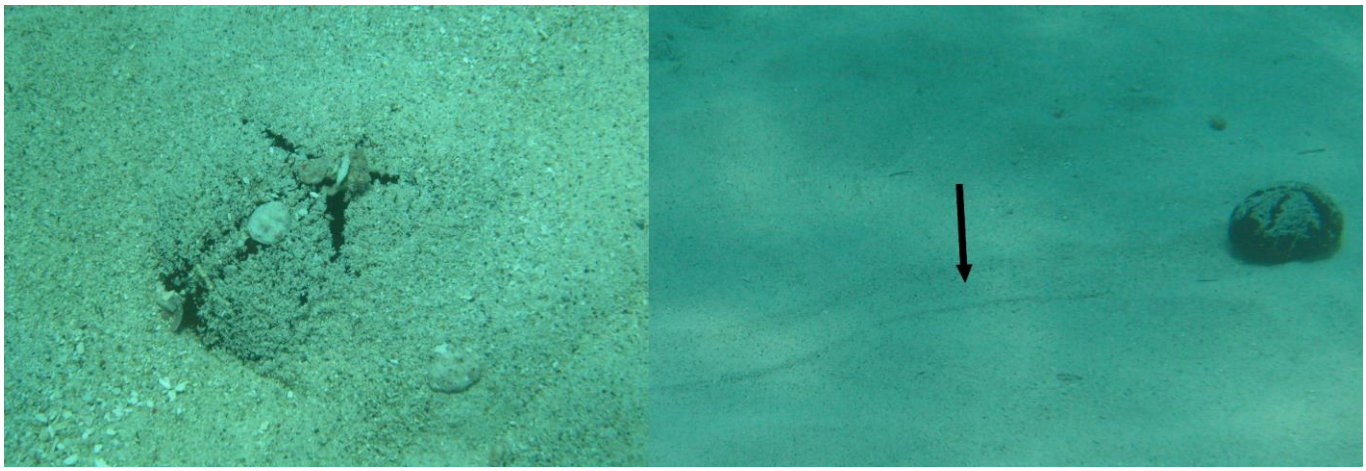


Fig. 15. Dôme de sable (gauche) et piste tracé dans le sable (droite) associés à un individu de *Meoma ventricosa* (Photo : C. De Ridder)

Pour chacun des sites, les oursins sont récoltés en les plaçant individuellement dans des sacs scellés sous l'eau et ramenés à bord d'un bateau. Ensuite, chaque oursin est mesuré (longueur, largeur) et examiné afin d'y prélever les crabes symbiotiques. L'ensemble des crabes d'un même oursin (ainsi que quelques piquants de ce dernier) sont disposés dans un microtube rempli d'éthanol absolu.

En 2012, une mission complémentaire réalisée par S. Motreuil (uB) a permis de récolter crabes et oursins à la Barbade (Tableau 2). Enfin, en 2013, une dernière mission a été effectuée dans un site au centre du Panama. La méthode utilisée lors de ces deux échantillonnages est la même que celle décrite précédemment.

Île	Site	Coordonnées	Profondeur (m)	Espèce(s)	Année(s)	Récolteurs
Jamaïque	Chalet Caribe (CC)	18°27'26.01"N / 77°57'40.26"O	7-9	*D. p*	2005	2, 4
	Pear Tree Bottom (PTB)	18°27'48.21"N / 77°21'14.16"O	12-18	*D. p*	2005, 2009	2, 3, 4, 9, 10
	Eastern Lagoon (EL)	18°27'58.20"N / 77°24'13.04"O	5-6	*D. p*	2007,2009	2, 3, 4, 9, 10
	Western Lagoon (WL)	18°28'3.15"N / 77°24'42.59"O	2-4	*D. p*, *M. v*	2006, 2007, 2009	1, 2, 3, 4, 7, 9, 10
Martinique	Point Borgnèse (MAR)	14°26'18.41"N / 60°54'54.74"O	10-15	*D. p*, *M. v*	2010	8, 9
Ste Croix	Kings Bay (SCRO-KB)	17°39'59.58"N / 64°48'56.59"O	8-9	*D. p*, *M. v*	2011	2, 4, 6, 8, 9
	Teague Bay (SCRO-TB)	17°46'4.04"N / 64°37'59.46"O	1-3	*D. p*, *M. v*	2011	2, 4, 6, 8, 9
St Barthélémy	Anse de Grand Cul de Sac (SBAR)	17°54'39.49"N / 62°48'5.31"O	1-2	*D. p*, *M. v*	2011	2, 4, 6, 8, 9
Antigua	Middle Reef (ANT)	17°0'23.33"N / 61°51'29.38"O	2-11	*D. p*, *M. v*	2011	2, 4, 6, 8, 9
Guadeloupe	Port-Louis (GUA-PL)	16°25'10.36"N / 61°32'31.75"O	11	*D. p*, *M. v*	2011	2, 4, 6, 8, 9
	Baie de Bouillante (GUA-BB)	16°7'52.79"N / 61°46'47.81"O	6-8	*D. p*, *M. v*	2011	2, 4, 6, 8, 9
	Les Saintes (GUA-SAI)	15°51'56.46"N / 61°36'0.15"O	10-17	*D. p*, *M. v*	2011	2, 4, 6, 8, 9
Bequia	Lower Bay (BEQ)	12°59'50.02"N / 61°14'51.11"O	7-9	*D. p*, *M. v*	2011	2, 4, 6, 8, 9
Canouan	Rameau Bay (CAN)	12°43'28.92"N / 61°19'58.42"O	7-8	*D. p*, *M. v*	2011	2, 4, 6, 8, 9
Barbade	Carlisle Bay (BARB)	13°4'26.84"N / 59°37'0.13"O	12-15	*D. p*, *M. v*	2012	5, 9
Panama	Isla Drake (PAN)	9°33'40.16"N / 79°41'2.27"O	9-22	*D. p*, *M. v*	2013	1, 6, 7

Tableau 2. Échantillonnages réalisés dans le cadre de ce travail. *D. p* : *Dissodactylus primitivus*, *M. v* : *Meoma ventricosa*. 1: Calderon A.; 2: David B.; 3: De Bruyn C.; 4: De Ridder C.; 5: Goodridge R.; 6: Jossart Q.; 7: Lessios H.; 8: Maréchal JP.; 9: Motreuil S.; 10: Rigaud T.. Le détail des tailles d'échantillonnages pour chaque analyse (génétique ou morphométrique) est repris dans la partie méthodologique de chacun des chapitres.

CHAPITRE 1

Chapitre 1 - Caractérisation de marqueurs génétiques

Ce chapitre comporte un résumé en français, un article (microsatellites de *M. ventricosa*) soumis au Journal of the Marine Biological Association of the United Kingdom ainsi que des résultats (non publiés) sur la caractérisation du COI chez les deux espèces.

A. Résumé

Huit locus microsatellites ont été caractérisés pour *Meoma ventricosa* au niveau d'un site des Îles Vierges américaines (St Croix). En effet, bien que des marqueurs moléculaires aient été développés pour cette espèce (16S rDNA, Cytochrome Oxidase I - COI, 28S rDNA), aucun ne possède un taux de mutation aussi élevé que celui des microsatellites.

Pour les 29 individus considérés, nous avons observé un nombre d'allèles moyen de 8,125, une hétérozygotie observée (Ho) de 0,640 ainsi qu'une une hétérozygotie attendue (He) de 0,747.

Deux locus ont présenté une déviation significative à l'équilibre de Hardy-Weinberg ce qui peut être lié à la présence d'allèles nuls sur ces deux locus.

De plus, nous avons montré que les locus pouvaient être amplifiés dans d'autres individus des Petites Antilles (Guadeloupe).

Globalement, les huit locus caractérisés ont un taux de polymorphisme moyen à élevé et semblent pertinents pour une étude de génétique des populations au sein de la mer des Caraïbes.

Enfin, nous avons testé efficacement plusieurs paires d'amorces (COI) sur *Meoma ventricosa* mais également sur *Dissodactylus primitivus*.

B. Characterization of eight microsatellite loci for the sea-urchin *Meoma ventricosa* (Spatangoida, Brissidae) through Next Generation Sequencing

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Abstract

Eight microsatellite loci were characterized for *Meoma ventricosa* (Lamarck, 1816), a burrowing sea-urchin that can be afflicted by a bacterial disease causing localized mass mortality. For the analyzed population (29 individuals from St. Croix, US Virgin Islands), we observed 8.125 mean number of alleles, 0.640 mean observed heterozygosity (H_o) and 0.747 mean expected heterozygosity (H_e). Two loci showed significant deviations from Hardy-Weinberg equilibrium. Moreover, we verified that these loci can be amplified in other individuals from the Antilles (Guadeloupe). Overall, the described loci were characterized by a high level of polymorphism; suggesting that these markers are useful for a population genetics study in the Caribbean Sea.

Introduction

Meoma ventricosa (Lamarck, 1816) (Spatangoida: Brissidae) is a common echinoid along the Caribbean and neighboring American coasts (Telford 1982; Hendler et al. 1995; De Bruyn et al. 2009). This large burrowing heart urchin (up to 20 cm in length) is found in sandy areas close to sea-grass beds or coral reefs as well as in deeper water. *M. ventricosa* lives in sediments ranging from fine sand to coral fragments (Chesher 1969; Hendler et al. 1995). It spends the daytime burrowed in the sediment and emerges at dusk to forage on the bottom (Kier & Grant 1965; Hammond 1982; Hendler et al. 1995). Chesher (1969) estimated that herds of *M. ventricosa* have a key influence on the disturbance of sand and consequently on the productivity of the areas it inhabits. Like the majority of echinoderms, *M. ventricosa* is gonochoric. Fertilization occurs in the

water column. The metamorphosis from blastula to first larval stage (echinopluteus) has been found to occur 20 hours later in the subspecies *M. ventricosa grandis* (Mortensen 1921). The larva is planktotrophic, but its pelagic larval duration (PLD) is unknown (Chesher 1969).

Although some molecular markers have been developed for this species (16S rDNA and Cytochrome Oxidase I (COI) mitochondrial genes, 28S rDNA nuclear gene) (Stockley et al. 2005), none have the high mutation rates of microsatellites. Microsatellites could provide information on genetic diversity, information relevant to conservation of this species, which is afflicted by a bacterial disease, sometimes causing localized mass mortality (Nagelkerken et al. 1999; Przeslawski et al. 2008). Microsatellites could also be helpful in evaluating the genetic structure of this species at a fine scale.

Material and methods

A gonad sample from a single individual of *Meoma ventricosa,* collected in San Blas, Panamá and preserved in high salt DMSO buffer (Seutin et al. 1991), was chosen from the research collection of the Smithsonian Tropical Research Institute. We extracted DNA from this sample using the DNeasy Blood & Tissue kit (Qiagen). The DNA was shipped to Genome Québec (Montréal, Canada) for library construction and sequencing using the GS FL Titanium method on a 454 instrument (Roche), at ¼ plate scale. This sequencing run yielded over 179,000 reads. These data were searched for simple sequence repeats (SSRs) with MSATCOMMANDER 1.0.8beta (Faircloth 2008), using a minimum search criterion of 8 di-nucleotide repeats. This search yielded 15,564 potential microsatellite loci. The sequences of these potential loci were processed with Primer3 (Rozen & Skaletsky 2000) for primer design using a CAG-tag (CAGTCGGGCGTCATC) (Boutin-Ganache et al. 2001), limiting the product size to 100-450 bp. Primer3 identified 913 potential primer pairs. Duplicates were eliminated, as were those primers with potential for hairpin formation, self-annealing, incomplete CAG-tag sequences, and any with a predicted pair penalty >2. The sequences of the remaining 149 loci were examined to eliminate any with sequence motifs likely to create problems during genotyping (sequences with long monomer strings and multiple SSRs). This reduced a potential list to 30 loci for which primers were ordered (Integrated DNA Technology).

Each of the 30 primer sets was tested for amplification using standard PCR conditions (described below) and an annealing temperature gradient of 48-60°C. Nine potential loci failed to amplify, and six yielded multiple products. The 15 remaining loci were amplified using a fluorescently labeled CAG-tag and genotyped using an ABI 3130Xl Genetic Analyzer. The resulting electropherograms were examined using GeneMapper 4.0 (Applied Biosystems). Seven additional loci were eliminated because they either resulted in uninterpretable multiple peaks or in allele sizes clearly outside the predicted range.

Twenty nine specimens of *Meoma ventricosa* were collected by SCUBA diving (May 2011) at one site in St. Croix, US Virgin Islands (17°39'N, 64°48'W). DNA was extracted from two spines of each individual using the Qiagen DNeasy Blood & Tissue Kit. Eight microsatellite loci (Table 3) were amplified in simplex according to the tagged primer-method (Schuelke 2000). Each reaction (ca. 12 µl) included: 1µl of extracted DNA, 0.030 µM of forward/reverse primers with CAG tail (CAGTCGGGCGTCATC), 0.5 µM of universal dye labelled CAG primer, 0.5 µM l of forward/reverse primers without CAG tail, 2.4 µl of Buffer (Promega), 1 µM of dNTP (Promega), 2.5 mM of MgCl2, 0.8M of Betaine (Affymetrix), 2.9 µl of sterile water and 0.04 U of Taq Polymerase (Promega). For loci CHCM and NLQK, 2.86 µl of water and 0.03 U of Taq were used.

The PCR conditions were: 14 cycles of 20s at 95°C (denaturation), 20s at 50-59°C (first annealing temperature, (Table 3) and 20s at 72°C (elongation); followed by 25 cycles of 20s at 95°C, 20s at 52.9°C (annealing temperature of the CAG primer) and 20s at 72°C. These cycles were preceded by 1 min at 95°C (first denaturation) and were followed by 5 min at 72°C (last elongation). Using band intensities on agarose gels as a rough estimate of product concentration, we mixed 0.5 - 2.5 µl of amplified DNA with 0.5 µl of GeneScan 500 LIZ size standard (Applied Biosystems) and 10.5 µl of formamide prior to genotyping with an ABI 3130xL Genetic Analyzer. Genotypes were deduced from electropherograms using the software Peak Scanner (Applied Biosystems). Allelic binning was done using the Excel macro Autobin (Guichoux et al. 2011) but each genotype was also checked by eye.

We used Micro-Checker (Van Oosterhout et al. 2004) to detect potential genotyping errors. Using GENEPOP 4.2.2 (Rousset 2008), we evaluated the observed (H_o) and

expected heterozygosities (H_e) as well as conformity to Hardy-Weinberg (HW) equilibrium, the presence of linkage disequilibrium and the number of alleles.

In order to determine whether these microsatellites are useful at a regional geographical scale, we genotyped 10 individuals from Guadeloupe (Port Louis, 16°25'N - 61°32'W). Although this is only a preliminary estimation, we calculated, with the program SMOGD, the actual differentiation (D value) between sea-urchins from St Croix and Guadeloupe (Jost 2008; Crawford 2010).

Results

In St Croix, for two loci (TCYO, 6SKB), Micro-Checker detected the probable presence of null alleles (Brookfield 1 estimator) with frequencies of 0.15 (TCYO) and 0.22 (6SKB) (Brookfield 1996). Average H_o and H_e were 0.640 (0.423- 0.923) and 0.747 (0.571-0.933), respectively (Table 3). Genotype frequencies in all loci, except for those with inferred null alleles, were not significantly different from HW expectations. For the loci with null alleles, F_{IS} values were equal to 0.338 (TCYO) and 0.503 (6SKB). Two loci (TCYO, CHCM) seem to be at linkage disequilibrium ($p < 0.0018$, Bonferroni-corrected). The number of alleles per locus varied between 3 and 17 with an average of 8.125 (Table 3).

All the individuals from Guadeloupe amplified for most of the eight markers with a percentage of missing genotypes equal to 10%. The average number of alleles for the small group from Guadeloupe was 6.5 (3-12).

The harmonic mean of D (actual differentiation calculated by SMOGD) between sea-urchins from St Croix and Guadeloupe was approximately equal to zero (-0.001).

Locus	Motif	Primer sequence	GenBank accession no.	Ta	Size range (bp)	Na	Ho	He
CHCM	$(CA)_{13}$	F: CCTGACAAGTTGACCACACG R: CAGTCGGGCGTCATCAGGGAACGAGCATAGAACCG	KJ875804	50°C	202-234	17	0.923	0.933
9901	$(AC)_{10}$	F: CAGTCGGGCGTCATCAGCATGTCAACAGCCTCACTC R: TTCGTTGCACCGTCTGTTTC	KJ875805	51°C	235-243	5	0.720	0.657
CHTO	$(GT)_{8}$	F: CAGTCGGGCGTCATCAGCAGCGTTGAGTTTGACTG R: AACGGAGCTAAGCCCTTCTG	KJ875806	59°C	314-326	5	0.517	0.612
93XX	$(AT)_{15}$	F: CAGTCGGGCGTCATCAACATTCATCAAGCGAGCCG R: GTTTCCTGTGGCGTGTTCAG	KJ875807	57°C	156-174	7	0.885	0.784
NLQK	$(CT)_{11}$	F: CAGTCGGGCGTCATCACTGTGCAATTCGTCACCTC R: AGCTCAGCGTGGACTCATAG	KJ875808	50°C	138-146	5	0.625	0.661
9U51	$(AC)_{9}$	F: GGCATTCGAGTTCTGACAGC R: CAGTCGGGCGTCATCATAGCTCTCATGTCCTTGGCC	KJ875809	56°C	169-173	3	0.448	0.571
6SKB	$(AC)_{10}$	F: GTCCATGGTTCGCAGTTGTC R: CAGTCGGGCGTCATCAATCTAGCCGTGGGATCTGG	KJ875810	59°C	321-341	10	0.423	0.851*
TCYO	$(CA)_{17}TA(CA)_{13}CT(CA)_{4}$	F: CAGTCGGGCGTCATCAGTGTCCTCAGCTCAGTTTGC R: GATGCATGGTTGTGGTAGGG	KJ875811	59°C	322-348	13	0.615	0.930*

Table 3. Characterization of eight microsatellite loci for *Meoma ventricosa*. Ta = annealing temperature, Na = Number of alleles, Ho = Observed heterozygosity, He = Expected heterozygosity, * indicates significant deviation from Hardy-Weinberg equilibrium. CAGTCGGGCGTCATC of primer sequence corresponds to the CAG-tail.

Discussion

Overall, this set of loci showed moderately high polymorphism and amplified efficiently for individuals collected at two distant islands (ca. 375km) of the Antilles. This suggests that the microsatellites can be useful for a broader population genetics study. The lack of genetic differentiation at this scale must be verified with a bigger sample from Guadeloupe. There may also be more pronounced structure at a larger geographical scale (other parts of Caribbean Sea). In other species of sea-urchins, previous phylogenetic analyses based on mitochondrial DNA (mtDNA) showed a great deal of homogeneity inside the Caribbean Sea (eg. Lessios et al. 2001; Lessios et al. 2003). The current characterization of microsatellites will permit an assessment of this pattern using molecular markers with higher mutation rates than mtDNA.

The characterization of microsatellites of *Meoma ventricosa* permits the comparison of genetic structure of *M. ventricosa* with that of its parasite, the crab *Dissodactylus primitivus* that could have different capacity of dispersal than its host (Yednock & Neigel 2011; Jossart et al. 2014). Microsatellite markers are already characterized and validated for this crab (Anderson et al. 2010; Jossart et al. 2013). This could reveal the factors that influence dispersal of each species and provide information about their potential for local adaptation (Greischar & Koskella 2007).

Acknowledgements

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C. Testing of Cytochrome Oxydase I marker (COI) in *D. primitivus* and *M. ventricosa*

COI in *D. primitivus*

For this testing, we focused on four studies that used COI marker in crustacean species (the pinnotherid crabs *Austinixa*: Harrison 2004; the gammarids *G. pulex* and *G. fossarum:* Rullman 2010; the mangrove crab *Perisesarma guttatum*: Silva et al. 2010a; the shore crab *Pachygrapsus marmoratus:* Fratini et al. 2011).

Three primers pairs were tested on 9 individuals (from different sites in the Antilles): DpCOIV1 (693 bp): S1718 5'-GGAGGATTTGGAAATTGATTAGTTC-3' // S2411 5'-GGGATAGCAATYATTATWGT-3' (Harrison 2004); DpCOIV2 (658 pb): COL6b 5'-ACAAATCATAAAGATATYGG-3' // HCO2198 5' TAAACTTCAGGGTGACCAAAAAATCA-3' (Fratini et al. 2011) and DpCOIV3 (651 pb): LCO1490 5′-GGTCAACAAATCATAAAGATATTGG-3′ // HCO2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994; Silva et al, 2010a ; Rullman 2010).

Preliminary PCR reaction (15 µl) included 7.5 µl of Master Mix Qiagen (Taq Polymerase, nucleotides), 1 µl of DNA, 0.3 µl (10 µM) of each forward or reverse primer and 5.9 µl of sterile water. Preliminary PCR conditions consisted of 35 cycles for each of the 3 temperature steps [45 s at 94°C (denaturation), 60 s at 47°C (annealing) and 90 s at 72°C (elongation)]. These cycles were preceded by a step of 3 min at 95°C (first denaturation) and were followed by a step of 6 min at 72°C (last elongation).

Samples amplified efficiently for all of these primers pairs (Fig. 16). We decided to use DPCOIV3 in further analyses because this primers pair presented the highest band intensities (Fig. 16).

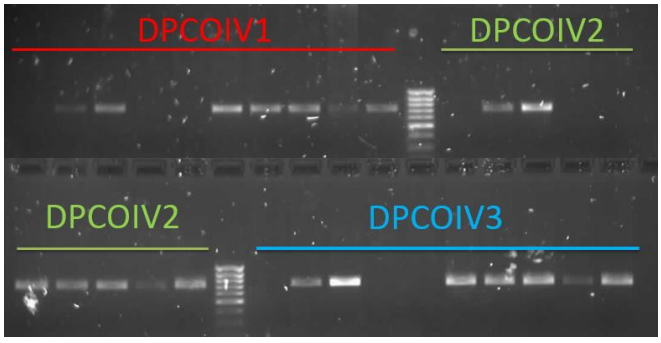


Fig. 16. Gel of COI amplification in *D. primitivus* using three primers pairs (DPCOIV1, DPCOIV2, DPCOIV3)

After several optimizations, the best amplification results were obtained for the following conditions: PCR reaction of 15 µl that included 7.5 µl of Master Mix Qiagen (Taq Polymerase, nucleotides), 2 µl of DNA, 0.6 µl (10 µM) of each forward or reverse primer and 4.3 µl of sterile water. PCR conditions consisted of 35 cycles for each of the 3 temperature steps [60 s at 94°C (denaturation), 60 s at 40°C (annealing) and 120 s at 72°C (elongation)]. These cycles were preceded by a step of 2 min at 94°C (first denaturation) and were followed by a step of 2 min at 72°C (last elongation).

COI in *M. ventricosa*

After a review of the literature and of the GenBank database, we focused on two works that used the same COI primers pairs on two irregular echinodis (*Meoma ventricosa*: Stockley et al, 2005; *Echinocardium cordatum*: Egea 2011).

The primer sequences were: 5'-GCYTGAGCWGGCATGGTAGG-3' and 5'-GCTCGTGCRTCTACRTCCAT-3') (Stockley et al. 2005; Egea 2011). After optimizations, PCR reaction (15 µl) included 7.5 µl of Master Mix Qiagen (Taq Polymerase, nucleotides), 1 µl of DNA, 0.3 µl (10 µM) of each forward or reverse primer and 5.9 µl of sterile water. PCR conditions consisted of 35 cycles for each of the 3 temperature steps [40 s at 94°C (denaturation), 30 s at 52°C (annealing) and 60 s at 72°C (elongation)]. These cycles were preceded by a step of 4 min at 95°C (first denaturation) and were followed by a step of 5 min at 72°C (last elongation).

The amplification step was done on 7 individuals (from different sites in the Antilles) that had different pretreatments (extractions). Two methods of extraction were used (Chelex resin method: Walsh et al. 1991; Qiagen DNeasy Blood & Tissue Kit) on whole sea-urchins spines or on their basal parts only.

DNA extracted using the Chelex method amplified very slightly or even not amplified (1-2 in Fig. 17). The Qiagen method showed a better amplification with no apparent difference among whole spines and basal parts (3-4 in Fig. 17).

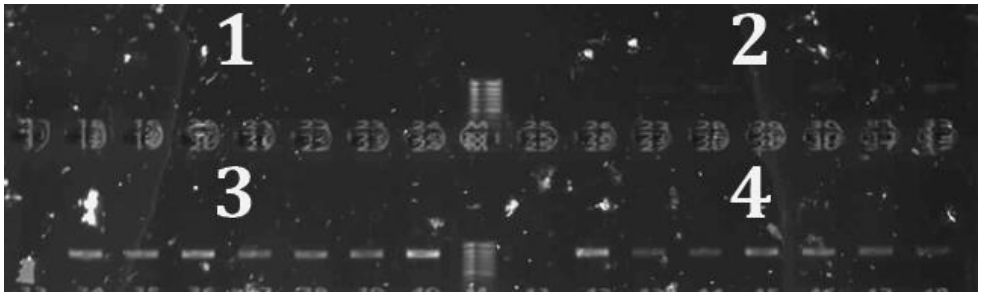


Fig. 17. Gel of COI amplification in *M. ventricosa* samples with four different pretreatments. 1: Chelex method on whole spines; 2: Chelex method on basal parts of spines; 3: Qiagen method on whole spines; 4: Qiagen method on basal parts of spines.

CHAPITRE 2

Chapitre 2 - Comparaison des structures génétiques hôtes-parasites

Ce chapitre comporte un résumé en français ainsi que des résultats (encore à soumettre) sur les structures génétiques de *M. ventricosa* et *D. primitivus*.

A. Résumé

Comparer les structures génétiques d'un couple hôte-parasite est un prérequis dans la prédiction de l'adaptation locale ainsi que dans la détermination de l'échelle à laquelle l'interaction peut évoluer. De plus, elle permet d'évaluer les facteurs environnementaux et les traits d'histoire de vie qui façonnent la dispersion de ces organismes.

A l'aide de marqueurs microsatellites et d'un marqueur mitochondrial (COI), nous avons analysé ces structures chez le couple symbiotique formé par l'oursin *Meoma ventricosa* et son crabe ectoparasite *Dissodactylus primitivus*. Cette étude a eu lieu dans la mer des Caraïbes (du Panama aux Petites Antilles) dont l'histoire géologique et la courantologie sont complexes.

Nous avons observé des patterns hautement contrastés : l'hôte montre de l'homogénéité génétique alors que le parasite est structuré génétiquement à différentes échelles géographiques (Mer des Caraïbes et au sein des Petites Antilles). Cette dissimilarité peut avoir plusieurs facteurs explicatifs comme la fécondité, l'habilité à la nage ou la disponibilité de l'habitat. Ce contraste aura des répercussions sur l'évolution de cette interaction (adaptation locale) ; des analyses complémentaires, comme des expériences d'infectivité, permettraient d'examiner ces répercutions.

Pour le crabe parasite, nous avons observé que la diversité génétique était répartie en deux groupes majoritaires (à la fois pour les microsatellites et le COI). Ceux-ci correspondent aux groupes Panama-Jamaïque-St Croix *vs* Est des Caraïbes. Certaines îles (Antigua, Guadeloupe, St Barthélémy) ont une situation intermédiaire au niveau de leur proximité génétique avec ces deux groupes. A une échelle plus réduite, une différenciation génétique a également été détectée (ex : comparaisons incluant la Barbade). Globalement, la distance géographique a une influence non négligeable sur le pattern observé pour ce crabe mais la courantologie actuelle ou encore des événements historiques (ex : glaciations) sont également à prendre en compte.

B. Highly contrasted genetic structures between Caribbean Sea urchins and their parasite crabs are patterned by historical and present processes

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Manuscrit à soumettre

Abstract

Comparing host and parasite genetic structures is a prerequisite in the prediction of local adaptation as well as determining the scale at which a parasitic interaction can evolve. Moreover, it allows to evaluate the environmental factors and life-history traits that have shaped the dispersal of these organisms. Using microsatellite and COI markers, we investigated the genetic structures of a host-parasite couple: the sea urchin host *Meoma ventricosa* and its parasitic crab *Dissodactylus primitivus*. This study took place in the Caribbean Sea (from Panama to Lesser Antilles) that presents a complex and interestingly framework (geology and currents patterns). We observed highly contrasted patterns: the host showed a genetic homogeneity while the parasite showed significant differentiation at various geographical scales (Caribbean Sea and within Lesser Antilles). This dissimilarity in genetic structures can have several explicative factors as fecundity, swimming capacities, or habitats suitability. It will affect the evolution of this interaction (local adaptation) and further investigations (infectivity experiments) are therefore needed to measure this local adaptation. For the parasitic crab, we observed that the genetic diversity was separated in two main groups (both for microsatellites and COI) that correspond to Panama-Jamaica-St Croix vs Easter Caribbean while some islands appeared to be admixed (Antigua, Guadeloupe, St Barthélémy). At a local scale, some islands appeared to be differentiated, as for example Barbados. Globally, while geographical distance has probably a strong influence on the observed pattern for the crab, the present marine currents and some historical events (as glacial periods) have also to be considered.

Introduction

Parasites and hosts reciprocally exert selective pressures on each other, due to their long-lasting interactions, the obligate dependency of parasites towards the host and the virulence exerted by parasites on their hosts (Poulin 2007). Adaptations in parasites favor the encounter with hosts and the survival once associated, while adaptations in hosts favor the avoidance and/or the elimination of parasites (Combes 1997; Tellier et al. 2014). The pattern of this "arms race" or "trench warfare" (Tellier et al. 2014) is nevertheless dependent of the ecological context in which it occurs. Notably, the distributions of host or of parasite populations are discontinuous and heterogeneous (Poulin 2007), and most, some parasite-host combinations do not coevolve in such a meta-population context. For instance, this can become a critical parameter in the discontinuous spatial framework of an archipelago. Consequently, the co-evolutionary pressures and adaptations are expected to occur at local scale (Kawecki & Ebert 2004; Blanquart et al. 2013). The effect of migration rate (and thus of gene flow; Hellberg 2009) on these local adaptations was assessed in several studies during the two last decades (Gandon et al. 1996; Storfer 1999; Gandon & Michalakis 2002; Kawecki & Ebert 2004; Sotka 2005; Greischar & Koskella 2007; Blanquart et al. 2013). Until the seminal work of Gandon et al. (1996), local adaptations were thought to be possible only under a regime of low gene flow of both partners of the association. It is now widely accepted that relatively high levels of gene flow does not automatically restrict local adaptation. By reviewing 54 case studies of aquatic and terrestrial host-parasite relationships, Greischar & Koskella (2007) confirmed theoretical predictions of Gandon et al. (1996) and Gandon & Michalakis (2002): the partner of the interaction displaying the higher gene flow is better locally adapted. In most cases, parasites have a higher dispersing ability and are therefore adapted locally to their hosts (Greischar & Koskella 2007). However, some experiments failed to detect parasite local adaptation (Dufva 1996; Oppliger et al. 1999; Reichard et al. 2011) suggesting that the parasite is not always being ahead in the "arms race" (Gandon & Michalakis 2002).

Gene flow directly influences the genetic structure of populations. Characterizing and comparing genetic structures is thus a prerequisite to predict local adaptations but also to determine the scale at which the interactions are evolving (McCoy et al. 2005; Poulin 2007). Congruences as well as incongruences of parasite and host population genetic

structures have been evidenced (eg. Mulvey et al. 1991; Delmotte et al. 1999; McCoy et al. 2005; Criscione & Blouin 2007). Ecological factors as well as factors related to organism life-history traits have shaped the history of populations of both partners (Criscione 2008; Hellberg 2009; Kochzius et al. 2009). This is particularly true in marine habitats, where relatively ancient phenomena such as changes in the sea level as well as recent processes such as sea currents, can shape the population genetic structures of organisms. For example, congruent population structures were found between two seastars (*Protoreaster nodosus, Linckia laevigata*) and their symbionts (*Periclimenes soror, Thyca crystallina*), for which synchronized demographic expansions were due to sea level fluctuation in the Coral Triangle during the Pleistocene (Crandall et al. 2008). In contrast, Kochzius et al. (2009) proposed that an extended breeding period combined with high dispersal of larvae could explain incongruence in genetic structures between the parasitic gastropod *T. crystallina* and its seastar host *L. laevigata* occurring in the Indo-Malay Archipelago.

This paper aims to understand how populations of a marine host-parasite couple are structured at the light of past (geology, sea level variations, climates) and present (current patterns) processes, by studying the irregular sea urchin *Meoma ventricosa* and its parasitic pinnotherid crab *Dissodactylus primitivus* (Telford 1982; De Bruyn et al. 2009). Both species are endemic to the Caribbean Sea and neighboring American coasts including Florida northward, and Brazil southward (Chesher 1969; Wirtz et al. 2009; Alvarado 2011). *M. ventricosa* is living between 1 to 200 m deep on soft substrates ranging from small coral pebbles to sandy or heavily silted sediments (Chesher 1969, Hendler et al. 1995). *D. primitivus* is an ectoparasite of *M. ventricosa* on which it reproduces, finds a shelter and feeds (Telford 1982). Prevalence is quite high, 75-100 % of the sea urchin being infected, and the mean burden fluctuates from 1 to 8 crabs per individual host according to localities (De Bruyn et al. 2009). The crab is consuming host tegument and spines (Telford 1982), producing wounds that induce fitness costs to its host (De Bruyn et al. 2009). No larval stages of the crab life-cycle were found on the host (De Bruyn et al. 2009), these being therefore strictly pelagic (Telford 1982). Larvae of *M. ventricosa* are pelagic too (Emlet et al. 1987).

Synthetically, the emergence of Caribbean islands followed five geological steps from the Cretaceous to the Pliocene: (*i*) the formation of the Central America Arc from 85 Ma to

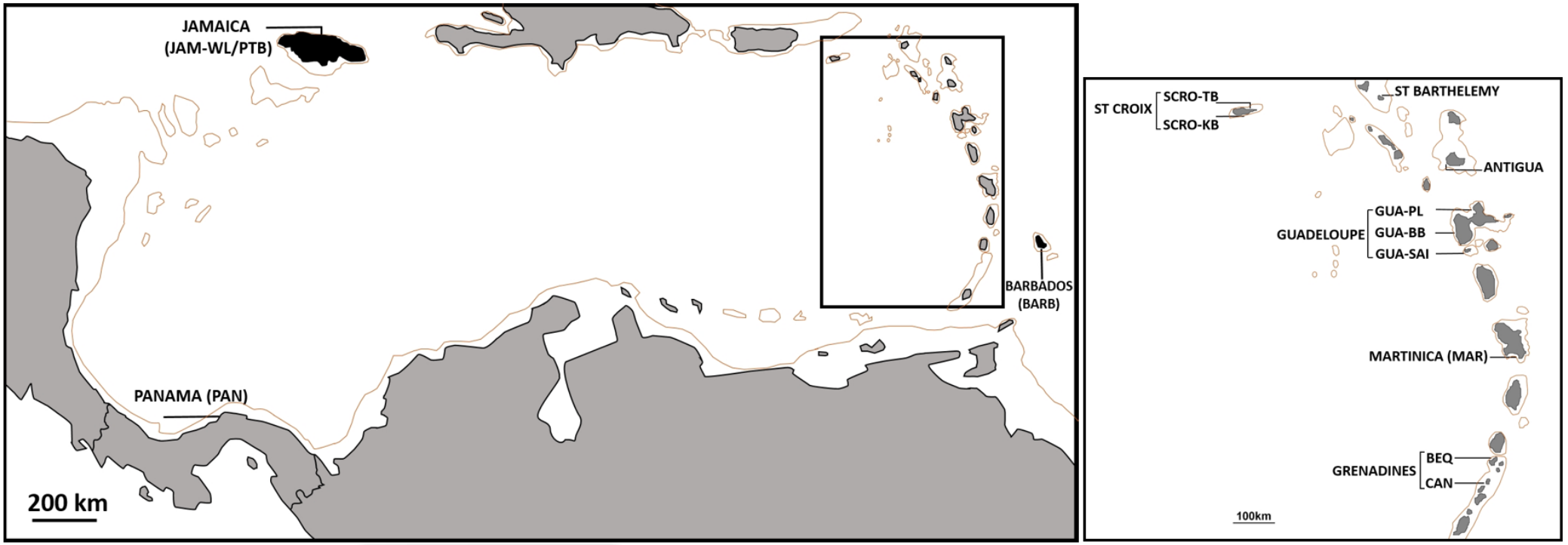


Fig. 18. Sites of samplings inside the Caribbean Sea with a focus on the Lesser Antilles. The orange line corresponds to the depth of 200 meters.

40 Ma ago on the western border of the Caribbean plate; (*ii*) the formation of the Greater Antilles and of the Aves Ridge, from 85 Ma to 40 Ma on the northern border of the Caribbean plate; (*iii*) the formation of the external arc of Lesser Antilles from 25 Ma to 20 Ma on the eastern border of the plate; (*iv*) more recently, after 6 Ma, the formation of the internal arc; (*v*) the formation of Barbados that corresponds to the emergence of an accretion prism from the Pliocene including an active phase during the last 700,000 years (Donovan 2005; Germa 2008). In addition, sea level fluctuations related to the Quaternary climatic oscillations since 2.5 Ma regularly changed the geography and ecology of the region. Eight climatic cycles have been recorded since 800,000 years (Waelbroeck et al. 2002; Pillans & Gibbard 2012). For example, the last glacial maximum (26 to 21 ka BP, Clark et al. 2009) induced eustatic sea level drop of c.a. 100-150 m (Fleming et al. 1998; Peltier 2002; Peltier and Fairbanks 2006; Clark et al. 2009, Fig. 18). Such a complex geotectonic and climatic history not only determined the chronology of the emergence of islands but also framed the extant of suitable habitats and of connections between regions in which the host-parasite system evolved. However, superimposed to those historical events, the marine currents regimes also play a key role that has to be considered.

In this region, circulation of present marine currents was profusely characterized from the sixties (see Molinari et al. 1981 and Gyory et al. 2013). Three main systems can be recognized. (*i*) The “Caribbean current” is the main surface current of the Caribbean Sea (Gyory et al. 2013, Fig. 19). It starts from the Lesser Antilles and flows to the Gulf of Mexico where it meets the “Loop current” (Lessios et al. 1984). The Caribbean current exits the Gulf in Florida where the Gulf Stream starts. (*ii*) Another water mass, the “Antilles current” flows along the Antilles to Bahamas (Silberman et al. 1994). (*iii*) A large eddy flows from Panama to Costa Rica (Lessios et al. 1984). Integrating these marine currents into a hydrodynamic model, Cowen et al. (2006) proposed four connectivity regions inside the Caribbean: Eastern Caribbean, Western Caribbean; Bahamas and Panama-Colombia (Fig. 19). Cowen's model predicts high dispersal potential within each region, but very limited ones across them. Some genetic data support these expected regions while others contradict their boundaries (Baums et al. 2005; Diáz-Ferguson et al. 2010; Johnston et al. 2012; Andras et al. 2013). Moreover, the

number of investigated taxa remains restricted and previous studies mainly focused on fishes and corals (Diáz-Ferguson et al. 2010).

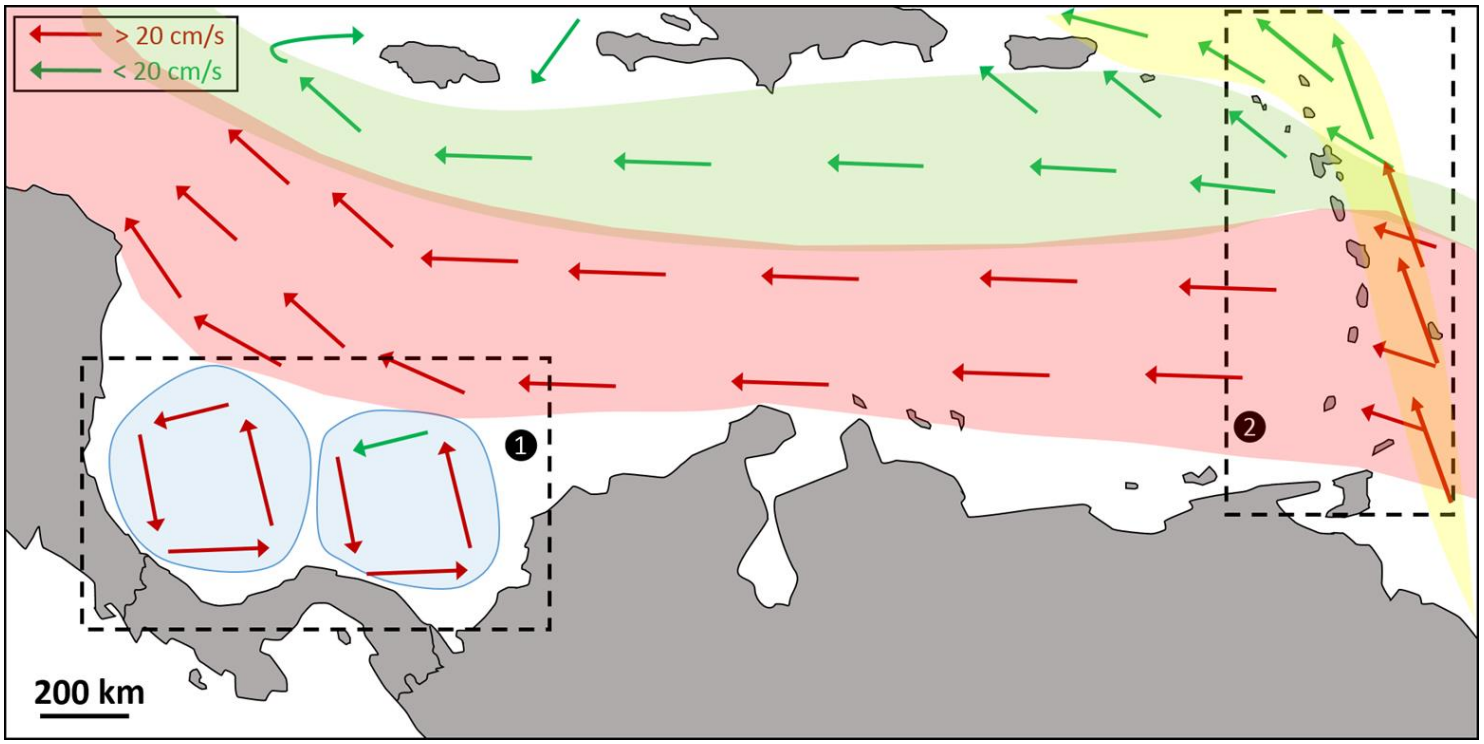


Fig. 19. Schematic illustration of the main currents in Caribbean region, and two of the connectivity regions defined by Cowen et al. (2006). Labels 1 to 2 denotes Panama-Colombia, Eastern Caribbean, respectively, delimited by dashed frames. Differences in the color of arrows denote differences in current speed. Current in yellow is the Antilles current, current in red and green is the Caribbean current (differences in color denote differences in current speed), currents circled in blue are Panama loops.

This complex pattern of currents provides an interesting environmental frame to our study. Water masses are expected to transport planktonic larvae of numerous organisms and through this, to shape population structures (White et al. 2010). *D. primitivus* and *M. ventricosa* have planctotrophic larvae but their respective abundances and swimming abilities differ sharply. The fecundity of *D. primitivus* is *ca.* 4000 times smaller than that of *M. ventricosa* (Emlet et al. 1987; Jossart et al. 2014). Swimming velocities of decapod larvae allow them to resist drifting (Yednock & Neigel 2011) while echinoid larvae are usually 'weak swimmers' and thus are susceptible to be drifted (Metaxas 2013).

The genetic structures of the parasite and host populations were analyzed using two types of molecular markers (COI - cytochrome c oxidase subunit I, microsatellites), permitting a population genetics approach as well as a phylogeographical one. The samplings were made along the Caribbean arc and at a point on the Central American coast. This will allow to answer several questions in this host-parasite system: (*i*) Do the

host and parasite exhibit an identical genetic structuration? (*ii*) Regarding these genetic structures, how can we predict local adaptations of these two species? (*iii*) Did the complex history of the Caribbean area had any influence on the crab and sea urchin genetic structures? (*iv*) What is the influence of present day currents? (*v*) Do the genetic structures match the two related connectivity regions (Eastern Caribbean, Panama-Colombia) identified by Cowen's model?

Material and Methods

Samplings

Crabs and sea urchins were sampled between 2006 and 2013 at 14 sites (13 for sea urchins) from 10 islands or Central America coastal area within the Caribbean Sea (Table 4; Fig. 18). These sites belong to the Lesser Antilles (Saint Barthélemy, Antigua, Guadeloupe, Martinique, Bequia, Canouan, Barbados), Greater Antilles (Jamaica, Saint Croix) or Central America (Panama) (Fig. 18).

State	Site	Coordinates	Depth (m)	Year	No. of individuals			
					D. p		*M. v*	
					µsats	COI	µsats	COI
Panama	Isla Drake (PAN)	9°33'40"N / 79°41'2"W	9-22	2013	20	17	17	16
Jamaica	Western Lagoon (JAM-WL)	18°28'3"N / 77°24'42"W	2-4	2006, 2009*	30	22	12	13
	Pear Tree Bottom (JAM-PTB)	18°27'48"N / 77°21'14"W	12-18	2009	30	20	-	-
Saint Croix	Kings Bay (SCRO-KB)	17°39'59"N / 64°48'56"W	8-9	2011	30	24	29	28
	Teague Bay (SCRO-TB)	17°46'4"N / 64°37'59"W	1-3	2011	30	22	26	23
Saint Barthélemy	Anse de Grand Cul de Sac (SBAR)	17°54'39"N / 62°48'5"W	1-2	2011	30	20	30	25
Antigua	Middle Reef (ANT)	17°0'23"N / 61°51'29"W	2-11	2011	30	23	26	26
Guadeloupe	Port-Louis (GUA-PL)	16°25'10"N / 61°32'31"W	11	2011	30	23	25	23
	Baie de Bouillante (GUA-BB)	16°7'52"N / 61°46'47"W	6-8	2011	30	26	27	25
	Les Saintes (GUA-SAI)	15°51'56"N / 61°36'0"W	10-17	2011	30	21	25	22
Martinica	Point Borgnèse (MAR)	14°26'18"N / 60°54'54"W	10-15	2010	30	21	20	18
Bequia	Lower Bay (BEQ)	12°59'50"N / 61°14'51"W	7-9	2011	30	23	30	28
Canouan	Rameau Bay (CAN)	12°43'28"N / 61°19'58"W	7-8	2011	30	21	30	23
Barbados	Carlisle Bay (BARB)	13°4'26"N / 59°37'0"W	12-15	2012	30	25	30	27
					410	**308**	**327**	**297**

Table 4. Sampling informations including state, site, GPS coordinates, depth, year and total number of samplings for COI and microsatellites (µsats) analyses. *D. p*: *Dissodactylus primitivus*; *M. v*: *Meoma ventricosa*. **D. p* was sampled in 2009 and *M. v* in 2006.

Samples were collected by SCUBA diving or snorkeling at depths ranging from 3 to 22m (Table 4). Sea urchins were sampled individually in plastic bags that were immediately

tied up after collection. Immediately on board, or in the laboratory, a sample of each sea urchin (2-3 spines) and all the crabs captured on the individual host were isolated and preserved in pure ethanol.

The total numbers of specimens for microsatellites analyses were 327 sea urchins and 410 crabs (Table 4). For COI analyses, we used 297 sea urchins and 309 crabs (Table 4).

DNA extraction

For each crab, DNA was extracted from two pereopods using the Chelex resin method (Walsh et al. 1991; see the detailed protocol in Jossart et al. 2014). For each sea urchin, DNA was extracted from two spines using the Qiagen DNeasy Blood & Tissue Kit, following manufacturer's instructions.

COI data collection and analysis

For crabs, a 652 base pairs fragment was amplified using the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Each PCR reaction included 7.5 µl of Master Mix Qiagen (Taq Polymerase, nucleotides), 2 µl of DNA, 0.6 µl (10 µM) of each forward or reverse primer and 4.3 µl of sterile water. PCR conditions consisted of 35 cycles for each of the 3 temperature steps [60 s at 94°C (denaturation), 60 s at 40°C (annealing) and 120 s at 72°C (elongation)]. These cycles were preceded by a step of 2 min at 94°C and were followed by a step of 2 min at 72°C. After amplification, 0.8 µl of sterile water were added, with 0.2 µl of Exonuclease I (Affymetrix) and 1 µl of Shrimp Alkaline Phosphatase (Affymetrix), to purify amplified DNA from dNTPs and primers. Samples were incubated 60 min at 37°C and 10 min at 80°C. The plates were then dried overnight in an oven at 37 °C. Finally, plates were sent to the MACROGEN sequencing service. Sequences edition and alignment were performed using MEGA 5.1 (Tamura et al. 2011).

For sea urchins, we amplified a 758 base pairs fragment using the primers AJ639919 (5'-GCYTGAGCWGGCATGGTAGG-3' / 5'-GCTCGTGCRTCTACRTCCAT-3') (Stockley et al. 2005; Egea 2011). Each PCR reaction (15 µl) included 7.5 µl of Master Mix Qiagen (Taq Polymerase, nucleotides), 1 µl of DNA, 0.3 µl (10 µM) of each forward or reverse primer and 5.9 µl of sterile water. PCR conditions consisted of 35 cycles for each of the three temperature steps [40 s at 94°C (denaturation), 30 s at 52°C (annealing) and 60 s at

72°C (elongation)]. These cycles were preceded by a step of 4 min at 95°C and were followed by a step of 5 min at 72°C. Purification and sequencing steps were as for crabs.

Arlequin 3.5 (Excoffier & Lischer 2010) was used to calculate the number of haplotypes, haplotype diversity, mean pairwise differences among haplotypes and the nucleotide diversity.

Haplotype networks (Minimum Spanning Networks) were constructed using MINSPNET (Excoffier & Smouse 1994) and HapStar 0.5 (Teacher & Griffiths 2011).

Using Arlequin 3.5, we evaluated demographic expansion or stability. Following Ramos-Onsins & Rozas (2002), two statistics were used. The first one was Fu's F_S statistic, testing for an excess of low-frequency haplotypes in an expanding population compared to a stable one (Fu 1997; Monceau et al. 2013). We also calculated the square differences statistic (SSD, mismatch distribution) that compare the observed distribution of the number of nucleotide differences between pairs of haplotypes with the distribution expected under the null hypothesis of population expansion (Schneider & Excoffier 1999; Monceau et al. 2013). Finally, a SSD calculation was also done to evaluate a potential spatial expansion (Ray et al. 2003; Monceau et al. 2013). This model assumes that gene flow between interconnected populations is part of the demographic expansion. Historical demographics analyses were performed for the whole data set in *M. ventricosa* and according to the two identified lineages in *D. primitivus* (see Results).

Arlequin 3.5 was also used to calculate sampling sites pairwise Φ_{ST} (Tajima & Nei 1984) and exact tests of population differentiation (significance evaluated using 10,000 and 100,000 permutations, respectively) (Goudet et al. 1996). Using SPADE, we evaluated the actual differentiation D (bootstrap replicates of 10,000) (Jost 2008; Chao & Shen 2010).

Finally, we tested isolation by distance (IBD) with a Mantel test (Φ_{ST} vs km), using the software Mantel 1.19 (life.bio.sunysb.edu/morph/soft-mult.html). The geographical distance corresponded to the shortest distance avoiding islands (barriers) and was calculated using the path tool in Google Earth. This analysis was performed both for all sampled sites and within the Lesser Antilles only.

Microsatellites data collection and analysis

For crabs, we used ten loci that were designed and utilized for the Jamaican populations (Anderson et al. 2010; Jossart et al. 2013; Jossart et al. 2014). These loci were amplified by PCR using the primers in four multiplex and genotyped with an AB 3730 DNA Analyzer (see Jossart et al. 2013 for detailed protocol and primers sequences).

For sea urchins, eight microsatellites loci characterized for *Meoma ventricosa* (Jossart et al. submitted). Microsatellites were amplified in simplex according to the tagged primer-method (Schuelke 2000) and genotyping step was done using an AB 3130xL Genetic Analyzer (see Jossart et al. submitted for detailed protocol and primers sequences). Genotypes were deduced from electropherograms using the software Peak Scanner (https://products.appliedbiosystems.com). Allelic binning was done using the program Autobin but each genotype was verified by eye (Guichoux et al. 2011).

We evaluated (using POWSIM 4.1) that these microsatellites had a statistical power (1–β) of 0.999 (associated with an F_{ST} of 0.0075) for the present data set. The retained parameter values were selected according to the instructions of POWSIM manual (Ne of 2000; 10 generations of drift; 1000 runs). For each locus, the frequency of null alleles as well as linkage disequilibrium were calculated using Genepop 4.2.2 (Rousset 2008). The software FSTAT 2.9.3.2 was used to estimate genetic variability i.e. number of alleles, allelic richness (AR) (Goudet 1995). Differences between sites in mean AR were tested using a Kruskal-Wallis test. Deviations from Hardy-Weinberg equilibrium (F_{IS}) were assessed using FSTAT. The significance of F_{IS} was evaluated using two permutation tests (2800 permutations for *D. primitivus* and 2080 permutations for *M. ventricosa*): one testing for heterozygote excess and the other testing for heterozygote deficiency.

Sampling sites pairwise F_{ST} (Weir and Cockerham' Theta, θ_{WC}) values were calculated using SPAGeDi 1.4 (Weir & Cockerham 1984; Hardy & Vekemans 2002). The significance of F_{ST} was evaluated using a permutation test (20,000 permutations). For *M. ventricosa*, we also evaluated adjusted F_{ST} values for null alleles with the software FreeNA (Chapuis & Estoup 2007). Using Genepop 4.2.2, we performed sampling sites pairwise exact tests of differentiation (Goudet et al. 1996). The sampling sites pairwise differentiation D of Jost (2008) was calculated using the software DEMEtics (Gerlach et al. 2010). We evaluated the possibility of isolation by distance (IBD) by a Mantel test (F_{ST} / (1-F_{ST}) vs ln

[km]) using the software Mantel 1.19. This analysis was performed both for all sampled sites and within the Lesser Antilles only.

Finally, Bayesian clustering approach was implemented to infer the most probable number (K) of genetic clusters using the software STRUCTURE 2.3.4 (Pritchard et al. 2000). For *M. ventricosa*, we used a putative value of K (1–13) and 10 independent simulations, using the following parameters: running lengths of 100,000, admixture model (indicating the sampling location to the software), alpha inferred, allele frequencies correlated among populations and potentiality of null alleles activated. For *D. primitivus*, we also used STRUCTURE with a putative value of K (1–14) and for 10 independent simulations, using the following parameters: running lengths of 100,000, admixture model (without indicating the sampling location to the software), alpha inferred and allele frequencies correlated among populations. The most likely value of K was determined using the original method (described in the STRUCTURE manual) and using the method of Evanno et al. (2005) implemented in the software STRUCTURE HARVESTER (Earl & vonHoldt 2012). The barplots were made using the software Distruct 1.1 (Rosenberg 2004). For the crabs, we also used BAPS 6.0 in complement of the STRUCTURE results (Corander & Marttinen 2006).

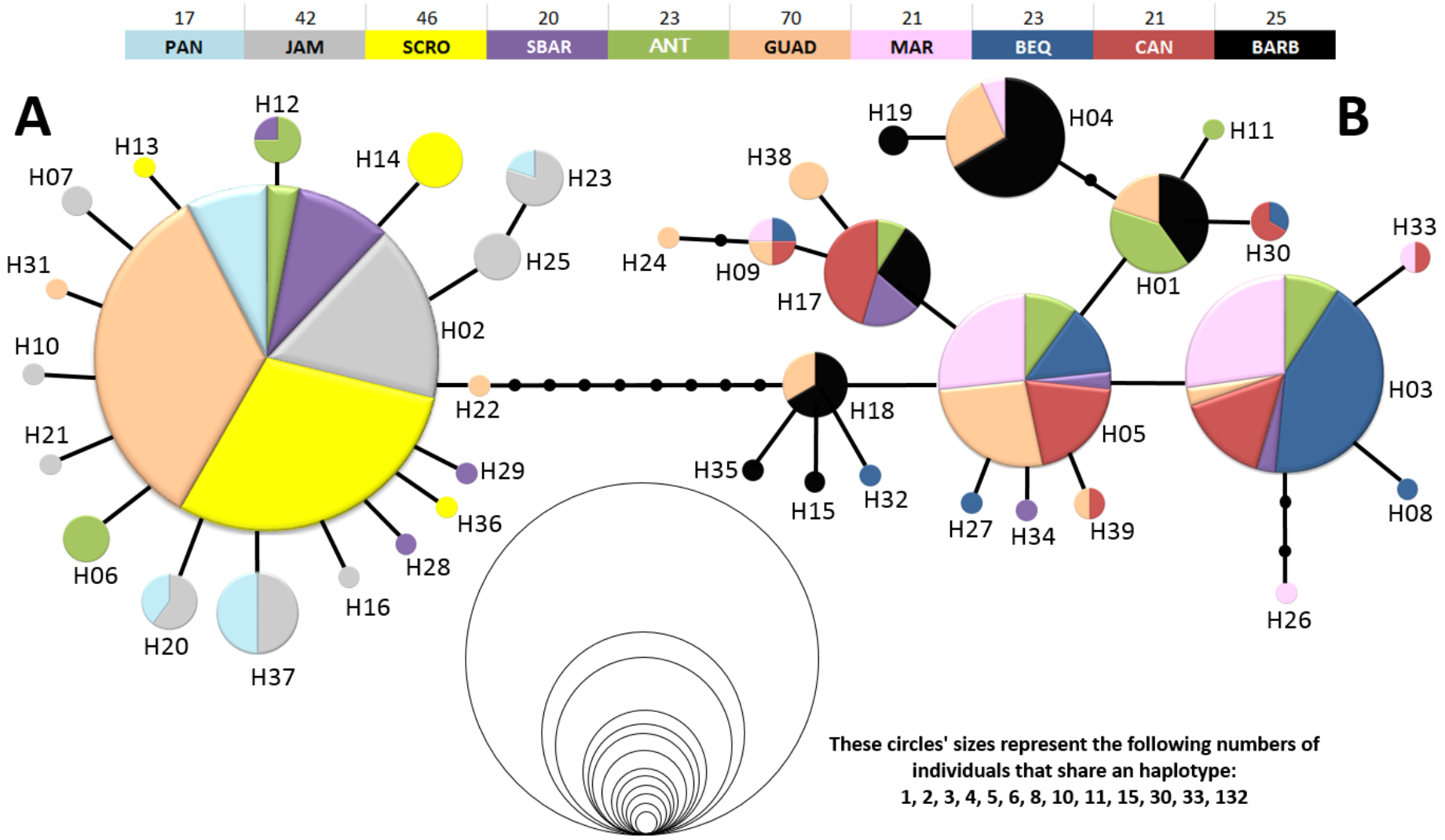


Fig. 20. Haplotype network for COI analysis in *D. primitivus*. Some sites (undifferentiated, see results) from the same island were pooled to improve visualization. Sampling size are indicated above the island's abbreviation.

Results

COI data

The total number of haplotypes for *D. primitivus* was 39 out of 308 individuals sequenced and they were distributed in two molecularly divergent groups (named A: 18 haplotypes and B: 21 haplotypes) separated by at least 10 nucleotides (Fig. 20). In addition, the haplotypic diversity was highly geographically structured along the Central America, Greater Antilles and Lesser Antilles. Panama, Jamaica and Saint Croix harbored crabs with haplotypes exclusively from the A group while Martinica, Bequia, Canouan and Barbados were only found in the B group (Fig. 20). The remaining islands (Guadeloupe, Antigua, Saint Barthélemy) harbored crabs with haplotypes from both groups (Fig. 20). The mean pairwise differences (MPD) among haplotypes were low (mean 2.82) but highly variable among sampled sites (SE ± 2.49, Table 5) and with highest values in islands where the two haplotype groups were present. The mean number of haplotypes per site was 6.21 (± 1.76) with a minimum of 2 (Saint Croix – Kings Bay) and a maximum of 8 (three sites) (Table 5). The nucleotide diversity was low in all sites (0.0043 ± 0.0038 in average) and the mean haplotype diversity was equal to 0.63 (±0.18).

In *M. ventricosa*, the total number of haplotypes was 38 out of a total of 297 individuals sequenced with three frequent haplotypes (H1 20%, H2 18% and H3 19%; Fig. 21). The MPD among haplotypes across sampling sites was low (3.23) and weakly variable across sampled sites (SE = ± 0.32) (Table 5, Fig. 21). The mean number of haplotypes per site was 10.46 (± 2.50) with a minimum of 7 (Panama) and a maximum of 17 (Bequia). The nucleotide diversity was low in all sites (mean = 0.0043 ± 0.0004) and the mean haplotype diversity was equal to 0.88 (± 0.04).

	Dissodactylus primitivus					*Meoma ventricosa*				
	N	**N_a**	**h**	**MPD**	**π**	**N**	**N_a**	**h**	**MPD**	**π**
PAN	17	4	0.62 ±0.11	0.84 ±0.63	0.0013 ±0.0011	16	7	0.85 ± 0.06	2.77 ± 1.54	0.0037 ± 0.0023
JAM-WL	22	6	0.69 ±0.10	1.00 ±0.70	0.0015 ±0.0012	13	9	0.94 ± 0.05	3.86 ± 2.07	0.0051 ± 0.0031
JAM-PTB	20	8	0.75 ±0.10	1.12 ±0.76	0.0017 ±0.0013	-	-	-	-	-
SCRO-TB	24	2	0.25 ± 0.11	0.25 ± 0.29	0.0003 ±0.0005	28	9	0.84 ± 0.05	2.85 ± 1.56	0.0038 ± 0.0023
SCRO-KB	22	4	0.31 ± 0.12	0.33 ± 0.34	0.0005 ± 0.0005	23	13	0.90 ± 0.04	3.39 ± 1.79	0.0048 ± 0.0026
SBAR	20	8	0.65 ± 0.12	5.03 ± 2.55	0.0077 ± 0.0044	25	9	0.84 ± 0.05	2.99 ± 1.61	0.0039 ± 0.0024
ANT	23	8	0.89 ± 0.03	7.04 ± 3.43	0.0108 ± 0.0059	26	9	0.84 ± 0.05	3.06 ± 1.65	0.0040 ± 0.0024
GUAD-PL	23	7	0.66 ± 0.10	6.11 ± 3.02	0.0094 ± 0.0052	23	10	0.85 ± 0.06	3.25 ± 1.74	0.0043 ± 0.0026
GUAD-BB	26	7	0.47 ± 0.12	4.73 ± 2.39	0.0073 ± 0.0041	25	11	0.89 ± 0.04	3.08 ± 1.66	0.0041 ± 0.0024
GUAD-SAI	21	6	0.61 ± 0.12	6.50 ± 3.20	0.0100 ± 0.0055	22	10	0.87 ± 0.05	3.43 ± 1.82	0.0045 ± 0.0027
MAR	21	6	0.70 ± 0.07	1.37 ± 0.88	0.0021 ± 0.0015	18	9	0.87 ± 0.06	3.05 ± 1.67	0.0040 ± 0.0025
BEQ	23	7	0.62 ± 0.11	1.17 ± 0.78	0.0018 ± 0.0013	28	17	0.96 ± 0.02	3.66 ± 1.91	0.0048 ± 0.0028
CAN	21	7	0.83 ± 0.05	1.50 ± 0.94	0.0023 ± 0.0016	23	11	0.91 ± 0.03	3.46 ± 1.83	0.0046 ± 0.0027
BARB	25	7	0.80 ± 0.06	2.43 ± 1.36	0.0037 ± 0.0023	27	12	0.88 ± 0.04	3.20 ± 1.70	0.0042 ± 0.0025
Average	**22 ± 2.29**	**6.21 ± 1.76**	**0.63 ± 0.18**	**2.82 ± 2.49**	**0.0043 ± 0.0038**	**23 ± 4.63**	**10.46 ± 2.50**	**0.88 ± 0.04**	**3.23 ± 0.32**	**0.0043 ± 0.0004**

Table 5. Diversity indexes for COI data in *D. primitivus* and *M. ventricosa*. N: number of samples, N_a: number of haplotypes, h: haplotype diversity, MPD: mean pairwise differences among haplotypes, π: nucleotide diversity.

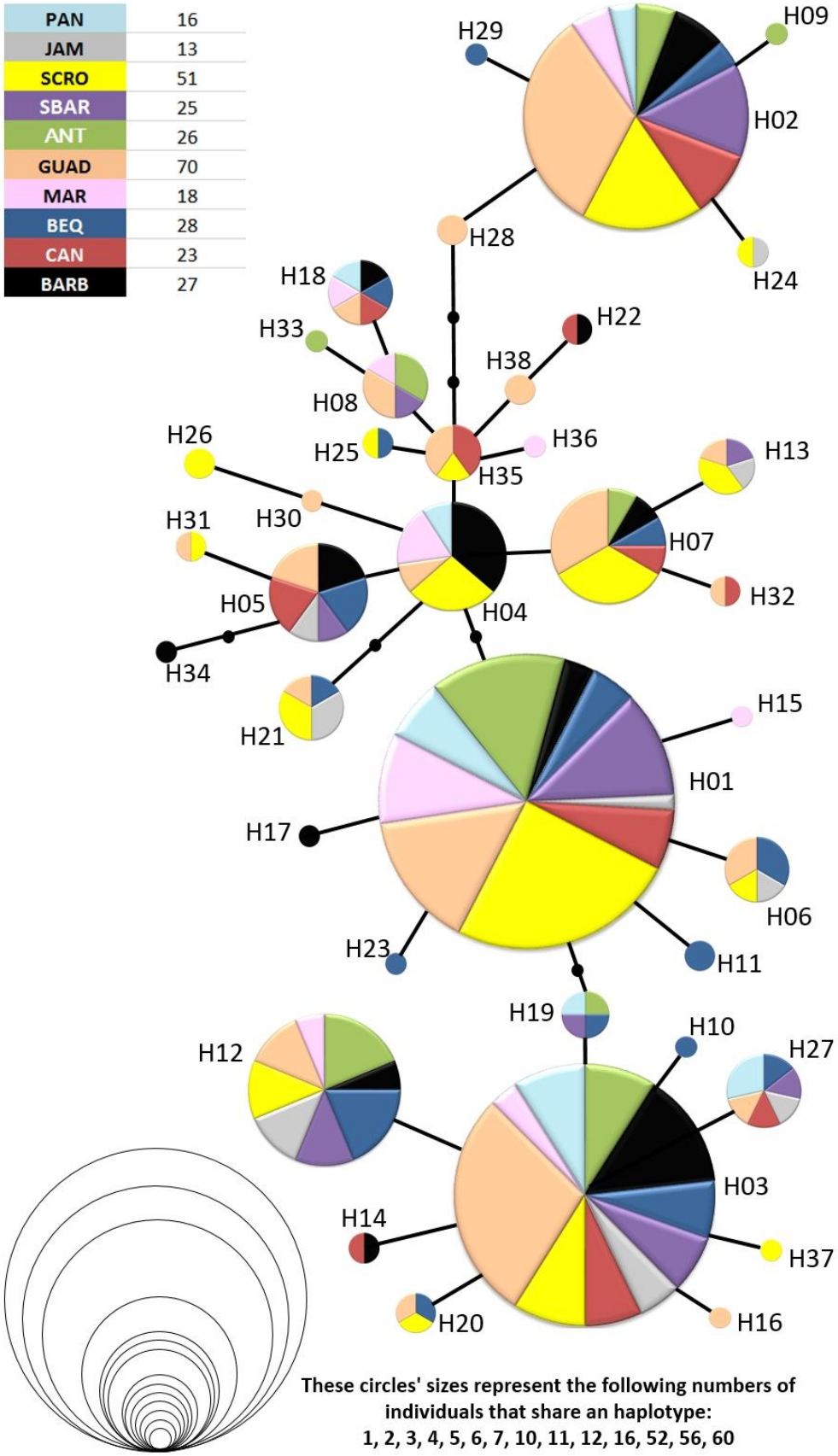


Fig. 21. Haplotype network for COI analysis in *M. ventricosa*. Some sites (undifferentiated, see results) from the same island were pooled to improve visualization. Sampling size are indicated next to the island's abbreviation.

	PAN	JAM-WL	JAM-PTB	SCRO-TB	SCRO-KB	SBAR	ANT	GUAD-PL	GUAD-BB	GUAD-SAI	MAR	BEQ	CAN	BARB
PAN		-0.022	0.022	**0.12**	**0.1**	0.159	**0.395**	**0.238**	0.169	**0.266**	**0.907**	**0.916**	**0.899**	**0.863**
JAM-WL	-0.017		-0.023	**0.089**	**0.075**	**0.17**	**0.415**	**0.254**	**0.179**	**0.283**	**0.903**	**0.911**	**0.896**	**0.865**
JAM-PTB	-	-		**0.08**	**0.067**	0.158	**0.401**	**0.238**	**0.168**	**0.266**	**0.898**	**0.906**	**0.891**	**0.858**
SCRO-TB	0.022	0.025	-		-0.022	**0.194**	**0.443**	**0.279**	**0.2**	**0.311**	**0.934**	**0.94**	**0.927**	**0.89**
SCRO-KB	0.063	0.028	-	-0.016		**0.198**	**0.451**	**0.285**	**0.204**	**0.318**	**0.932**	**0.938**	**0.926**	**0.891**
SBAR	-0.007	0.017	-	-0.011	0.01		0.093	-0.017	-0.042	-0.009	**0.663**	**0.681**	**0.648**	**0.649**
ANT	-0.019	0.008	-	-0.012	0.022	-0.02		0.023	0.093	0.007	**0.367**	**0.389**	**0.347**	**0.382**
GUAD-PL	0.001	-0.019	-	0.044	0.062	0.034	0.025		-0.018	-0.041	**0.552**	**0.573**	**0.534**	**0.534**
GUAD-BB	0.026	0.025	-	-0.022	-0.013	-0.024	0.002	0.031		-0.01	**0.651**	**0.667**	**0.637**	**0.639**
GUAD-SAI	0.034	0.04	-	0.004	0.004	-0.019	0.013	0.038	-0.027		**0.524**	**0.547**	**0.503**	**0.509**
MAR	0.032	0.047	-	-0.028	-0.009	-0.003	-0.018	0.059	-0.006	-0.001		-0.014	0.032	**0.337**
BEQ	-0.028	-0.027	-	-0.007	0.019	-0.01	-0.022	0.005	0.002	0.018	0,000		0.101	**0.402**
CAN	0.01	0.003	-	-0.012	0.001	-0.004	0.002	-0.005	-0.019	-0.018	-0.009	-0.006		**0.3**
BARB	-0.002	-0.018	-	0.018	0.032	0.016	0.013	-0.018	0.013	0.019	0.028	-0.009	-0.023	

Table 7. Sampling sites pairwise Φ_{ST} values for COI analysis in *D. primitivus* (above diagonal) and *M. ventricosa* (below diagonal). Bold values differ significantly from zero (permutation tests with 10,000 permutations). The p-threshold was corrected by Benjamini-Yekutieli method (Narum 2006) and was 0.0094 for *D. primitivus* and 0.0099 for *M. ventricosa*.

In *D. primitivus*, Fu's F_S was negative and significant for both haplotype groups (A & B). Mismatch analyses detected a demographic expansion as well as a spatial expansion in both groups (Table 6). *In M. ventricosa* (whole dataset), Fu's F_S was negative and significant. Mismatch analyses did not detect a demographic expansion but detected a spatial expansion (Table 6).

		Fu's *Fs*			**Mismatch distribution analysis**					
					Demographic expansion			**Spatial expansion**		
		F_S	p	↑→	SSD	p	↑→	SSD	p	↑→
Mv	All	-18.31	0.003	↑	0.0329	0.012	→	0.0272	0.062	↑
Dp	A	-21.16	0.001	↑	0.0004	0.68	↑	0.0004	0.29	↑
	B	-8.01	0.015	↑	0.0043	0.28	↑	0.0043	0.22	↑

Table 6. Fu's F_S statistic and mismatch distribution analysis. SSD: square differences statistic. An up arrow indicates expansion while a horizontal arrow indicates stability.

Sampling sites pairwise Φ_{ST} values were highly contrasted between the host and its parasite (Table 7). None was significantly different from 0 for *M. ventricosa*, while most of them where significant for *D. primitivus* (Table 7). In *D. primitivus*, all Φ_{ST} values were close to 0 for sites from the same island. Moreover, the Φ_{ST} were also very low between the sites from Panama and Jamaica. All Φ_{ST} pairwise comparisons involving sites from Martinica, Bequia, Canouan and Barbados were significant and even close to 1 for some of them (Table 7). D values and exact tests of differentiation led to the same trends (Annexe 1).

The Mantel test, performed for the crabs, detected an isolation by distance for the whole data set ($r = 0.262$, $p < 0.04$). However, the r value increased to 0.524 ($p < 0.002$) when only the Lesser Antilles were considered (Fig. 22).

Microsatellites data

For *D. primitivus*, the frequencies in null alleles were low for each locus for the large majority of sampling sites. Contrastingly, two loci in *M. ventricosa* (NLQK, 6SKB) had frequencies in null alleles higher than 0.10 in most of the sites investigated. In both *D. primitivus* and *M. ventricosa*, no linkage disequilibrium was detected between each pair of loci in each population (600 and 364 pairwise comparisons, p threshold Benjamini-Yekutieli corrected to 0.0071 and 0.0076, respectively). In *D. primitivus*, no F_{IS} values per site were associated to a heterozygote deficiency but five sites showed heterozygote

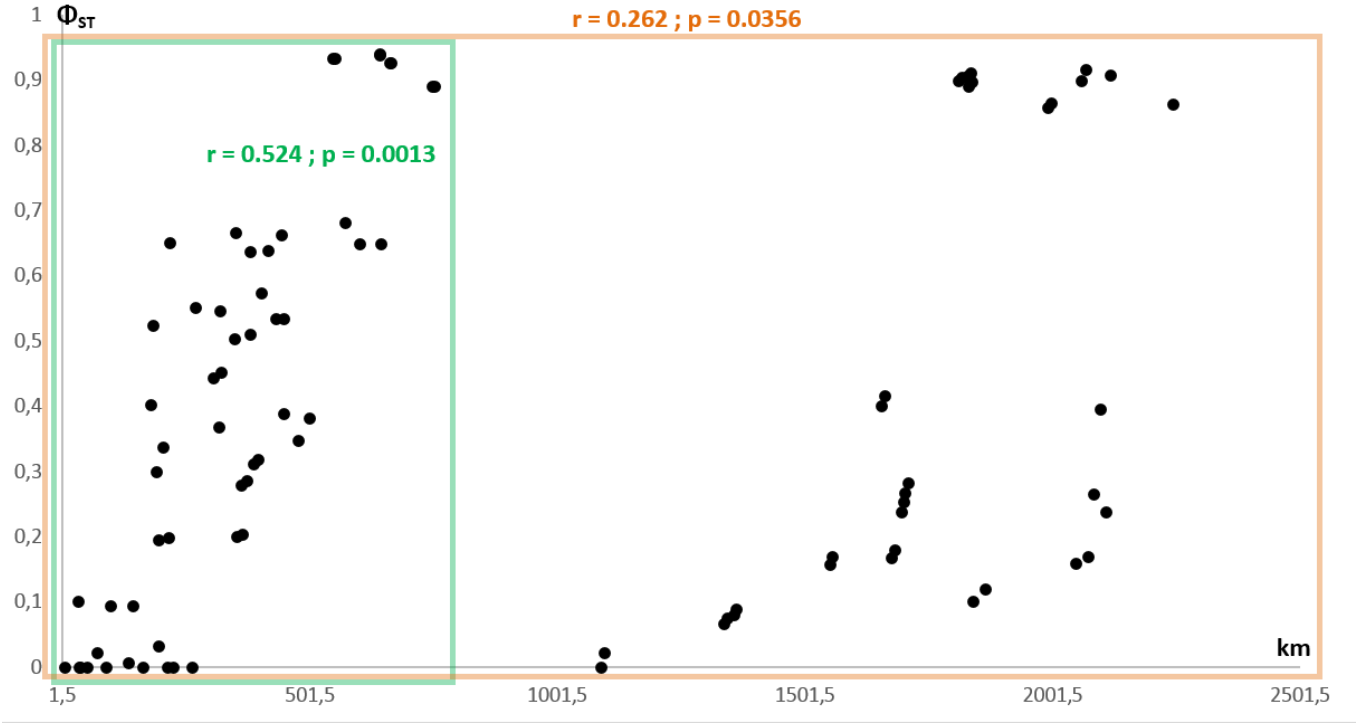


Fig. 22. Isolation by distance (IBD) by a Mantel test (ΦST vs km) for *D. primitivus.* The orange frame correspond to the whole data set and the green frame to the Lesser Antilles only.

	Dissodactylus primitivus				*Meoma ventricosa*			
	N	**N_a**	**AR**	***F_{IS}***	**N**	**N_a**	**AR**	***F_{IS}***
PAN	20	7.0 ± 1.2	6.5 ± 1.3	-0.104*	17	7.0 ± 3.9	6.1 ± 3.0	0.122**
JAM-WL	30	7.8 ± 2.0	6.7 ± 1.7	-0.076*	12	6.9 ± 4.0	6.7 ± 3.9	0.090
JAM-PTB	30	8.5 ± 1.9	7.4 ± 1.6	-0.127*	-	-	-	-
SCRO-TB	30	9.7 ± 3.6	7.7 ± 2.6	0.025	29	8.0 ± 5.0	6.2 ± 3.3	0.165**
SCRO-KB	30	9.9 ± 4.0	8.0 ± 2.8	0.059	26	8.1 ± 4.8	6.3 ± 3.2	0.140**
SBAR	30	9.1 ± 2.6	8.0 ± 2.2	-0.098*	30	8.8 ± 5.3	6.6 ± 3.5	0.145**
ANT	30	8.3 ± 2.8	7.1 ± 2.3	-0.077*	26	7.3 ± 4.3	6.0 ± 3.1	0.040
GUAD-PL	30	9.2 ± 2.9	7.5 ± 2.2	0.034	25	8.6 ± 4.7	6.6 ± 3.2	0.150**
GUAD-BB	30	8.5 ± 2.6	7.3 ± 2.3	0.021	27	7.6 ± 3.9	6.2 ± 2.9	0.061
GUAD-SAI	30	9.4 ± 2.5	7.8 ± 2.2	-0.006	25	7.9 ± 4.4	6.0 ± 3.0	0.113**
MAR	30	9.1 ± 2.8	7.3 ± 2.5	-0.007	20	7.4 ± 3.5	6.3 ± 2.8	0.092
BEQ	30	9.3 ± 3.1	7.5 ± 2.2	-0.030	30	7.9 ± 3.9	6.3 ± 3.0	0.198**
CAN	30	8.3 ± 2.6	7.0 ± 2.1	0.025	30	7.9 ± 4.8	6.2 ± 3.1	0.168**
BARB	30	6.7 ± 3.1	5.6 ± 2.4	-0.049	30	8.0 ± 4.3	6.2 ± 3.0	0.205**
Average	**29 ± 2.67**	**8.6 ± 1.0**	**7.2 ± 0.7**	**-0.029 ± 0.059**	**25 ± 5.63**	**7.8 ± 0.6**	**6.3 ± 0.2**	**0.130 ± 0.050**

Table 8. N (number of samples), Number of alleles (N_a), Allelic Richness (AR) and F_{IS} for microsatellites data in *D. primitivus* and *M. ventricosa*. *indicates heterozygote excess (p<0.02, Benjamini-Yekutieli corrected) and ** indicates heterozygote deficiency (p<0.02, Benjamini-Yekutieli corrected) (Narum 2006).

excess (Table 8). In *M. ventricosa*, most of the sites showed a heterozygote deficiency that can be linked to the presence of null alleles in NLQK and 6SKB (Table 8). The average number of alleles was 8.6 (± 1.0) in *D. primitivus* and 7.8 (± 0.6) in *M. ventricosa*. In *D. primitivus*, the mean Allelic Richness (AR) was equal to 7.2 (± 0.7) and did not significantly differ among sites (Kruskal-Wallis test: H = 10.00; p = 0.69). In *M. ventricosa*, the mean AR was 6.3 (± 0.2) and did not significantly differ among sites (Kruskal-Wallis test: H = 0.97 p = 1).

Like for COI analysis, F_{ST} analyses were highly contrasted between the two species (Table 9). The large majority of F_{ST} were close to 0 for *M. ventricosa* whatever the calculation method (adjusted or non-adjusted for null alleles) (Table 9, Annexe 2). Only three of them were significantly different from zero namely Guadeloupe (Les Saintes) / Guadeloupe (Baie de Bouillante): 0.0187; Barbade / Martinica: 0.0322 and Barbade / Canouan: 0.0171. In *D. primitivus*, all F_{ST} pairwise values were significantly different from 0 except comparisons between sites from the same island, between Martinica and Grenadines (Bequia *vs* Canouan) or between Guadeloupe (Baie de Bouillante) and Bequia (Table 9). Highest F_{ST} were observed when Barbados was involved, even for comparisons with geographically close islands. D values and exact tests of differentiation led to the same trends (Annexe 3). Isolation by distance of crab populations was detected by Mantel tests, both for the whole data set ($r = 0.657$; $p<0.00001$) and within the Lesser Antilles ($r = 0.524$; $p < 0.005$).

For *M. ventricosa*, STRUCTURE identified that the most probable number (K) of genetic clusters was 1. In *D. primitivus*, the most probable K was 4 for the original method and 2 for the Evanno method. The barplot for K = 2 (Fig. 23) showed that the individuals from Panama Jamaica and St Croix were assigned in majority to one genetic cluster. On the other hand, Martinica, Bequia, Canouan and Barbados were highly associated to the other cluster, while remaining islands (St Barthélemy, Antigua, Guadeloupe) had more intermediate assignments. For K = 4 (Fig. 23), the same situation was observed except that Barbados segregated in a single genetic cluster. The most probable K value in BAPS was 6 (Fig. 23) and the same subdivisions as those obtained with STRUCTURE are observed, with refinements. Panama and Jamaica were still highly assigned to a single cluster (green color on down plot in Fig. 23), cluster also shared by most samples from

	PAN	JAM-WL	JAM-PTB	SCRO-TB	SCRO-KB	SBAR	ANT	GUAD-PL	GUAD-BB	GUAD-SAI	MAR	BEQ	CAN	BARB
PAN		**0.0159**	**0.0196**	**0.0379**	**0.0342**	**0.0478**	**0.0658**	**0.0628**	**0.0745**	**0.0754**	**0.0807**	**0.0721**	**0.0926**	**0.1086**
JAM-WL	0.0014		0.0056	**0.0258**	**0.0266**	**0.0393**	**0.0613**	**0.0480**	**0.0523**	**0.0491**	**0.0671**	**0.0555**	**0.0742**	**0.0950**
JAM-PTB	-	-		**0.0273**	**0.0235**	**0.0327**	**0.0567**	**0.0503**	**0.0495**	**0.0540**	**0.0677**	**0.0583**	**0.0675**	**0.0949**
SCRO-TB	0.0020	-0.0083	-		**-0.0013**	**0.0154**	**0.0332**	**0.0145**	**0.0229**	**0.0224**	**0.0471**	**0.0302**	**0.0509**	**0.0790**
SCRO-KB	0.0114	0.0024	-	-0.0021		**0.0205**	**0.0375**	**0.0202**	**0.0246**	**0.0285**	**0.0526**	**0.0400**	**0.0601**	**0.0947**
SBAR	-0.0019	0.0064	-	0.0001	0.0030		**0.0214**	**0.0212**	**0.0207**	**0.0211**	**0.0391**	**0.0251**	**0.0363**	**0.0654**
ANT	0.0124	-0.0024	-	0.0018	-0.0015	0.0011		0.0186	0.0222	**0.0253**	**0.0347**	**0.0229**	**0.0349**	**0.0715**
GUAD-PL	0.0050	0.0038	-	-0.0076	-0.0094	-0.0069	-0.0076		-0.0019	**-0.0005**	**0.0286**	**0.0198**	**0.0240**	**0.0636**
GUAD-BB	0.0115	0.0091	-	0.0062	-0.0071	0.0037	-0.0035	-0.0051		**-0.0015**	**0.0194**	0.0100	**0.0158**	**0.0618**
GUAD-SAI	-0.0053	-0.0056	-	0.0042	0.0094	0.0045	0.0076	0.0011	**0.0187**		**0.0328**	**0.0195**	**0.0291**	**0.0701**
MAR	0.0066	0.0079	-	-0.0051	0.0095	0.0104	0.0166	0.0018	0.0084	0.0171		0.0008	0.0014	**0.0636**
BEQ	0.0049	0.0009	-	0.0067	0.0005	0.0005	-0.0050	-0.0078	-0.0030	-0.0025	0.0129		0.0009	**0.0592**
CAN	0.0113	-0.0025	-	0.0050	0.0098	0.0143	0.0132	-0.0004	0.0089	0.0044	0.0105	-0.0021		**0.0587**
BARB	0.0134	0.0251	-	0.0180	0.0008	0.0044	0.0046	-0.0031	-0.0041	0.0148	**0.0322**	0.0035	**0.0171**	

Table 9. F_{ST} (θ_{WC}) values for microsatellites analysis in *D. primitivus* (above diagonal) and *M. ventricosa* (below diagonal). Bold values differ significantly from zero (permutation tests with 20,000 permutations). The p-threshold was corrected by Benjamini-Yekutieli method (Narum 2006) and were 0.0094 for *D. primitivus* and 0.0099 for *M. ventricosa*.

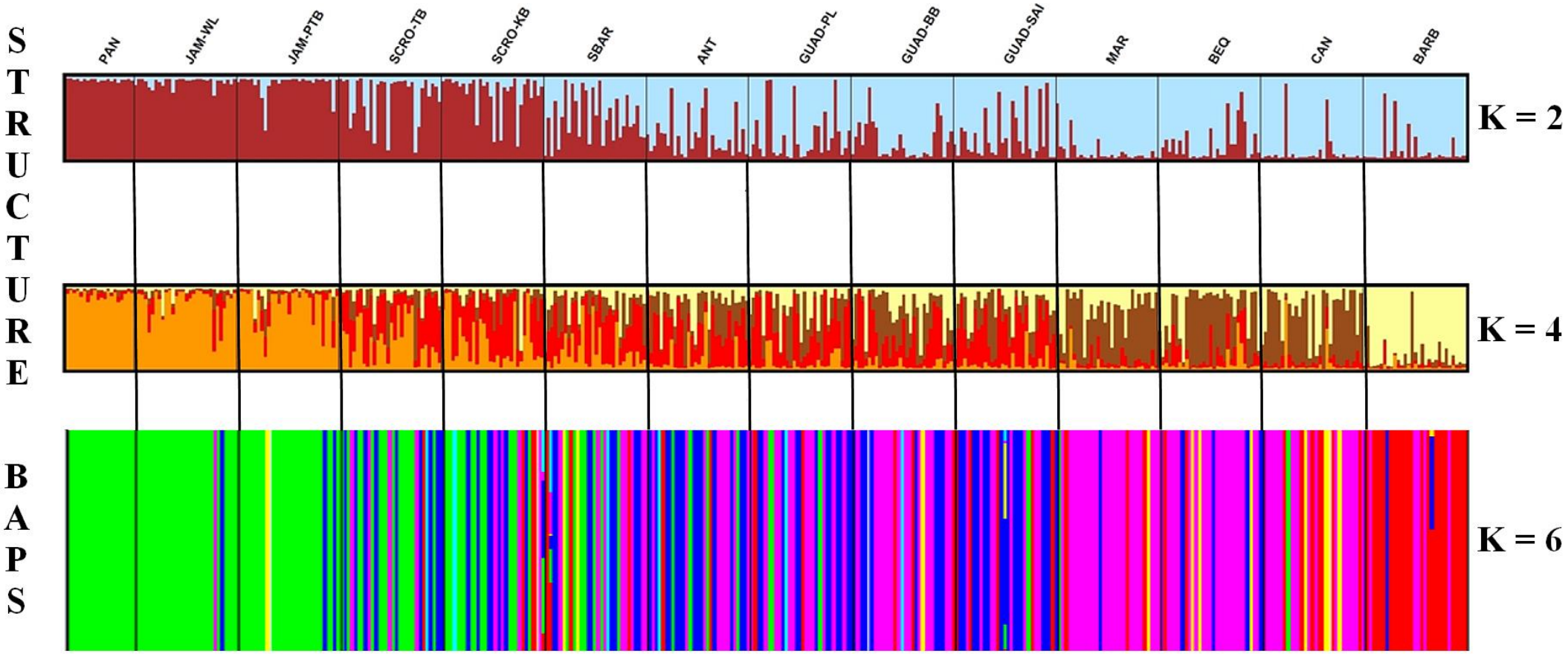


Fig. 23. STRUCTURE barplots for K = 2 (up), K = 4 (middle) and BAPS barplot for K = 6 (down) in *D. primitivus*. Each line corresponds to an individual that was assigned with a certain probability to each genetic cluster.

St Croix. Martinica, Bequia and Canouan were mostly assigned to a distinct cluster (pink), and Barbados to another (red). Few rare specimens from Martinica and Grenadines were attributed to a small cluster (orange). Most crabs from Guadeloupe sites, as well as most from St Barthélemy and Antigua belong to a fifth genetic cluster (blue), albeit numerous crabs from these islands were assigned to one of the three main clusters, mostly pink and red, with few greens. Finally, the sixth cluster (yellow) is distributed among most populations, probably reflecting crabs with genotypes for which assignment was difficult.

Discussion

Host-parasite comparison

The main finding of this study is that the two interacting species exhibited highly contrasted genetic structures within the Caribbean Sea. While the sea urchin host *Meoma ventricosa* showed a homogenous distribution of its genetic diversity (both mitochondrial and nuclear) across the entire geographic zone investigated, the parasite crab *Dissodactylus primitivus* variation was subdivided in several structured populations. The crab haplotypic COI diversity is divided in two main groups, one mostly found in the western part of the region, the other mostly found in the eastern part (groups A and B, Fig. 20). Variation in the nuclear genetic diversity, estimated here by microsatellites, provides refinements on this east-west dichotomy. Four main geographical groups were identified (groups I to IV). A "western" group includes Panama, Jamaica and St Croix (group I); a "central" group includes St Barthélemy, Antigua and Guadeloupe islands (group II); a "southern" group comprises Martinica and Grenadine islands (III), and Barbados Island forms the group IV. Although relatively well separated, group II is not genetically original as it consists in a mixture of microsatellites types from other groups, particularly from groups I and III. At a local scale (i.e. among sites from the same islands), crab populations can be considered as homogenous, confirming previous results obtained in Jamaica (Jossart et al. 2013). However, at a wider spatial scale, all sites are genetically differentiated from others and there was a significant isolation by distance even at relatively small geographic scale (e.g. within Lesser Antilles).

Several factors could explain the contrasted patterns between crabs and their sea urchin hosts. Differences in gene flow probably reflect differences in dispersion ability of the

animals. While both adult crabs and sea urchins are able to move (De Bruyn 2010; personal observations), such movements are made at very local scale (dozens of meters) and cannot explain the genetic homogeneity observed in *M. ventricosa* at the Caribbean scale. Therefore, dispersal capacity has to be considered at larval pelagic stages. It is conventional to consider pelagic larval duration (PLD) as a proxy of dispersal distance although the relation of PLD with genetic structure it is still under debate (Shulman & Bermingham 1995; Selkoe & Toonen 2011, Faurby & Barber 2012). The PLD of *M. ventricosa* is unknown but data for a temperate spatangoid (*Echinocardium cordatum*) indicate a PLD of 2 to 6 weeks (Nichols 1962; Kashenko 2007). However, it is recognized that the PLD is longer in temperate zone (due to lower temperatures) and could highly vary among related species (Emlet et al. 1987). Regarding the PLD of tropical sea urchins species (Emlet et al. 1987), it is probable that the PLD of *M. ventricosa* is at least equal to the PLD of *D. primitivus* which is equal to two weeks but this remains to be verified. Owing this uncertainty regarding *M. ventricosa* PLD, we will not farther consider the putative influence of the PLD on the observed genetic structures.

Other factors may explain the contrasted patterns of gene flow between *M. ventricosa* and *D. primitivus*. First, as suggested for various marine taxa, fecundity has a positive influence on gene flow by increasing the number of potential migrants (Palumbi 1994; Ni et al. 2010; Johnston et al. 2012). *D. primitivus* produces around 300 larvae per clutch, while we can expect a magnitude of million(s) larvae for *M. ventricosa* (there are no data available for this species, but see Emlet et al. 1987 and Lawrence 2013, for estimates in other planktotrophic sea urchins with similar egg size). Second, swimming capacities of the larvae between the two species are different. Indeed, sea urchins larvae are weak swimmers and can be dispersed far from the breeding site (Metaxas 2013). On the contrary, crabs larvae are able to resist drifting, therefore favoring local recruitment of larvae (Yednock & Neigel 2011; see also below). Third, differences in the availability and suitability of settlement habitats could also explain differences in gene flow (Treml et al. 2008; Cowen & Sponaugle 2009). Because the hosts are discrete and distributed in small patches, the total area for recruitment is restrained for crab larvae, a parameter that could decrease dispersal success. However, the distribution of favorable habitats is also patchy for the sea urchin (soft substrate banks do not have a continuous distribution), and the magnitude of differences in gene flow can hardly be explained by this trait alone. Such a more local recruitment for *D. primitivus*, in addition with a non-negligible effect of

currents would explain the observed pattern of genetic structures, where isolation by distance takes an important part.

Whatever the best explicative factor, the sharp differences in gene flow have implications in the evolution of the parasitic interaction. From both theoretical models and meta-analysis data (Gandon 2002; Greischar & Koskella 2007), since gene flow is higher in the host than in the parasite we can predict that crabs cannot be locally adapted to their host, but, conversely, that the hosts would be locally adapted to limit the impact of their parasites. There are no data available for comparing parasitic traits among populations of the pair *M. ventricosa* - *D. primitivus* from different localities. It would be interesting to acquire such data and compare them with those obtained in Jamaica (De Bruyn et al. 2009, 2010). Nevertheless, very high levels of gene flow may induce gene swamping and prevent local adaptation for the host (Lenormand 2002; Blanquart et al. 2013). The genetic homogeneity of *M. ventricosa* across Caribbean Sea suggests such a gene swamping. Therefore, infectivity measures and host resistance to crabs, among other parasitic traits, are needed across host and parasite populations to understand the magnitude of host local adaptation to their parasites (Greischar & Koskella 2007).

Are genetic structures of *M. ventricosa* and *D. primitivus* compatible with the connectivity model of Cowen et al. (2006)?

Homogeneity of *M. ventricosa* populations was observed across the Panama region and the Eastern Caribbean. Moreover, preliminary analyses also detected a lack of differentiation with sea urchins from Bahamas (COI analysis, results not shown), which indicates that *M. ventricosa* is probably panmictic inside the whole Caribbean region. This ascertainment was previously observed in other taxonomic groups: the snail *C. abbreviata* (Johnston et al. 2012), the fish *T. bifasciatum* (Purcell et al. 2006) and the lobster *P. argus* (Silberman et al. 1994). In sea urchins, previous phylogenetic analyses also suggested homogeneity within the Caribbean (Lessios et al. 2001; Lessios et al. 2003) but the present study assesses this statement using molecular markers with higher mutation rates (microsatellites) and using a larger sample size.

The observed patterns indicated that Panama is differentiated from Eastern Caribbean, as it was demonstrated in numerous other taxa (Anthozoa: Baums et al. 2005; Foster et al. 2012; Andras et al. 2013; Gastropoda: Diáz-Ferguson et al. 2010; Actinopterygii:

Purcell et al. 2006). Cowen et al. (2006) suggested that Jamaica represents a zone of mixing among the connectivity regions. However, our microsatellites results (Bayesian approaches) suggest that crabs from Jamaica are rather associated to those of Panama. Although these zones are not totally isolated regarding currents patterns (Fig. 19), it is probable that occasional oceanographic events (eg. hurricanes) promote dispersal between them (Foster et al. 2012).

We also identified heterogeneity in genetic variation of *D. primitivus* within the Lesser Antilles, a phenomenon not detected in any other animal species to our knowledge. Globally, microsatellites data showed that Martinica-Grenadines comparison yielded F_{ST} values non-significantly different from zero, while there was a significant differentiation between Martinica and Guadeloupe (0.0194 - 0.0328). This cannot be explained by geographical distance because the distance between Martinica and Grenadines is comparable to the distance between Martinica and Guadeloupe. This contrast can be rather clarified by the different current velocities between Guadeloupe and Martinica (Lessios et al. 1984; Baums et al. 2005, Gyory et al. 2013, Fig. 19). It is probable that *D. primitivus* can resist (or at least decrease) drifting in water velocities of several tens of centimeters per second, as it was demonstrated in other decapod larvae (Luckenbach & Orth 1992; Fernandez et al. 1994; Yednock & Neigel 2011). However, in the zone South to Guadeloupe, the Caribbean east-west current was recognized to be at very high velocity, potentially improving connection between these islands. We also observed an isolation of Barbados (in both COI and microsatellites analyses) in spite of its geographical proximity with some other islands. The explanation is probably related, again, to the surrounding currents, notably the northward Antilles Current. Emery (1972) proposed that the pelagic larvae could be retained by a local gyre. Roberts (1997) also evaluated that the import of larvae (in coral reefs) of this island is one of the smallest in the Caribbean and that a high self-recruitment can be expected. However, because we did not observe a lower genetic diversity in this island, an external input must be considered, probably from South America (Shulman & Bermingham 1995) where the presence of *M. ventricosa – D. primitivus* was suggested (Wirtz et al. 2009). This potential input could be facilitated by the Antilles current (Fig. 19).

In conclusion, we observed a different concordance with Cowen's connectivity regions for the two species. Indeed, *M. ventricosa* does not respect the predictions of this model whereas *D. primitivus* is more in accordance with it.

Deep history and genetic structuration

For *M. ventricosa*, ancient divergence was simply not detected and obviously, a scenario of colonization events followed by isolation through the temporal sequence of geological build-up of the Caribbean is not valid. Historical demography analyses are in favor of a demographic expansion associated with a spatial one. The timing of such a historical expansion remains unknown. Further analyses such as Bayesian Sky Plots (Drummond & Rambaut 2007) might give an insight into the temporal evolution of the effective population size.

For *D. primitivus,* it exists some similarity between the main steps of formation of the different parts of the Caribbean region and the genetic structure. The oldest parts, formed between 85 Ma and 40 Ma, fit rather well with the clade A of the COI analysis, and the group I of the microsatellites. The external arc of the Lesser Antilles, formed between 25 Ma and 20 Ma, corresponds to the microsatellites group II, while the more recent internal arc (6 Ma) corresponds to the group III. The Pliocene emergence of Barbados fits well the group IV. All together the last three events are coincident with the COI clade B. However, those geological events are by far too old to be regarded has influential in the present genetic structure of *D. primitivus*. Indeed, the two haplotype groups (A-B) were separated by 10 nucleotides, corresponding to a Kimura 2-parameter distance of 1.6%. In marine crustaceans, the molecular clock was estimated to vary between 0.9% and 3.1% per million years for COI (Sturmbauer et al. 1996; Cassone & Boulding 2006). These values lead to a possible separation date of the population of the Greater and Lesser Antilles of about 0.52 Ma to 1.78 Ma, absolutely not compatible with the time frame of island emergences.

On the other hand, it is likely that Quaternary climatic oscillations (glacial - interglacial periods) had an influence of COI genetic structures in *D. primitivus*. Indeed several sea level drops are related to the succession of glacial episodes (starting at 2.7 Ma, more important at 0.8 Ma when the glacial periods became much stronger, and ending with the last glacial maximum at 0.02 Ma; Pillans & Gibbard 2012). For example, during the last glaciation event, which occurred between 20,000 and 25,000 years ago, the sea level

was at least 100-150 m below the present level (Waelbroeck et al. 2002; Miller et al. 2005; Bowen et al. 2006; Peltier & Fairbanks 2006). At that time, Panama and Jamaica were much more connected by an archipelago (Fig. 18), which would have probably created more suitable habitats and modification of current patterns that could have promote a stepping stone dispersal between Panama and Jamaica (see Fig. 18). This hypothesis explains rather well the lack of differentiation observed between them (COI marker). In the Lesser Antilles, the increasing of connections among islands during the glacial maximums was also possible even if this is less obvious genetically (Table 7) and cartographically (Fig. 18). An increase of suitable habitats and connectivity is in concordance with the historical demographic and spatial expansion detected inside each haplotype group for *D. primitivus*. Globally, the glacial periods corresponds to episodes of genetic homogenization for these geographical zones, while interglacial times likely promote more differentiation, mainly inside the haplotype groups A and B. We can expect that these two groups were highly separated since 0.52 Ma/1.78 Ma. Such a differentiation could have been regularly enhanced during each interglacial period, even if partly blurred during glacial episodes, it remained effective up to the present interglacial.

Conclusion

We observed contrasted genetic structures in this host-parasite system. Whereas the host exhibited a global genetic homogeneity, the parasite showed significant differentiation at various geographical scales (Caribbean Sea and within Lesser Antilles). This distinction in genetic structures could have several explicative factors as fecundity, swimming capacities, or habitats suitability. It will probably have consequences on the evolution of the interaction (local adaptation) and further investigations are therefore necessary. For the parasitic crab, while geographical distance is an important factor, present currents and historical events have also to be considered to explain the observed patterns of genetic variation.

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CHAPITRE 3

Chapitre 3 - Différenciation locale en Jamaïque

Ce chapitre comporte un résumé en français ainsi qu'un article publié dans Marine Ecology Progress Series (2013). Dans celui-ci, les parties en orange, postérieures à la publication, sont insérées dans le texte de l'article qu'elles complètent dans le cadre de la thèse. A noter que l'étude de différenciation génétique et morphologique inter-hôtes des crabes avait été amorcée durant mon mémoire de Master 2 (ULB).

A. Résumé

Le crabe Pinnotheridae *Dissodactylus primitivus* parasite deux échinides fouisseurs, *Meoma ventricosa* et *Plagiobrissus grandis*. La fécondité des crabes femelles varie selon l'espèce d'hôte considérée ; celle-ci étant plus importante pour les femelles de *P. grandis* que pour celles de *M. ventricosa*. De plus, les hôtes ont une morphologie contrastée (taille et densité des piquants). Au vu de ces caractéristiques, nous avons testé l'existence d'une différenciation des crabes en fonction de l'espèce hôte.

Nous avons étudié la différenciation génétique (microsatellites, cytochrome oxydase I-COI) et morphologique (analyse de contour du céphalothorax) du crabe entre ses deux espèces hôtes issus d'un même site jamaïcain (Western Lagoon, Discovery Bay). Nous avons ensuite examiné la différenciation géographique des crabes associés à *M. ventricosa* dans quatre sites se succédant le long de la côte Nord de la Jamaïque. Dans ce contexte, une plus grande différenciation des crabes entre hôtes qu'entre sites pourrait indiquer une spécialisation d'hôte (en anglais : « host-differentiation, host specialization ou host race formation »).

Les analyses génétiques n'ont mis aucune différenciation géographique en évidence, ni aucune différenciation en fonction de l'espèce hôte. Cette absence de différenciation entre les hôtes peut être expliquée par la mobilité des crabes adultes qui passent d'une espèce d'hôte à l'autre ainsi que par une attirance relativement semblable des crabes pour ses deux espèces hôtes (kairomones non discriminantes).

Cependant, nous avons mesuré une différenciation morphologique légère (mais significative) entre les crabes femelles issus d'hôtes différents. Cette différence morphologique pourrait refléter des contraintes différentes liées à la morphologie de chaque hôte.

B. No evidence of host specialization in a parasitic pea-crab exploiting two echinoid hosts

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Abstract

The pinnotherid crab *Dissodactylus primitivus* lives parasitically on 2 burrowing echinoid species, *Meoma ventricosa* and *Plagiobrissus grandis*. The fecundity of female crabs varies between hosts, and is higher when parasitizing *P. grandis* than *M. ventricosa*. Moreover, the hosts present great variations in morphology (size and density of spines). These characteristics suggest the potential to differentiate crabs according to host species. We investigated the genetic (microsatellites, cytochrome oxydase I-COI) and morphometric (outline analysis) differentiation of this parasitic crab between 2 host species at 1 Jamaican site (Western Lagoon, Discovery Bay), and compared it with geographic differentiation among 4 sites along the north coast of Jamaica. Greater genetic differences between parasites of the 2 sympatric hosts than between parasites of a single host at different geographic locations would indicate host differentiation. Genetic analyses (microsatellites, cytochrome oxydase I-COI) did not detect spatial differentiation (probably due to local hydrography) or differentiation according to host species. This lack of host differentiation could be explained by mobility of adult crabs between hosts. However, there was weak but significant morphological differentiation between female crabs from the 2 hosts. This morphological difference may reflect constraints due to host morphology.

Introduction

The habitat of a parasite is, by definition, discontinuous and variable in time and space (Price 1980). Parasites can be associated with several hosts and can also, during free stages of their life-cycle, pass across various surrounding environments. Hence, the differentiation among parasite populations can be variable, according to their degree of host specialization, and the complexity of their life cycles. Parasite populations can be characterized along different spatial scales: between host individuals (infrapopulations), between host populations and even between host species when parasites are not strict specialists (Combes 2001; Poulin 2007). For instance, colonization of a new host species can lead to host-specialization (host-race formation) (McCoy 2003). Drès and Mallet (2002) gave a definition of host-races which are *"genetically differentiated, sympatric populations of parasites that use different hosts, and between which there is appreciable gene flow"*. Parasite differentiation according to host species (with variable degrees of sympatry) has been detected in different taxa during the last decades (e.g. the frugivorous fly *Rhagoletis pomonella*: Bush 1969 & Feder et al. 2003; the tick *Ixodes uriae*: Kempf et al. 2009; the barnacle *Wanella milleporae* associated with fire corals: Tsang et al. 2009; the crab *Pinnotheres novaezelandiae* associated with bivalves: Stevens 1990a). On the contrary, other studies failed to demonstrate genetic differentiation according to host species as in the parasitic fish *Rhodeus amarus* (Reichard et al. 2011), or in three lice species of shearwaters (Gómez-Díaz et al. 2007). Therefore, multi-hosts parasites provide a variety of case-studies to explore alternative mechanisms influencing the degree of observed differentiation, such as host specialization and spatial differentiation in a single model (Bouzid et al. 2008).

The pea crab *Dissodactylus primitivus* (Brachyura, Pinnotheridae) is an ectoparasite of two burrowing echinoids (*Meoma ventricosa*, *Plagiobrissus grandis*) along the Caribbean and neighboring American coasts (Telford 1982; Hendler et al. 1995; De Bruyn et al. 2009). *M. ventricosa* is a relatively sluggish echinoid with uniformly short dense spines, spending daytime burrowed in the sediment. It emerges at dusk to forage on the sediments surface for the whole night (Kier & Grant 1965; Hammond 1982; Hendler et al. 1995). In contrast, *P. grandis* is a highly active, fast moving, echinoid covered in less dense spines and two aboral sets of long spines. Its nyctohemeral rhythm is less pronounced than that of *M. ventricosa* (Kier & Grant 1965; Hammond 1982; Hendler et

al. 1995). The crab damages host tegument and negatively affects host fecundity (De Bruyn et al. 2009). *D. primitivus* infects its two hosts asymmetrically. Juveniles only occur on *M. ventricosa*, while adult crabs are found with similar mean burden and sex-ratios on the two echinoid hosts (De Bruyn et al. 2010). Parasite fecundity differs slightly according to host species, the females brooding more eggs (+17%) on *P. grandis* (De Bruyn et al. 2010). The adult crabs are also differentially attracted by their two host species. Crabs collected on *M. ventricosa* showed a marked preference for chemical cues from this host in situation of choice (imprinting), a factor that could promote specialization (De Bruyn et al. 2011). However, crabs collected on *P. grandis* do not show such a preference (De Bruyn et al. 2011). It still remains unclear if *D. primitivus* is on the way of an incipient specialization between the two hosts. On the one hand, factors promoting differentiation between hosts could be the imprinting phenomenon, the higher fecundity for females on *P. grandis* and the different living conditions provided by the host species having contrasting morphologies and behaviors. On the other hand, factors that may prevent differentiation could be shift of adult crabs from one host to the other and the loss of imprinting in crabs infecting *P. grandis*.

The study of genetic differentiation is often useful to understand parasite life cycles (de Meeûs et al. 2007) and necessary to detect host specialization. Many studies have analyzed population genetics of crustaceans (e.g. Weber et al. 2000; Cassone & Boulding 2006; Herborg et al. 2007; Silva et al. 2009; Silva et al. 2010a, b; Fratini et al. 2011), but fewer dealt with parasitic or symbiotic species (Stevens 1990a, b; de Meeûs et al. 1992; Harrison 2004; Sotka 2005; Tsang et al. 2009).

In this study, we investigated the genetic differentiation of *D. primitivus* in relation to its two hosts within a single location, and compared it with local scale geographic genetic differentiation in one host. A higher genetic structure between the two hosts living in sympatry than between host populations from different geographic locations would be a good indicator of host-race formation.

In addition to population genetic analyses, morphology may also provide interesting clues to explore patterns of differentiation among populations (Magniez-Jannin et al. 2000; Navarro et al. 2004; Pinceel et al. 2004; Garnier et al. 2005; Silva et al. 2010a, b). Recent developments in morphometrics allow quantifying morphological disparity by

building morphospaces in which distances are unbiased (notably for the size of the measured object), therefore facilitating comparison with population genetic data and distances (Laffont et al. 2011). For example, Silva et al. (2010a) detected congruence between the two data sets in the crab *Perisesarma guttatum*, both attesting the subdivision of this species into two main clades on the East African coast. However, other studies found morphological differentiation without any genetic differentiation (eg. Nice & Shapiro 1999, Magniez-Jannin et al. 2000). Therefore, we combined our population genetic survey with a morphometric analysis of morphological variation in *D. primitivus*.

Material and methods

Sites description

Four sites were sampled on the northern coast of Jamaica (Fig. 24). Two sites are located within the lagoon of Discovery Bay (Western Lagoon: WL, Eastern Lagoon: EL) and two are outside (Pear Tree Bottom: PTB, Chalet Caribe: CC) (Fig. 24). Discovery Bay (180°28'N, 77°24'W) is partially closed by a fringing reef pierced by a 12 m deep channel to allow shipping traffic (Gayle & Woodley 1998). This channel extends into the lagoon so that WL and EL are located on opposite sides of the channel.

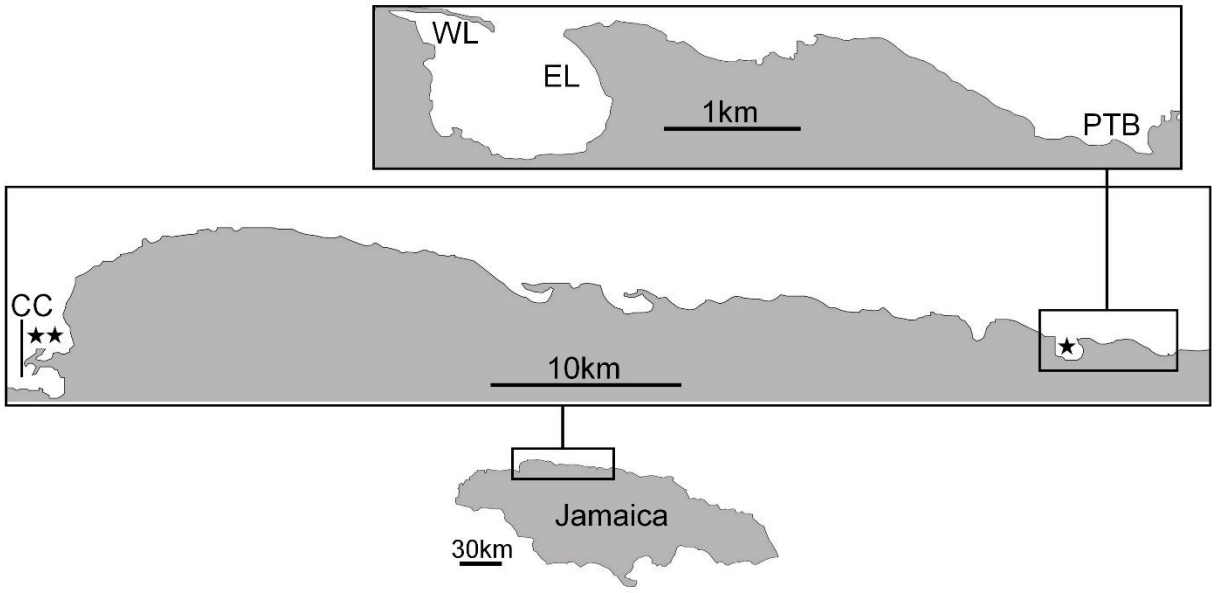


Fig. 24. Sampling sites on the North coast of Jamaica. WL: Western Lagoon, EL: Eastern Lagoon, PTB: Pear Tree Bottom, CC: Chalet Caribe. Simple black star indicates Discovery Bay and double black stars indicate Montego Bay

Sample collections

In WL, crabs were sampled both from sympatric *M. ventricosa* and *P. grandis* (the two host species may be found within the same 10 m^2). In EL, PTB, and CC, *P. grandis* was very rare or absent and crabs were only collected on *M. ventricosa*.

For population genetic analyses, samples were collected by SCUBA diving or snorkeling at depths ranging from 2 to 4 m at WL, 5 to 6 m at EL, 12 to 18 m at PTB and 7 to 9 m at CC. In WL and EL, each individual host specimen (= infrapopulation) was kept separately in a plastic bag that was immediately tied up after collection. In CC and PTB, crabs were directly collected under water without counting the number of sea-urchins. In the laboratory, crabs were individually isolated and preserved in pure ethanol. We sampled 407 crabs, 169 from WL (82 from 33 *P. grandis* and 87 from 26 *M. ventricosa*), 132 from 32 hosts at EL, 83 at PTB and 23 at CC. Sampling years were 2009 for WL and EL, 2005 for CC, and 2005 and 2009 for PTB. For COI analysis, the sampling was reduced to 21 crabs from *M. ventricosa* and 22 crabs from *P. grandis* (all individuals from WL).

For morphometric analyses, crabs were collected in 2007 (WL, EL) and 2005 (PTB, CC). The total number of specimens was 137: 70 crabs at WL (32 from *P. grandis* and 38 from *M. ventricosa*), 39 crabs at EL from 28 *M. ventricosa*, 13 from PTB and 15 from CC.

Population genetics data collection

The cephalothorax of each crab was removed and the muscles at the basis of pereopods were collected. The muscles were dried during two hours at ambient temperature and frozen at -80° C. DNA extractions were performed using a Chelex chelating resin method (Walsh et al. 1991). First, each sample was crushed using one tungsten ball (3 mm diameter) and a mixer mill (1 min at 18Hz). After adding 100 µl of "Chelex solution" (1 g of Chelex into 20 ml of sterile water), a new crushing was done during 1 min. The samples were then placed at 85° C during 90 min and mixed every 30 min. Finally, after centrifugation (3 min at 12 000 rpm), the supernatant with DNA was collected.

Ten microsatellite loci (Anderson et al. 2010) were amplified by Polymerase Chain Reaction using primers in four PCR multiplex (Table 10). Each reaction in multiplex (15 µl) includes 7.5 µl of Master Mix Qiagen (Taq Polymerase, nucleotides), 1 µl of DNA, 0.3 µl (10µM) of each forward/reverse primer and a variable volume of sterile water (5.3 µl for multiplex a and d, 4 µl for multiplex b, 4.7 µl for multiplex c). The PCR

conditions consisted of 40 cycles of 30 s at 94° C (denaturation), of 90 s at 51° C (annealing) and of 30 s at 72° C (elongation). These cycles were preceded by a step of 15 min at 95° C (first denaturation) and were followed by a step of 10 min at 72° C (last elongation). Finally, 1 µl of amplified DNA was mixed with 0.4 µl of the size standard LIZ (AB) and 10 µl of formamide prior to electrophoresis with an AB 3730 DNA Analyzer. Genotypes were deduced from electropherograms using the software Peak Scanner (https://products.appliedbiosystems.com). Allelic binning was done using the program Autobin but each genotype was verified by eye (Guichoux et al. 2011).

Locus	PCR Multiplex	Fluorochrome	Size range (bp)	A
DpC115	a	NED	162-188	7
DpC118	a	VIC	265-291	12
DpA5	b	VIC	210-238	11
DpA113	b	VIC	108-134	12
DpC4	b	FAM	152-184	14
DpA101	c	NED	217-259	16
DpD110	c	PET	236-276	17
DpD111	c	FAM	251-309	11
DpC9	d	FAM	178-220	8
DpC110	d	PET	134-168	11

Table 10. Ten microsatellite loci used in the present study. A denotes the total number of alleles. The fluorochromes are part of the DS33 Applied Biosystems Standard Dye Set.

For COI analysis, we amplified a 652 base pairs fragment using the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Each PCR reaction (15 µl) included 7.5 µl of Master Mix Qiagen (Taq Polymerase, nucleotides), 2 µl of DNA, 0.6 µl (10 µM) of each forward or reverse primer and 4.3 µl of sterile water. PCR conditions consisted of 35 cycles for each of the 3 temperature steps [60 s at 94°C (denaturation), 60 s at 40°C (annealing) and 120 s at 72°C (elongation)]. These cycles were preceded by a step of 2 min at 94°C (first denaturation) and were followed by a step of 2 min at 72°C (last elongation). After amplification, we did an ExoSAP purification. In each well, we added 0.8 µl of sterile water, 0.2 µl of Exonuclease I (Affymetrix) and 1 µl of Shrimp Alkaline Phosphatase (Affymetrix). Each plate was then putted back in a thermocycler with the following program: 60 min at 37°C and 10 min at 80°C. The plates was then dried in an oven at 37 °C during the whole night. Finally, we added 10µl of forward

primer (2.5 µM) in each well and sent the plate to the MACROGEN sequencing service. The alignment of sequences was done using MEGA 5.1 (Tamura et al. 2011).

Statistical analyses of population genetics data

Microsatellites

The software FSTAT (2.9.3.2) was used to estimate genetic variability i.e. allele frequencies, number of alleles, allelic richness (AR, a measure of the number of alleles adjusted for sample size), number of private alleles (alleles only found in a single group) and expected and observed heterozygosities (Goudet 1995). Differences between sites in mean AR averaged over loci were tested using non parametric statistics (Kruskal-Wallis test). Deviations from Hardy-Weinberg (F_{IS}) and linkage disequilibrium were also assessed using FSTAT. In WL, F_{IS} was tested both for separate host populations and for pooled data.

POWSIM (4.1) was implemented to measure the statistical power (1–β) of detecting differentiation, taking into account the number of loci, the level of polymorphism and numbers of individuals in our study (Ryman & Palm 2006). The retained parameter values were selected according to the advices of POWSIM manual (N_e of 2000; 10 generations of drift; 1000 runs). A power of 0.75 was considered enough to detect differentiation in our samples (Beninger et al. 2012; Olsson et al. 2012).

We measured differentiation using different complementary methods. Weir and Cockerham (1984) estimator of multilocus F_{ST} (θ_{wc}) was calculated using FSTAT and was statistically tested against the null hypothesis of $F_{ST} = 0$, using 120 permutations of alleles between populations. The p value of this test corresponds to the proportion of permutations leading to a F_{ST} greater (or equal) than the observed one (Fratini & Vannini 2002). Nominal level (5%) was adjusted by standard Bonferroni correction for multiple comparisons (Rice 1989). Following the recommendation of Waples and Gaggiotti (2006), we also applied a contingency tests of allele frequency heterogeneity (hereafter Fisher test of differentiation) using Arlequin 3.5 (Excoffier & Lischer 2010). This approach follows the method of Raymond and Rousset (1995). Finally, we also used Arlequin to run an analysis of molecular variance (AMOVA) to assess the relative variance contributions of genetic differences within and between groups (Excoffier & Lischer 2010).

In addition, two Bayesian clustering approaches were implemented to infer the more probable number (K) of genetic clusters. For putative value of K (1-5) and for 10 independent simulations, parameters were set in STRUCTURE 2.3.3 (Pritchard et al. 2000) as: running lengths of 100,000, admixture model (with prior sampling location), alpha inferred and allele frequencies correlated among populations. We also used DPART software which uses Dirichlet Process to infer K values (Onogi et al. 2011). The parameter set for 5 independent simulations was: alpha equal to 0.51, lambda equal to 0, length of burn-in of 400,000 and length of iterations after burn-in of 100,000.

COI

We calculated the number of haplotypes, the haplotype diversity, the mean pairwise differences among haplotypes and the nucleotide diversity using Arlequin 3.5 (Excoffier & Lischer 2010). Haplotype network (Minimum Spanning Networks) was constructed using the software MINSPNET (Excoffier & Smouse 1994). Using Arlequin 3.5, we calculated pairwise ΦST (Tajima & Nei 1984). The significance of ΦST was evaluated using a permutation test (10,000 permutations).

Morphometric data collection

Body shape (cephalothorax outline) was assessed using the Discrete Fourier Analysis (DFA) (Moellering & Raynor 1981). A Nikon Measuring Microscope (MM60) was used to take pictures of all individuals with the same capture method (same positioning, same point of reference). Then, outlines were extracted from cephalothorax pictures using the software Optimas 6.5 (www.mediacy.com). Each outline could be approximated by a sum of sine and cosine functions (= harmonics). DFA was performed to determine the parameters (amplitude, phases, Fourier coefficients) of each harmonic (H) (software CDFT, Dommergues et al. 2007).

Twenty cephalothoraxes were measured twice to assess Measurement Error (ME) (Bailey & Byrnes 1990). The amplitude parameter for the first 40 harmonics (20 conjugates) has been retained. This allowed keeping the maximum ME reasonably low. Indeed, ME were always lower than 20% except for three harmonics ($ME_{conjugate}$ of H2= 42%; $ME_{conjugate}$ of H16= 33%; $ME_{conjugate}$ of H18= 20%). The first measure was kept for analyses for these 20 crabs. For other crabs, each cephalothorax was measured only once to obtain harmonic's parameters.

Statistical analyses of morphometric data

Statistical analyses were made using STATISTICA 7.0 (www.statsoft.com). Two Principal Component Analyses (one for inter-hosts comparisons, another for inter-sites comparisons) were performed to reduce the number of variables, the initial 40 amplitudes being transformed into seven components. These seven components explained 91% of the total variance for the two analyses. The seven components followed a normal distribution in each group (non-significant Kolmogorov-Smirnov tests). Potential allometric effects were estimated using Spearman correlations between area of cephalothorax (taken as a proxy of size) and each of these components. Since all data met the homoscedasticity conditions (non-significant Levene tests), multivariate analyses of variance (MANOVAs) were made on the seven components to appraise morphometric differentiation between hosts or sites. Two factors were simultaneously considered in the MANOVAs: host and crab gender for differentiation by host species; site and crab gender for the differentiation among locations. Gender of crabs was always taken in account as a factor because the crabs are sexually dimorphic. Interactions between these factors were also evaluated. Finally, multiple discriminant analyses (MDA) were performed on the seven components to infer Mahalanobis distances and posterior probabilities between groups. Divergences expressed by MDA were visualized by reconstituting the outlines of individuals that were representative of the shape changes along the canonical axis.

Results

Population genetic analysis

Variability

The ten microsatellite loci were highly polymorphic, allele numbers per locus ranging from 7 to 17 (mean: 11.9). The minimum average number of alleles per site (7.6) was observed at CC (Table 11). Allelic richness per locus ranged from 5 to 10.6, with an average of 7.5 (Table 11), and was not significantly different between hosts or sites (Kruskal-Wallis test: H = 0.358, p = 0.986). There were only 10 private alleles (2.1% of all alleles) and these were found in WL-P, EL and PTB (Table 11). The mean observed and expected heterozygosities per locus were 0.83 (0.71-0.93) and 0.78 (0.63-0.89), respectively. Multilocus F_{IS} (including that of the pooled data for WL) ranged between -0.003 (CC) and -0.114 (EL). These values were not significantly different from zero

	A					**AR**					***F*$_{IS}$**				
Locus	WL-*P*	WL-*M*	EL	PTB	CC	WL-*P*	WL-*M*	EL	PTB	CC	WL-*P*	WL-*M*	EL	PTB	CC
DpC115	7 (1)	6	6	6	6	5.4	5	5	4.8	5.6	-0.113	-0.184	-0.267	-0.173	0.093
DpC118	12	10	11	10	8	8.9	8.4	8.2	8.7	7.7	0.024	-0.035	0.005	0.010	0.061
DpA113	10	9	9	10 (1)	8	7.7	8.1	7.3	7.4	7.9	-0.157	-0.060	-0.068	-0.036	-0.038
DpC4	7	10	11 (1)	10 (1)	6	5.3	6.6	7.4	7.4	5.8	-0.102	-0.097	-0.171	-0.167	0.044
DpA5	12 (2)	12	12	10	9	8.6	8.6	8	7.5	9	0.003	-0.085	-0.107	-0.165	-0.171
DpA101	15	13	15	14 (1)	10	10.3	10.6	10.4	10.7	9.9	0.017	-0.061	-0.027	0.031	0.163
DpD111	14	14	14 (1)	15 (1)	9	10.3	10.3	9.6	10.8	8.9	0.018	0.023	0.018	-0.007	0.205
DpD110	8	9	11 (1)	9	7	6.5	7	7.1	7.1	6.9	0.049	0.001	0	-0.060	-0.071
DpC110	7	8	7	8	6	5.6	6.2	5.7	5.9	5.9	-0.195	-0.209	-0.343	-0.244	-0.214
DpC9	9	11	10	8	7	5.5	6.1	6	6.1	6.7	0.002	-0.036	-0.271	-0.085	-0.159
All loci	**10.1**	**10.2**	**10.6**	**10**	**7.6**	**7.4**	**7.7**	**7.5**	**7.6**	**7.4**	**-0.040**	**-0.069**	**-0.114**	**-0.083**	**-0.003**

Table 11. Number of alleles (A) including the number of private alleles in parenthesis, Allelic richness (AR), and F_{IS} for 10 microsatellite loci in *D. primitivus* from four sites (WL: Western Lagoon, EL: Eastern Lagoon, PTB: Pear Tree Bottom, CC: Chalet Caribe). *D. primitivus* is present on two hosts: *M. ventricosa* (M) and *P. grandis* (P) in WL, while only M is present in EL, PTB, and CC.

(Table 11; 5% nominal threshold Bonferroni corrected to p = 0.0010) and hence no deviations from HWE were observed. No linkage disequilibrium was detected between pairs of loci (45 pairwise comparisons, 5% nominal threshold Bonferroni corrected to p = 0.0011).

For COI, the total number of haplotypes was 12 (6 in WL-M and 11 in WL-P; Fig. 25). The haplotype diversity was equal to 0.66 in WL-M (+/- 0.11) and 0.76 (+/-0.10) in WL-P. The mean pairwise differences among haplotypes was 0.95 for WL-M (+/-0.68) and 1.41 for WL-P (+/- 0.90). The nucleotide diversity was low in both groups (0.0015+/-0.0012 in WL-M and 0.0022+/- 0.0015 in WL-P).

Genetic differentiation of crabs among hosts

For microsatellites, population genetic differentiation of crabs between hosts was considered only at WL, where the two hosts are abundant. The results of the software POWSIM gave statistical powers (1–β) of 0.752 for Fisher test of differentiation, associated with an F_{ST} equal to 0.0025. F_{ST} between crabs from the different hosts species was very low (F_{ST} = -0.0015) and not significant (p = 0.80). In addition, Fisher test of differentiation did not find any differentiation (p = 1). AMOVA (results not shown) identified that all genetic variance was due to within host variation. Bayesian clustering confirmed this absence of differentiation. STRUCTURE identified the highest posterior probability (p = 0.99) when K = 1 (Table 12). Other posterior probabilities (K = 2, 3, 4) were close to 0. Results from DPART (data not shown) and STRUCTURE were congruent.

For COI, ΦST was equal to -0.019 (p = 0.74). ΦST among females only was equal to -0.012 (p = 0.59, N = 22) and -0.027 among males only (p = 0.54, N = 21).

Analysis	K	Ln Pr(X/K)	Variance Ln Pr(X/K)	Pr(K)
A	**1**	-5029.8	50.2	0.99
	2	-5076.5	172	5×10^{-21}
	3	-5080.8	182.2	7×10^{-23}
	4	-5049.7	119.8	2×10^{-9}
B	**1**	-12242.5	55.5	1
	2	-12324	284.1	4×10^{-36}
	3	-12344.4	337.9	5×10^{-45}
	4	-12360.9	373.7	4×10^{-52}

Table 12. STRUCTURE results for A: crabs from different hosts within Western Lagoon (WL) and B: All samples from all sites. K is the number of genetic clusters, X represents the genotypes of individuals and Pr is the posterior probability. For each K value, Ln Pr(X/K), associated variance and Pr(K) were calculated. Ten runs were done for each value of K and only the average values are shown in this table.

	WL	EL	PTB	CC
WL		1.00	1.00	0.77
EL	0.0021^{ns}		1.00	0.78
PTB	0.0022^{ns}	0.0028^{ns}		0.67
CC	0.0037^{ns}	0.0095^{ns}	0.0028^{ns}	

Table 13. Pairwise F_{ST} values (below the diagonal) and probabilities of Fisher tests of differentiation (above diagonal) among the four sites (WL: Western Lagoon, EL: Eastern Lagoon, PTB: Pear Tree Bottom, CC: Chalet Caribe). NS indicates a F_{ST} value no significantly different from zero after Bonferroni correction (5% nominal threshold corrected to p = 0.0083). These probabilities associated with F_{ST} are: 0.017 (WL-EL), 0.217 (WL-PTB), 0.083 (WL-CC), 0.100 (EL-PTB), 0.017 (EL-CC), 0.050 (PTB-CC).

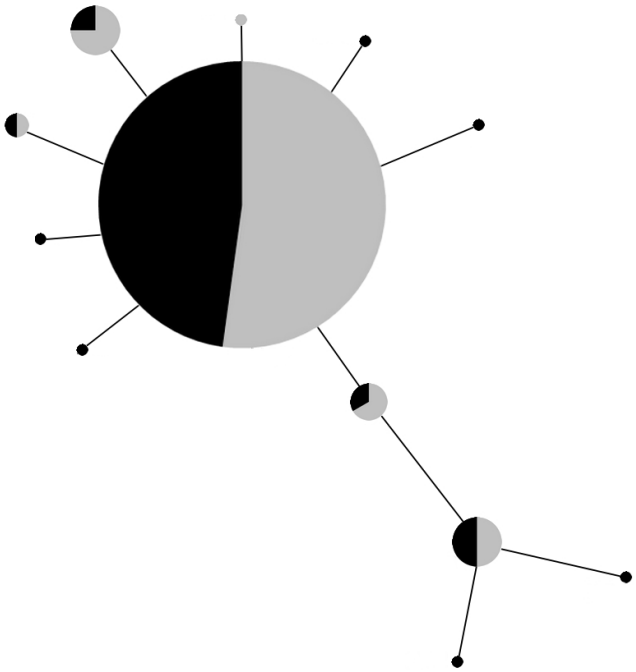


Fig. 25. Haplotype network (COI analysis) for *D. primitivus* among its two hosts (*M. ventricosa*: grey; *P. grandis*: black).

Population genetic differentiation among sites (only microsatellites)

At WL, the samples from the two hosts were pooled since they showed no differentiation (see above). The statistical power (1–β) from POWSIM was 0.994 (associated with an average F_{ST} of 0.0025) for Fisher differentiation test. F_{ST} between the crabs coming from two different sampling years in PTB (2005, 2009) was equal to 0.0038 and was not significant (p = 0.35). Fisher test of differentiation was congruent with this (p = 1). Consequently, a temporal variation was not detected and samples from PTB 2005 and 2009 were pooled for further analyses.

Overall F_{ST} between the four sites was equal to 0.003. Pairwise F_{ST} values ranged from 0.0021 (EL-WL) to 0.0095 (EL-CC), but none were significantly different from zero (Table 13). Fisher tests of differentiation were all non-significant (Table 13). The global Fisher test of differentiation yielded a p value of 0.28. The AMOVA revealed that almost all of the genetic variance (99.76%) occurred within sites (details not shown).

STRUCTURE detected no genetic structure. The highest posterior probability (p almost equal to 1) appeared when K = 1 (Table 12). DPART also found only one genetic cluster (result not shown).

Morphometric analysis

Morphological differentiation among hosts

Morphometric differentiation between crabs of different host species was considered only at WL. Spearman correlations between components of the PCA and crab size (cephalothorax area) detected no strong allometric effect: 6/7 of the correlations were non-significant and never exceeded 0.27. Therefore, the observed differences are not size dependent. Moreover, component 4, for which the Spearman correlations are significant, does not contribute to the results of the MANOVA for host species effects.

The MANOVA revealed a highly significant effect of crab gender (Wilks' lambda = 0.403; F = 12.495; p < 0.0001) and a marginally significant effect of the host factor (Wilks' lambda = 0.796; F = 2.198; p = 0.047). Interaction between these two factors was not significant (Wilks' lambda = 0.862; F = 1.347; p = 0.245) indicating that the variation between hosts were independent of gender. In order to discard gender related differences, multiple discriminant analyses were performed separately for male and female crabs. For males, there was no significant effect of host (Squared Mahalanobis distance = 1.299; F = 1.371; p = 0.256), but for female crabs, there was a marginally significant difference among host species (Squared Mahalanobis distance = 2.662; F = 2.481; p = 0.043). Projecting specimens along the canonical axis of the multiple discriminant analysis showed that the small difference between female crabs of the two hosts concerns the anterolateral margin of the cephalothorax (Fig. 26). Female crabs from *P. grandis* tend to have a more rounded margin than females from *M. ventricosa* which have a more angular margin (Fig. 26).

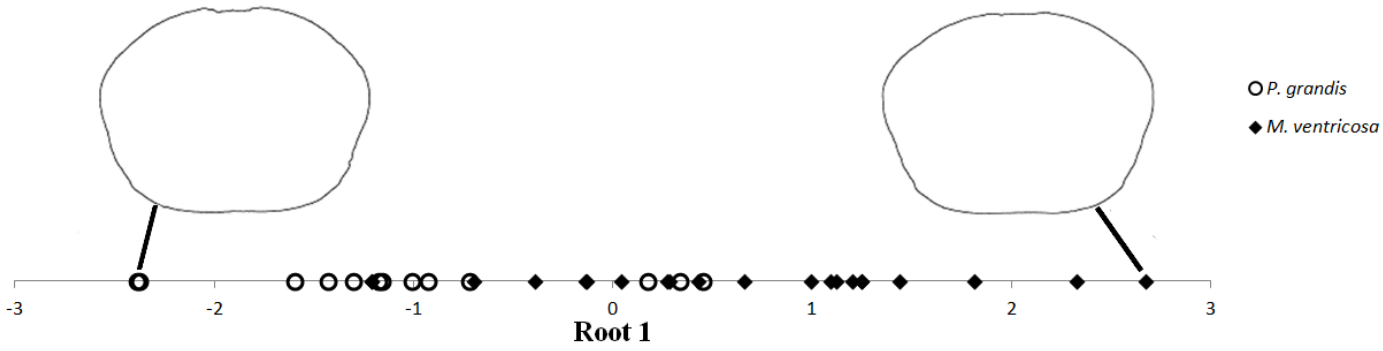


Fig. 26. Females distribution on the canonical axis (Root 1) of a multiple discriminant analyses (MDA). Crabs coming *from P. grandis* are represented by open circles while crabs from *M. ventricosa* are labeled by black diamonds. True outlines of extreme individuals showed the deformation along the axis.

Morphological differentiation among sites

Morphometric differentiation among sites was studied for crabs on *M. ventricosa* only. As for inter-hosts comparisons, Spearman coefficients did not detect strong correlations between PCA components and size: they never exceeded 0.37 and 5/7 of them were non-significant.

There were no morphological differences among crabs on *M. ventricosa* from different sites. The MANOVA revealed that site had no significant effect (Wilks' lambda = 0.716; F = 1.538; p = 0.065) but gender did (Wilks' lambda = 0.612; F = 8.257; p < 0.0001), though the interaction had no significant effect (Wilks' lambda = 0.788; F = 1.077; p = 0.373).

Discussion

The question of differentiation according to host species of the parasitic crab *Dissodactylus primitivus* came from observations of its life cycle, the asymmetrical exploitation of its two echinoid host species, and the uncertainty on the rate and direction of adult crab movements between these two hosts (De Bruyn et al. 2009, 2010, 2011). The main question concerning differentiation of *D. primitivus* according to host species was whether young mature crabs issued from parents living on *P. grandis* would be returning to this host, or whether random movement between hosts occurred.

All our analyses revealed that population genetic differentiation of *Dissodactylus primitivus* among its two hosts species was not significant. This suggests random mating and high gene flow between the crabs from the two hosts. At least two factors could explain this situation: an insufficient chemical preference for at least one of the two host species, and a high mobility of adult crabs between host species. De Bruyn et al. (2011) showed that crabs from *M. ventricosa* were more attracted by this host species than by *P. grandis*, but crabs from *P. grandis* were equally well attracted by both host species. These results, together with our new results, suggest that chemical attraction is insufficient to provoke a genetic divergence between crabs living on different host species. The lack of genetic differentiation according to host species could also be triggered by adult mobility. This situation contrasts with that of another pea-crab, *Pinnotheres novaezelandiae*, that lives in the shell cavity of bivalves. *P. novaezelandiae* spends most of its adult life within a single individual host; females are strictly host-

bound and males show a low mobility (Stevens 1990a). This low mobility between hosts is associated with a host race differentiation.

Nevertheless, there was a small morphological differentiation between females *D. primitivus* infecting the two different echinoid hosts. Two hypotheses (selection, phenotypic plasticity) could be associated with this situation of slight morphological variation associated with an absence of genetic differentiation for microsatellite markers (which are theoretically neutral).

As it was suggested in theoretical and review studies, adaptation could emerge in the presence of gene flow for neutral markers (Gandon et al. 1996; Gandon & Michalakis 2002; Greischar & Koskella 2007; Blanquart et al. 2013). This could be explained by the lack of physical linking between neutral and selective markers. Moreover, if the gene flow is intermediate (rather than low), adaptation could be even favored in a changing environment (Gandon 2002; Blanquart et al. 2013). In the case of *D. primitivus*, the gene flow between its two hosts could be nonetheless high enough to induce gene swamping and thus erases adaptation to host (Lenormand 2002; Blanquart et al. 2013).

Consequently, if selection cannot be refuted with the current data, phenotypic plasticity (associated with host constraints during development) could represent a parsimonious explanation. *M. ventricosa* is densely covered by short stiff spines, while *P. grandis* has less dense, longer, and more flexible spines (Hendler et al. 1995). It is therefore possible that, during crab's molts, spine characteristics of *M. ventricosa* lead to more compressed carapaces. The fact that differences exist only for females can be related to differential mobility between sexes. Indeed, male crabs tend to move more frequently than females the later spending probably more time on a given host (De Bruyn 2010). This was not confirmed by COI analysis ($\Phi ST = 0$ among males and females), a situation presumably due to similar larval dispersal whatever the gender. The hypothesis of phenotypic plasticity should be verified in aquarium experiments (maintaining individuals from juvenile to adult stages on one or the other host species).

Among the four sites investigated, there was no overall genetic differentiation, hence the crabs on *M. ventricosa* constitute a single panmictic population. In addition, the absence of morphological differentiation strengthens this homogeneity and may be indicative of similar environmental conditions between locations. Former studies have shown

contrasted genetic structures (using various molecular markers) in crabs at similar spatial scale. For example, low but significant differentiation was detected in free-living crabs: in the spider-crab *Inachus dorsettensis* between two sites of the Isle of Man (Weber et al. 2000) and in *Pachygrapsus marmoratus* along sites of the Lusitanian and Italian coasts spaced by less than 50 km (Silva et al. 2009, Fratini et al. 2011). In symbiotic species, genetic structuring was also detected in *Pinnotheres atrinicola* between locations distant from less than 100 km (Stevens 1990b). However, population homogeneity was found in *Pachygraspus crassipes* in the North Pacific (Cassone & Boulding 2006) and in *Perisesarma guttatum* from the African mangrove (Silva et al. 2010a). Genetic homogeneity over tens of kilometers, as observed for *D. primitivus*, is likely due to dispersal of pelagic larval stages.

D. primitivus has a pelagic larval duration (PLD) of around 15 days (Pohle & Telford 1983), which should favour dispersal, matching the classical hypothesis (still under debate) that a long PLD could decrease population genetic structure (Bohonak 1999; Kochzius et al. 2009; Selkoe & Toonen 2011; Faurby & Barber 2012). *D. primitivus* females have small brood sizes (less than 300 eggs per brood) compared to other crab species (Christensen & McDermott 1958; Telford 1978; Mantelatto & Fransozo 1997). Like large PLD, the release of numerous pelagic small eggs, which is frequent in marine organisms (Bohonak 1999; Ni et al. 2011), is thought to favour dispersal by increasing the probability of settlement in many different locations. The present study shows that a small brood size does not necessarily represent a limiting factor to an efficient gene flow. Finally, many decapod species larvae have a good active mobility and, despite their small size, are able to resist drifting due to currents (Bradbury & Snelgrove 2001; Yednock & Neigel 2011). This life history trait might reduce dispersal (Yednock & Neigel 2011). To our knowledge, no data exists on swimming capability for *D. primitivus* larvae. However, we could speculate that active swimming is not effective enough to prevent genetic homogenization as observed in the present study. In spite of these characteristics *a priori* limiting dispersal, and despite the discontinuous habitat associated with its symbiotic life, *D. primitivus* showed no population structuring, suggesting a major effect of current. We therefore propose that the east-west Caribbean Current, that sweeps the northern coast of Jamaica (Gayle & Woodley 1998), could promote dispersal of pelagic organisms among the different sites investigated here.

The overall absence of genetic and morphological differentiation according to host species or between crabs from *M. ventricosa* in four sites suggests a strong homogeneity within and among the North Jamaican locations investigated. Future work will consider crabs coming for other Caribbean islands with a hierarchical approach, ideally including sites with *P. grandis*. This would allow assessing the scale needed to observe a significant differentiation and if population genetic structure correlates with that of the hosts, with the hydrography or with the history of Caribbean islands.

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CHAPITRE 4

Chapitre 4 - Système de reproduction de *D. primitivus*

Ce chapitre comporte un résumé en français ainsi qu'un article publié dans Plos One (2014). Les résultats de cette partie sont issus d'une collaboration avec Chedly Kastally lors de son stage approfondi de Master 1 (ULB).

A. Résumé

Les systèmes de reproduction (« mating systems ») sont très diversifiés dans le règne animal, notamment chez les crustacés ; ils sont cependant inférés à partir d'un nombre limité de paramètres. Baeza et Thiel (2007) ont proposé un modèle prédisant le système de reproduction des crustacés symbiotiques à partir de trois caractéristiques des hôtes ainsi que du risque de prédation. Cinq systèmes de reproduction sont envisagés par ce modèle, allant de la monogamie à de la polygynandrie (où l'accouplement multiple a lieu chez les deux sexes).

A l'aide de locus microsatellites, nous avons testé le système de reproduction supposé du crabe ectoparasite *Dissodactylus primitivus*. Nous avons déterminé les fréquences d'accouplements des mâles et des femelles, l'assignement parental (logiciels COLONY et GERUD) ainsi que le contenu des spermathèques des femelles.

Nos résultats sont globalement cohérents avec le modèle de Baeza & Thiel et montrent, en combinaison avec des expériences antérieures (en aquarium), que cet ectoparasite a évolué vers un système de reproduction polygame où les mâles et les femelles se déplacent entre les hôtes à la recherche de partenaires sexuels. Les analyses de parenté ont révélé que la polyandrie est fréquente et concerne plus de 60% des pontes aux seins desquelles on peut retrouver jusqu'à six mâles différents. La polygynie est suggérée par la détection de huit mâles ayant contribué à deux pontes différentes. Nous avons également détecté un biais de paternité dans 92% des pontes multipaternelles. De plus, le biais réel est probablement plus élevé que dans cette estimation issue des pontes car des allèles supplémentaires ont été détectés dans la plupart des spermathèques. Ce haut biais peut être expliqué par plusieurs facteurs comme de la compétition spermatique ou un choix cryptique des femelles. Nos données génétiques, combinées à des analyses anatomiques préalables, fournissent des arguments cohérents suggérant de la précédence spermatique chez *D. primitivus*.

B. Genetic evidence confirms polygamous mating system in a crustacean parasite with multiple hosts

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Abstract

Mating systems are diverse in animals, notably in crustaceans, but can be inferred from a limited set of parameters. Baeza and Thiel (2007) proposed a model predicting mating systems of symbiotic crustaceans with three host characteristics and the risk of predation. These authors proposed five mating systems, ranging from monogamy to polygynandry (where multiple mating occurs for both genders). Using microsatellite loci, we tested the putatively mating system of the ectoparasite crab *Dissodactylus primitivus.* We determined the mating frequencies of males and females, parentage assignment (COLONY & GERUD software) as well as the contents of female spermathecae. Our results are globally consistent with the model of Baeza and Thiel and showed, together with previous aquarium experiments, that this ectoparasite evolved a polygamous mating system where males and females move between hosts for mate search. Parentage analyses revealed that polyandry is frequent and concerns more than 60% of clutches, with clutches being fertilized by up to 6 different fathers. Polygyny is supported by the detection of eight males having sired two different broods. We also detected a significant paternity skew in 92% of the multipaternal broods. Moreover, this skew is probably higher than the estimation from the brood because additional alleles were detected in most of spermathecae. This high skew could be explained by several factors as sperm competition or cryptic female choice. Our genetic data, combined with previous anatomic analyses, provide consistent arguments to suggest sperm precedence in *D. primitivus*.

Introduction

The knowledge of mating systems is of primary importance to determine the factors that have shaped evolutionary trends in a given taxon. In return, phylogenetic or ecological constraints may also explain why a mating system could be predominant in a biological group. Mating systems are diverse in animals, but can be predicted using a limited set of parameters that determine the intensity of sexual selection, such as anisogamy, operational sex ratio and the spatial and temporal distribution of ready-to-mate individuals (especially receptive females) (Emlen & Oring 1977; Shuster & Wade 2003; Baeza & Thiel 2007).

Crustaceans are extraordinarily diverse in morphology, life history traits and habitat distribution (Ross 1983; Thiel & Baeza 2001; Baeza & Thiel 2007). They are ideal biological models for the study of the selective forces affecting the evolution of mating systems, because closely related species can evolve different lifestyles and mating strategies. This prospect is reinforced by the variability of many traits linked with sexual selection, including internal vs. external fertilization, presence vs. absence of sperm storage, intensive vs. no parental care, semelparity vs. iteroparity (Sainte-Marie 2007; Vogt 2013). Surprisingly, however, their mating systems remain largely unexplored, with the noticeable exception of commercially - exploited species (Streiff et al. 2004; McKeown & Shaw 2008; Baggio et al. 2011).

Several crustacean taxa, including isopods, amphipods, shrimps and crabs have evolved a symbiotic life history strategy. Symbiotic crustaceans live with various other invertebrates (sponges, anthozoans, sea urchins, among others), with relationships ranging from parasitism to commensalism (Ross 1983). For these crustacean symbionts, the hosts are discrete habitats providing food, shelter and mating site. Such a discrete distribution of breeding habitats may deeply influence mating systems, because the distribution and abundance of host affect symbiont behavior and, hence, the rate and number of interactions between potential mates. Based on the framework of Shuster & Wade (2003) and on life history strategies, Baeza and Thiel (2007) proposed a model predicting mating systems in symbiotic crustaceans as a function of three host characteristics, namely the relative size of host vs. symbiont, host morphological complexity, and host abundance, as well on the predation risk off hosts (Thiel & Baeza 2001). According to their model, these traits affect directly the tendency of symbiotic

crustaceans to either monopolize their hosts (host guarding behavior, if hosts are not too large, rare and if predation risk while changing host is high) or, rather, to roam among them (host switching behavior, if hosts are too large and too complex to be guarded, abundant, and if predation risk off hosts is low). Monopolizing hosts or changing between them are the extremes of a continuum, where the rate and number of interactions between individuals directly influence mating opportunities. Baeza and Thiel's model comprises five mating systems, ranging from strict monogamy to polygynandry (i.e., mating occurs between multiple males and females with higher variance in mate numbers for males). Surprisingly, polyandrogyny, which is characterized by a higher variance in mate numbers for females, is not considered in this theoretical model (Shuster & Wade 2003). Interestingly, because the model focuses on mating systems in discontinuous habitat, it could be extended to other aquatic or terrestrial organisms living in discrete refuges, like parasitoid insects, litter-associated amphibians or sea-grasses bed associated species. While the authors provided some examples illustrating their model, they also claimed that empirical studies are lacking to confirm its ubiquity (Baeza & Thiel 2007; De Bruyn et al. 2009). Over the last decade, a number of works explored the mating systems of ectosymbiotic crustaceans to test Baeza and Thiel's model. Most relied, on observations of life-cycles or of life histories (Thiel & Baeza 2001; Baeza & Thiel 2007; Baeza 2008; De Bruyn et al. 2009; De Bruyn et al. 2010; Caulier et al. 2012; Ocampo et al. 2012; Baeza et al. 2013). However, due to the aquatic lifestyle of ectosymbiotic crustaceans, direct observations are not easy and the knowledge on the species' mating system is often incomplete. Though polygamy or monogamy was suspected in a number of cases, there was a lack of direct evidence (Baeza 2008; De Bruyn et al. 2009: Ocampo et al. 2012). More recently, mating systems have been investigated using molecular tools, especially microsatellite loci (Streiff et al. 2004; McKeown & Shaw 2008; Angeloni et al. 2003; Yue & Chang 2010). These studies showed that multiple mating occurs rather frequently in crustaceans (Yue & Chang 2010; Avise et al. 2011) but none of them concerns ectosymbiotic species.

In the present work, we used the ectosymbiotic crab *Dissodactylus primitivus* to test the prediction of the model of Baeza and Thiel (2007) that mating system can be inferred from host and crustacean life history traits*. D. primitivus* is a pea-crab (Pinnotheridae), ectoparasite of two burrowing sea urchins (*Meoma ventricosa* and *Plagiobrissus grandis*)

distributed along the Caribbean and neighboring American coasts (Telford 1982; Hendler et al. 1995; De Bruyn et al. 2009). A higher fertility in pea-crab females living on *P. grandis* was observed (De Bruyn et al. 2010). A population genetics analysis revealed no genetic differentiation among crabs living on the two different host species, suggesting a lack of host specialization (Jossart et al. 2013). Consequently, the higher fertility of females living on *P. grandis* remained unexplained. Considering Baeza and Thiel's model, the authors hypothesized that *D. primitivus* could use a strategy of "pure-search polygynandry of mobile females" whereby both males and females move between hosts to find several mates at the period of reproduction. Indeed, individual hosts are relatively close to each other with population densities of 0.2 individuals / m^2 for *M. ventricosa* and 0.02 individuals / m^2 for *P. grandis*. Host sizes are also particularly large compared to their symbiotic crabs (*ca*. 225 times larger in area). Finally, predation risk undergone by the crabs when changing hosts could be low because they display the same color as the surrounding coral sands. Using demographic analyses, De Bruyn et al. (2009, 2010) showed that *D. primitivus* assemblages living on a same host are variable in composition. Crabs can be found alone, in pairs (heterosexual or homosexual), and up to 6 adult crabs can infect a single host (De Bruyn et al. 2009; unpublished data). Furthermore, both genders could move among host individuals, whatever the host species (De Bruyn et al. 2009; De Bruyn et al. 2010). If a "pure-search polygynandry of mobile females" mating system actually occurs, a high rate of polyandry and polygyny should be genetically detected.

Using microsatellite marker loci, we tested the "pure-search polygynandry of mobile females" hypothesis in *D. primitivus.* We determined the mating frequencies of males and females, as well as parentage assignment by genotyping eggs from gravid females and the contents of female spermathecae. In addition we addressed the following two questions: (i) does mating system of *D. primitivus* vary between the two hosts? This could account for the difference in female fertility if mating frequency is positively associated with fitness on *P. grandis* or, conversely, if females suffer sexual harassment on *M. ventricosa*; and (ii) is multiple mating associated to an unequal contribution of the females' mates to offspring production.

Materials and Methods

Sampling

Crabs were sampled in one site, located into the lagoon of Discovery Bay (180°28'N, 77°24'W, Northern coast of Jamaica) in March-April 2009 by SCUBA diving or snorkeling at depths ranging from 2 to 4 m. All samples were obtained under the University of the West Indies Collecting Permit from the National & Environmental Planning Agency.

Fifteen hosts were sampled separately in plastic bags that were immediately tied up after collection. Back in the laboratory, crabs were individually isolated and preserved in pure ethanol. We collected 39 gravid crab females (for a total of 64 females), among which 18 were chosen for molecular analyses (9 from 8 *M. ventricosa* and 9 from 7 *P. grandis*) (Table 14). All collected male crabs at the same site (55 in total) were used for molecular analyses, taking in account their host of origin (labeled "M" or "P" crabs sampled on *M. ventricosa* or *P. grandis*, respectively). Gravid females carried 203 eggs ± 34 (mean ± SD, N = 9). Genotyping was performed on an average of 40.56 ± 4.67 eggs per clutch, totaling 758 eggs that were randomly sampled in each clutch (Table 14). For 14 of these 18 gravid females, we also collected a spermatheca.

Tissue and DNA extractions

For each adult crab, two legs were removed and dried during two hours at ambient temperature. The clutch of each gravid female was isolated and eggs were placed individually in a microtube and dried during two hours. Then, the legs and eggs were frozen at -80°C pending DNA extraction step.

DNA extractions were performed using a Chelex chelating resin method (Walsh et al. 1991). Each sample was crushed using one tungsten ball (3 mm diameter) and a mixer mill (1 min at 18Hz). After having added 100 µl of "Chelex solution" (1 g of Chelex into 20 ml of sterile water), another crushing was made during 1 min. The samples were then placed at 85°C during 90 min and mixed every 30 min. Finally, after centrifugation (3 min at 12 000 rpm), the supernatant with DNA was collected.

Spermathecae were dried for one hour and then directly placed into Chelex solution without any crushing step.

Mother's (clutch) ID (a)	No. of eggs genotyped	No. of fathers (b)	Adult infrapop. (c)		Fatherhood (d)		
			f	m	m1	m2	m3
M12	46	2/2	2(1)	1	M15		1
M16	44	1/2	1(1)	1			**2*** 3
M22	31	1/1	2(1)	1	M24		
M32	39	1/1	2(1)	1	M31		
M39	47	1/1	1(1)	1	M38		
M49	37	2/2	2(1)	0			**4*** 5
M64	36	4/6	1(1)	1	M63	M18 **M84***	**6* 7* 8***
M69	35	2/3	2(2)	1		**M84***	**2* 7***
M70	45	3/3	2(2)	1	M71		9 10
P3	42	2/2	1(1)	1			**6* 8***
P5	45	2/3	1(1)	0			11 12 **13***
P11	41	2/2	1(1)	2			14 15
P26	38	1/1	1(1)	3	P27		
P46	37	1/1	1(1)	1	P45		
P58	43	1/1	5(2)	3			**16***
P59	35	2/2	5(2)	3			**16*** 17
P69	45	3/4	3(2)	0			**13*** 18 19 20
P70	44	3/3	3(2)	0			**4*** 21 22
Mean (SD)	40.56 (4.67)	1.89 (0.90)/ 2.22 (1.31)					

Table 14. Characteristics of clutches, host infrapopulation composition and estimated fathers number and identity. (a) M = *M. ventricosa* and P = *P. grandis*, M69&M70, P58&P59, P69&P70 were found on the same host individual; (b) No. of fathers determined by GERUD/COLONY; (c) composition of the adult infrapopulation (individuals on the same host individual) including the studied mother, f = females (including gravid females in brackets) and m = males; (d) Fatherhood (COLONY analyses), Males were subdivided in three classes: m1 = fathers present with the mother on the host, m2 = males sampled in Discovery Bay and m3 = inferred fathers, not sampled. Males in bold and shown by a star were contributing to two clutches.

Amplification and genotyping

Four highly informative microsatellite loci (Anderson et al. 2010) were amplified in one multiplex (DpA113-VIC, DpA101-NED, DpD110-PET, DpD111-FAM) (Table 15). PCR reactions were made in a volume of 15 µl that includes 7.5 µl of Master Mix Qiagen (Taq Polymerase, nucleotides), 1 µl of DNA, 0.3 µl (10µM) of each forward/reverse primer and 4.1 µl of sterile water. The PCR conditions consisted of 40 cycles of 30 s at 94°C (denaturation), of 90 s at 51°C (annealing) and of 30 s at 72°C (elongation). These cycles were preceded by a step of 15 min at 95°C (first denaturation) and were followed by a step of 10 min at 72°C (last elongation). Finally, 1 µl of amplified DNA was mixed with

0.4 µl of the size standard LIZ (AB) and 10 µl of formamide prior to electrophoresis with an AB 3730 DNA Analyzer.

Genotypes were deduced from electropherograms using the software Peak Scanner (products.appliedbiosystems.com). Allelic binning was done using the program Autobin but each genotype was checked by eye (Guichoux et al. 2011).

Locus	Motif	Primers	Fluorochrome	Size range (bp)	A
DpA113	AC	F : GCGTAGTTCTCCTCCCGTAG R : GCGCTACCCATCAGTCTTG	VIC	108-134	12
DpA101	CA	F : CTCTCCGTCACTTTGTGTAGGT R : GTGTTCTTGTGTGCGTGTATTC	NED	217-259	16
DpD110	TAGA	F : GAAGGGTTGCTTATAGACGTG R : CCTCCTTGTTTACCGTGAGT	PET	236-276	17
DpD111	CTAC	F : CTTGACCTGACCTGTCTATCA R : CGGTGGACTACATAAGTAAAGG	FAM	251-309	11

Table 15. The four microsatellite loci used in this study. A denotes the total number of alleles evaluated from a previous population genetics study (Jossart et al. 2013). The fluorochromes are part of the DS33 Applied Biosystems Standard Dye Set.

Parentage analyses

Using FSTAT (Goudet 1995), we assessed deviation from Hardy-Weinberg equilibrium using F_{IS} and randomization of alleles between individuals for 169 previously genotyped specimens, 82 and 87 individuals from *P. grandis* and *M. ventricosa*, respectively (Jossart et al. 2013). Then, we tested if the four loci were adequate for parentage analysis. First, using the GERUD (2.0) software, we calculated an exclusion probability (PE), namely, the probability to exclude a candidate parent if this candidate is effectively unrelated to the offspring (Marshall et al. 1998; Jones 2005) This PE was estimated taking into account that one parent is known with certainty (here the mother) and using the allelic frequencies based on all adult individuals collected in Discovery Bay (this study and Jossart et al. 2013). We also calculated the probability to detect multiple paternity in a sample of the offspring (PrDM) using the software PrDM (Neff & Pitcher 2002). This software allows calculation of a set of PrDM according to the number of putative fathers, their relative contribution to offspring and the number of offspring analysed by clutch (Neff & Pitcher 2002; McKeown & Shaw 2008). For example, given the level of informativeness of our set of loci, we may have increased the number of offspring in

order to detect rare contributions. Parentage assignment was analyzed using two software: GERUD 2.0 and COLONY 2.0.2.1 (Jones 2005; Jones & Wang 2010; Karaket & Poompuang 2012). While GERUD infers the minimum number of fathers, COLONY infers the most likely number of fathers.

Mother's and genotyped offspring's alleles were compared to the set of alleles observed from her spermatheca. Extra alleles were taken as a clue of additional inseminating males for which contribution to clutch was not detected. The number of matings was estimated by summing the number of newly males detected to the minimum number of fathers (GERUD data).

We evaluated how the paternal contributions in each clutch deviated from an equilibrate contribution (G-test for goodness-of-fit on COLONY data, p-value threshold after Bonferroni correction = 0.004) using an Excel Macro developed by McDonald (2009). In addition, skewness (S) in paternity was calculated according to Pamilo and Crozier (1996): $S = (M_p - M_{e,p})/(M_p - 1)$ where M_p is the total number of fathers and $M_{e,p}$ is the effective number of fathers (Pearcy et al. 2009). We also evaluated the correlation between relative contribution of fathers in a clutch and the genetic similarity of the two partners. Genetic similarity (GS) was calculated as $GS_{ij} = 2\ N_{ij}/(N_i + N_j)$ with N_{ij} the number of alleles in common between the partners (i and j) and N_i and N_j the number of alleles of the individuals i and j, respectively (Nei & Li 1979; Yue & Chang 2010).

Other classical statistical tests (Mann-Whitney tests, Spearman correlations, Wilcoxon signed-rank tests, G-tests) were performed using STATISTICA 7.0 (statsoft.com), PAST (folk.uio.no/ohammer/past) or Excel Macro developed by McDonald (2009).

Results

Overall F_{IS} was equal to -0.025 for adult crabs from *M. ventricosa* and -0.017 for crabs from *P. grandis* and were non-significantly different from zero. This result is in agreement with a previous population genetics study on a larger data set from Discovery Bay (Jossart et al. 2013).

None of the genotypes of the 73 adults used in our study were similar except for two individuals (M16, M17), leading to a probability of exclusion (PE), when one parent is known of 0.991. This indicates that the four microsatellite markers were variable

enough to discriminate candidate parents. The probability to detect multiple paternity (PrDM) increased rapidly with sample size, indicating that the selected egg sample size ranging from 35 to 47 was high enough to detect multiple paternity even with unequal contributions of the fathers (Table 16).

		Fathers' contributions			
		50:50	**90:10**	**33:33:33**	**80:10:10**
		2 fathers		**3 fathers**	
Number of eggs genotyped per clutch	**10**	0.995	0.635	0.999	0.882
	20	0.999	0.867	0.999	0.986
	30	0.999	0.950	0.999	0.998
	40	0.999	0.981	0.999	0.999
	50	0.999	0.992	0.999	0.999

Table 16. Probabilities to detect multiple paternity (PrDM) for different number of eggs per clutch and either 2 or 3 fathers contributing and different relative contribution. (e.g. 50:50 is an equal contribution of both fathers).

Clutch analysis: searching for evidence of polyandry and polygyny in *D. primitivus*

Multiple paternity was detected in 12 clutches out of 18 in COLONY (i.e., 66.7%) and 11 clutches in GERUD (i.e., 61.1%; Table 14). Using GERUD, the minimal mean number of fathers per clutch was 1.89 when combining data from both hosts (Table 14, *M. ventricosa* SD = 1.05 and range = 1-4 and *P. grandis* SD = 0.78, range = 1-3). Using COLONY, the estimated number of fathers per clutch was 2.22 for the whole data set (Table 14), 2.33 for females from *M. ventricosa* (SD = 1.58, range = 1-6) and 2.11 for females from *P. grandis* (SD = 1.05, range = 1-4). These values (between software) were non-significantly different (Mann-Whitney test, U = 142.5, p = 0.54). Unless specified, most of the results described in the forthcoming paragraphs will refer to results obtained with COLONY, because the use of sampled fathers in parentage assignment was possible with this software.

The estimated rate of polyandry in *D. primitivus* was the same on both host species (6/9), and the number of fathers per clutch was not significantly different between the two hosts (Mann-Whitney test, U = 40.0, p = 0.96). The total number of fathers for the whole data set was 32 (Table 14). Among them, COLONY detected eight males that contributed to two different clutches (Table 14), one being among our sampled males (M84) and seven inferred by COLONY. Three fathers contributed to two clutches

sampled from the two different host species (males 4; 6 and 8 on Table 14). Three pairs of females (M69-M70, P58-P59, P69-P70) were collected on the same host individual (Table 14). Two of these pairs were sired by different males, but the two females P58-P59 were sired by the same male (male 13, not captured in our sampling).

There was a significant deviation from an equal contribution among fathers in 11 out of 12 multipaternal clutches (Fig. 27, Annexe 4), with a skewness of paternity (S) ranging from 0.44 (P70) to 0.96 (M12) with an average of 0.81 (SD = 0.16) (G-tests all significant, all p <0.004). For 8 clutches out of 18, one father was found on the same individual host than the mother (M15, M24, M31, M38, M63, M71, P27, P45; Table 14; Fig. 27). Among these 8 fathers, 7 were the principal (or the only one) contributors of the clutch (Fig. 27). The mean number of fecundated clutches by a successful male is 1.25 (SD = 0.43, N = 32). The mean number of offspring produced were 114.19 (SD = 98.37, N = 32) for males and 203 (SD = 34, N = 9) for females (Annexe 5).

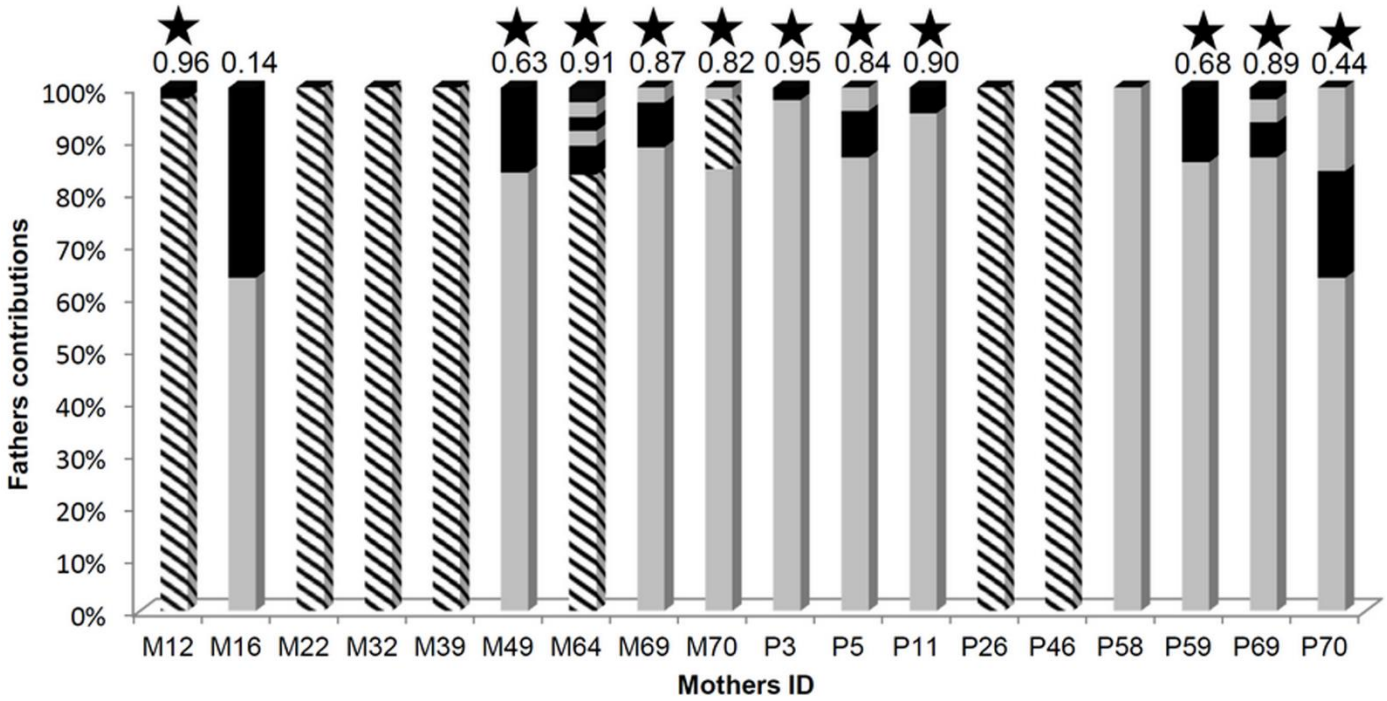


Fig. 27. Fathers' contributions within each clutch (COLONY analyses). Fathers are shown by alternate shadings. Stripped bars correspond to fathers sampled on the same host individual as the mother (see also Table 14). The values above bars correspond to the skewness (S) in paternity. The stars indicate that paternal contributions deviated significantly from equality (G-test for goodness-of-fit, Bonferroni adjusted p-value threshold = 0.004). M = *M. ventricosa* and P = *P. grandis*.

There was no association between the number of eggs per clutch (a correction for female size was included by dividing the number of eggs per clutch by the square of the cephalothorax width) and the mating frequency (Spearman correlation, r_s = 0.39, p =

0.30, N=9). Moreover, no correlation was detected between genetic similarity of the two parents and the relative contribution of fathers to the clutch (r_s= 0.02, p=0.90, N=40).

Spermatheca analysis: searching for mates that did not contribute to the clutch

The total number of additional alleles detected was different between loci (DpA113: 4, DpA101: 12, DpD111: 23, DpD110: 7). For the 14 spermathecae investigated, nine contained at least a non-parental allele for two loci or more (Table 17). Moreover, two of them had three non-parental alleles for at least two loci (Table 17). There were significantly more mates than the minimum number of fathers (estimated with GERUD) (2.71 *vs* 1.93, Wilcoxon signed-rank test, z = 18.00, p = 0.008).

Mother's ID	DpA113	DpA101	DpD111	DpD110	Min. no. of fathers	Min. no. of matings
M12	0	1	2	0	2	3
M22	0	0	1	1	1	2
M32	1	0	0	0	1	1
M39	0	1	2	0	1	2
M49	0	1	2	0	2	3
M64	0	0	0	0	4	4
M69	0	0	0	0	2	2
M70	0	0	2	0	3	3
P5	0	1	1	1	2	3
P11	2	2	4	3	2	4
P26	0	0	1	0	1	1
P58	1	2	3	2	1	2
P59	0	3	4	0	2	4
P69	0	1	1	0	3	4
Mean (SD)					1.93 (0.92)	2.71 (1.07)

Table 17. Number of non-parental alleles detected in each female's spermatheca for the four loci (DpA113, DpA101, DpD111, DpD110).The minimum number of matings was calculated in the same way than the Minimum number of fathers (GERUD data) to which we added the number of new males deduced from spermatheca analysis.

Discussion

Our results are globally consistent with the model of Baeza and Thiel (2007), indicating that mating system can be predicted from host and crustacean life history traits. They show, together with the experiments of De Bruyn et al. (2009, 2010), that the ectoparasite crab *D. primitivus* evolved a polygamous mating system where both males and females move between hosts for mate search. Other studies inferred mating systems of symbiotic crustaceans from this predictive model (Baeza 2008; Caulier et al. 2012;

Ocampo et al. 2012). However, our study is the first that directly links such predictions with genetic measurements. Parentage analyses reveal that polyandry is frequent in *Dissodactylus primitivus* and concerns more than 60% of clutches, with clutches being fertilized by up to 6 different fathers. Moreover, the number of matings was greater than the number of fathers, indicating that some male mates did not contribute to the offspring. Because we did not genotyped the whole clutches, the difference between the minimum number of fathers and the minimum number of matings could be lower than our estimation. Anyway, our estimation of the minimum number of matings was most likely conservative, because (i) we considered an additional mating only when a new allele was detected at two different loci, and (ii) a competition during amplification step may occur, leading to the non-amplification of some alleles (e.g. short allele dominance, Van Oosterhout et al. 2004), therefore minimizing the number of detected additional alleles. Polygyny is supported by the detection of eight males having sired two different broods. Only twenty five percent of the 32 detected fathers occurred simultaneously with the investigated females on the same individual host. This suggests that the fathers and/or the mothers regularly leave the host after mating. In line with this hypothesis, a few inferred males have mated with females collected on distinct host species, indicating that the crabs could move from one to another host whatever the host species. Overall, these results corroborate those of De Bruyn et al. (2009), showing that crabs move between hosts in the field, and Jossart et al. (2013) reporting a lack of genetic differentiation between crabs found on the two host species. The model of Baeza & Thiel does not consider the variance in mate numbers between genders. Our results (from clutches) suggest a higher variance in females (mean = 2.22; variance = 1.31; N=18) than in males (mean = 1.25; variance = 0.44; N=32). Therefore, according to Shuster & Wade (2003), the mating system of *D. primitivus* could be considered as polyandrogyny rather than polygynandry. However, our study probably underestimates the real values, especially for males, because we lack data on the set of females they mated at a given time.

Our data also indicate that the difference in brood size reported by De Bruyn et al. (2009) for *D. primitivus* collected on *P. grandis* and *M. ventricosa* does not imply a difference in multiple paternity: the number of mates is not related to the number of eggs occurring in a brood/clutch, conversely to what was observed in some other

species where multiple paternity occurs (e.g. the shrimp *Caridina ensifera*, Yue & Chang 2010).

We detected a significant paternity skew in 92% of the multipaternal broods. This confirms that high skew could occur in various taxa among decapod crustaceans (Yue et al. 2010; Bailie et al. 2011). The paternal skew is probably higher than the estimates inferred from the brood since additional alleles were detected in most of spermatheca (64%). High skews in sperm usage could be explained by several factors like sperm competition or cryptic female choice (Parker 1970; Eberhard 1991; Arnaud 1999). Our study was not designed to test the proximate causes for the sperm use bias. Nevertheless, some explanations can be discussed in the light of our data. First, cryptic female choice can be associated with a negative relationship between reproductive success of sires and their relatedness to mothers, allowing the avoidance of genetic incompatibility or inbreeding (Bretman et al. 2004). Yet, we found no correlation between genetic similarity of the two parents and sire success, nor an advantage in brood size in polyandrous females. Second, females of *Dissodactylus sp.* could produce more than one clutch during a breeding period or during their entire life (Pohle & Telford 1981; Bell & Stancyk 1983). They are able to store sperm, but are also potentially receptive to mating before each new clutch. In pea-crabs, the spermatheca forms a pouch with the oviduct opening at the basis of the vagina where sperm intromission takes place (Becker et al. 2011). Consequently, if several spermatophores are inserted successively, the last male sperm would be: (i) in higher numbers than the preceding stored sperm (e.g. due to sperm mortality), (ii) in favorable position, at the basis of the spermatheca close to the oviduct (Jensen et al. 1996; Sainte-Marie 2007; Takami 2007). The importance of such "stratification" in paternity insurance was notably proposed for the snow crab *Chionoecetes opilio* (Urbani et al. 1998; Sainte-Marie et al. 2000) and the importance of position of stored sperm has been noted in fertilization success of isopods (Moreau et al. 2002). Moreover, our results show that 83% (5/6) of fathers present on the same host than female had the skewness of paternity in their favor (in clutch or because some alleles in spermatheca were not used). It is likely that this father was the last male that mated with the female. Therefore, anatomic and genetic data provide consistent arguments to suggest sperm precedence (Parker 1970) for *D. primitivus*.

Future researches should test, for contrasted conditions, which factors particularly affect multiple paternity (e.g. predation pressure off hosts). Spermathecae should be further examined to characterize the degree of sperm stratification after short-term and long-term sperm storage, in order to estimate sperm precedence pattern. Moreover, a complementary experiment in aquarium (where all mating individuals are known) could also be done in order to quantify post-mating sexual selection and the sex difference in the opportunity for selection (Shuster & Wade 2003; Shuster et al. 2013).

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DISCUSSION

DISCUSSION

La génétique des populations permet d'analyser l'évolution des espèces en suivant la distribution des fréquences alléliques et en confrontant les 'patrons génétiques' obtenus aux conditions environnementales actuelles et passées. Par cette approche, les populations peuvent être caractérisées à différentes échelles géographiques. Dans le cadre de symbioses, l'approche génétique affine aussi la compréhension des interactions symbiotiques tout en fournissant des indices sur l'adaptation locale des protagonistes (McCoy et al. 2005 ; Greischar & Koskella 2007 ; Poulin 2007). En se focalisant sur deux espèces symbiotiques, le crabe parasite *Dissodactylus primitivus* et son hôte, l'oursin *Meoma ventricosa*, nous avons analysé la structuration génétique de leurs populations à l'échelle régionale (entre les îles de l'arc antillais et la côte panaméenne) et à l'échelle locale (entre différents sites d'une même île), et nous avons examiné cette structuration à la lumière de plusieurs paramètres biologiques et environnementaux, notre but étant de 'visualiser' l'intégration de ces espèces au sein du biota caribéen.

La discussion générale est organisée selon quatre pistes de réflexion centrées sur la structuration des populations de ce couple; elle intègre de ce fait les principaux résultats à différentes échelles spatiales (des Caraïbes à un lagon jamaïcain de dimension kilométrique).

Rappelons brièvement, avant d'envisager ces pistes, que la première étape de ce travail a été technique, puisqu'il s'agissait de caractériser plusieurs marqueurs moléculaires **(Chapitre 1)**. Dix locus microsatellites ayant déjà été validés préalablement pour *D. primitivus* (Anderson et al. 2010), aucune mise au point n'a été nécessaire pour cette espèce. Au contraire, huit locus microsatellites ont été caractérisés pour *M. ventricosa* avec l'aide de chercheurs du STRI (Panama) par séquençage nouvelle génération. Bien que deux locus aient des fréquences élevées en allèles nuls, ces huit microsatellites montrent un polymorphisme élevé et se présentent comme pertinents pour une étude de génétique des populations. Enfin, nous avons testé avec succès l'utilisation d'un marqueur mitochondrial (cytochrome oxydase I - COI) chez *D. primitivus* et *M. ventricosa*.

Empreinte des courants et de l'histoire géologique

L'oursin *M. ventricosa* et le crabe *D. primitivus* présentent tous deux un développement indirect avec des stades larvaires planctoniques susceptibles d'être véhiculés par les courants d'une île à l'autre (Pohle & Telford 1983 ; Emlet et al. 1987 ; Yednock & Neigel 2011). Cependant, leurs populations, alors que ces espèces vivent en symbiose et occupent les mêmes sites, ont une signature génétique différente. Les populations de *M. ventricosa* montrent une homogénéité complète à l'échelle régionale tandis que les populations de *D. primitivus* sont hétérogènes montrant une différenciation marquée d'Ouest en Est. Il est vraisemblable que cette situation traduise les capacités de dispersion qui diffèrent entre ces deux espèces. En effet, comparé au crabe parasite, l'oursin hôte présente une fécondité beaucoup plus élevée, des larves moins habiles à la nage (donc plus facilement emportées par les courants) et bénéficiant d'habitats plus accessibles (Emlet et al. 1987; Treml et al. 2008; Cowen & Sponaugle 2009; Yednock & Neigel 2011 ; Lawrence 2013 ; Metaxas 2013).

D'autres espèces montrent des différenciations très variées entre les zones de Panama et des Petites Antilles, deux régions du modèle hydrodynamique de Cowen et al. (2006, voir **Introduction**). Huit études menées dans ces deux régions entre 1994 et 2013 indiquent tant de l'homogénéité que de la différenciation génétique, soulignant l'absence de tendance commune (Tableau 18). Les cas d'homogénéité sont notamment ceux d'un échinide (*Diadema antillarum* : Lessios et al. 2001), d'un mollusque gastéropode (*Coralliophila abbreviata* : Johnston et al. 2012), d'un crustacé décapode (*Panulirus argus* : Silberman et al. 1994) ou encore d'un poisson (*Holocentrus ascensionis* : Bowen et al. 2006). Les cas de différenciation concernent des anthozoaires (*Acropora palmata* : Baums et al. 2005 ; *Montastraea annularis* : Foster et al. 2012 ; *Gorgonia ventalina* : Andras et al. 2013) ainsi qu'un mollusque gastéropode (*Cittarium pica* : Díaz-Ferguson et al. 2010).

Taxon	Étude	PLD	Marqueur(s) moléculaire(s)	Différenciation Panama-Petites Antilles
Anthozoaire	Baums et al. 2005	3-27 jours	µsats	OUI
Anthozoaire	Foster et al. 2012	?	µsats	OUI
Anthozoaire	Andras et al. 2013	?	µsats	OUI
Échinide	Lessios et al. 2001	?	COI	NON
Gastéropode	Diáz-Ferguson et al. 2010	4 jours	COI, 16S	OUI
Gastéropode	Johnston et al. 2012	30 jours	µsats, COI	NON
Malacostracé	Silberman et al. 1994	6-12 mois	Enz. rest. sur mtDNA	NON
Poisson	Bowen et al. 2006	40-56 jours	Cyt b	NON

Tableau 18. Inventaire des études de connectivité (1994-2013) entre Panama et les Petites Antilles. PLD : durée de vie larvaire pélagique (« pelagic larval duration »).

On notera qu'au sein d'un même groupe taxonomique, ici les gastéropodes (*Coralliophila abbreviata vs Cittarium pica*) ou encore les décapodes (notre étude *vs Panulirus argus*), la différenciation peut être très contrastée. Il a d'ailleurs été démontré qu'une hétérogénéité génétique existait également entre plusieurs espèces de poissons coralliens à l'échelle des Caraïbes (Shulman & Bermingham 1995 ; Purcell et al 2006 ; Puebla et al. 2009 ; Salas et al. 2010). La fécondité, la disponibilité de l'habitat, le marqueur moléculaire utilisé ou encore la durée de vie larvaire (PLD) sont des facteurs à prendre en compte dans l'analyse des résultats (Palumbi 1994; Purcell et al. 2006; Cowen & Sponaugle 2009; Fratini et al. 2011; Ni et al. 2011; Faurby & Barber 2012). Parmi ces facteurs, la PLD fait encore débat : la relation entre la PLD et le flux de gènes est incertaine (Shulman & Bermingham 1995; Selkoe & Toonen 2011, Faurby & Barber 2012). Ainsi, lors d'une comparaison de structures génétiques, il n'est légitime de considérer cette PLD que si celle-ci diffère fortement entre les espèces considérées. On remarquera ici que les trois espèces de l'inventaire (Tableau 18) ayant une PLD supérieure à 1 mois ne montrent pas de différenciation.

La différenciation Ouest-Est observée chez plusieurs espèces de la Mer des Caraïbes montre deux zones de 'rupture', l'une aux alentours du canal de la Mona (entre Puerto Rico et la République Dominicaine) et l'autre au niveau de Curaçao (Baums et al. 2005 : Foster et al. 2012). La localisation de la rupture « du canal de la Mona » est encore débattue même si elle est la plus généralement citée (Baums et al. 2005). Le courant à ce niveau est orienté Nord-Ouest et pourrait jouer un rôle de barrière à la dispersion entre les îles réparties à l'Ouest et à l'Est de ce canal. Nos données indiquent cependant que cette 'rupture' se situe plus à l'Est pour *D. primitivus* puisque les crabes de l'île de St

Croix sont plus apparentés génétiquement à ceux des sites de l'Ouest qu'à ceux des Petites Antilles. La seconde 'rupture' aux alentours de Curaçao s'expliquerait par un gradient de salinité le long de la côte vénézuélienne. En effet, les fleuves (Magdalena, Orénoque) qui se jettent le long de cette côte pourraient affecter la dispersion des larves (Foster et al. 2012). Notons que dans le cas de *D. primitivus*, une différenciation génétique (parfois importante, cf. Barbade) se marque à l'Est des Caraïbes, donc au sein d'une des zones de connectivité proposées par Cowen et al. (2006). Ainsi, le découpage proposé par le modèle de Cowen et al. (2006) n'est pas systématiquement conforté par les analyses génétiques récentes. De nouveaux exemples devraient être étudiés pour mieux évaluer la connectivité entre et au sein de ces zones et éventuellement aboutir à la redéfinition de certaines d'entre elles.

Deux catégories d'événements dans l'histoire de géologique des Caraïbes sont susceptibles d'avoir influencé la structure les populations étudiées ici: l'émergence des îles et les variations glacio-eustatiques. L'émergence des îles ne semble pas avoir joué un rôle dans les structures génétiques de l'oursin et du crabe. Concernant *M. ventricosa*, aucune divergence ancienne n'a été détectée tandis que chez *D. primitivus*, le cadre temporel n'est pas compatible avec le temps de divergence estimé (voir « Deep history and genetic structuration » du **Chapitre 2**).

Par contre, il est fort probable que les variations glacio-eustatiques du Quaternaire aient influencé la dispersion de *D. primitivus* au sein de la Mer des Caraïbes. La diminution du niveau marin (100-150 m) (Pirazolli 1993 ; Miller et al. 2005; Bowen et al. 2006) a pu favoriser la dispersion entre certains sites (continuité côtière accrue, Fig. 18), tout particulièrement entre Panama et la Jamaïque.

De nouvelles approches permettraient d'affiner la lecture de la différenciation régionale et constituent de nouvelles perspectives de recherche. Un calcul de distance océanographique (plutôt que d'une distance géographique) permettrait par exemple de vérifier s'il existe une relation plus forte entre celle-ci et la distance génétique comme cela a été proposé par White et al. (2010). Cependant, cette approche n'apporte pas nécessairement de meilleurs résultats puisqu'une étude récente réalisée sur l'étoile de mer *Linckia laevigata* indique que les distances géographiques (même euclidiennes) sont mieux corrélées aux distances génétiques (marqueur mitochondrial) que les

distances océanographiques (Crandall et al. 2014). Selon ces auteurs, ces résultats traduiraient un plus grand effet des événements historiques que contemporains sur cette espèce. Par ailleurs, d'autres techniques statistiques pourraient également être testées comme le logiciel MIGRATE (Beerli & Felsenstein 2001) qui estime directement le flux de gènes. Ceci semble particulièrement pertinent puisque l'analyse de la structure génétique, comme mesurée dans ce travail, ne permet pas de distinguer l'influence du flux de gènes et de la taille efficace des populations (Crandall et al. 2014). La génétique des larves pourrait également être comparée à celles des populations d'adultes installées sur les îles, ce qui permettrait de relever les zones de recrutement et contribuerait à l'explication de certaines connectivités (Hellberg 2009). L'approche génétique pourrait en outre être complétée par une approche morphologique. Existe-t-il ou non une différenciation morphologique entre nos populations et celle-ci est-elle corrélée à la différenciation génétique ? En effet, cette relation n'est pas évidente et plusieurs études indiquent l'existence de variations morphologiques associées à une homogénéité génétique (ex : Nice & Shapiro 1999, Magniez-Jannin et al. 2000). De plus, étendre ce travail à toute l'aire de distribution du couple symbiotique *M. ventricosa – D. primitivus* (Bahamas, Golfe du Mexique, Bermudes, Brésil) permettrait d'avoir une vision plus complète des connectivités entre populations et de compléter l'histoire évolutive de ce couple.

Enfin, 'retrouver' et analyser les groupes frères de *M. ventricosa* et *D. primitivus* de part et d'autre de l'isthme de Panama permettrait de caler nos données dans un contexte évolutif et phylogénique plus large. Avant la fermeture de l'isthme de Panama (2.8 Ma ; Lessios 2008), *M. ventricosa* formait une large population transocéanique (Chesher 1970). A la fermeture de l'isthme, cette population a été subdivisée en deux sous-populations (vicariance) ce qui a conduit à l'apparition des deux sous-espèces actuelles : *M. ventricosa ventricosa* (Caraïbes) et *M. ventricosa grandis* (Pacifique) (Chesher 1970 ; Schulz 2009). Parallèlement, une étude de Griffith (1987) mentionne l'existence de *D. schmitti*, une espèce sœur de *D. primitivus* dans le Pacifique qui pourrait être associée à *M. ventricosa grandis*. Mener une étude similaire à celle de cette thèse, de l'autre côté de l'isthme offrirait un beau sujet d'étude phylogéographique.

Prévisibilité de l'adaptation locale

L'adaptation locale peut être discutée tant à l'échelle régionale **(Chapitre 2)** qu'à l'échelle locale d'une île **(Chapitre 3)**.

L'étude la plus probante sur l'adaptation locale des couples hôte-parasite est la synthèse réalisée par Greischar & Koskella (2007). Ces auteurs ont démontré qu'au sein d'une relation hôte-parasite, l'adaptation locale se met en place prioritairement chez l'espèce présentant le flux de gènes le plus élevé. Cependant, la situation n'est pas aussi simple à envisager puisqu'un flux de gènes très important va contraindre l'adaptation locale via un phénomène d'envahissement de gènes (« gene swamping ») (Lenormand 2002; Blanquart et al. 2013). Gandon (2002) et Blanquart et al. (2013) proposent plutôt que l'adaptation locale dans une relation hôte-parasite soit tributaire d'un flux de gènes 'intermédiaire'. Cette prédiction est issue d'un modèle théorique où la migration peut posséder une valeur allant de 0 à 1, la valeur intermédiaire étant dès lors 0,5. Cependant, ceci semble difficilement transposable à des résultats empiriques (Blanquart, comm. pers.).

Selon les attendus exprimés par McCoy et al. (2005); Greischar & Koskella (2007); Blanquart et al. (2013) ; et au vu des résultats de l'approche régionale, il est acquis que *M. ventricosa* et *D. primitivus* n'auront pas la même tendance à s'adapter localement. Dans le modèle *M. ventricosa*-*D. primitivus*, peut-on considérer le flux de gènes entre populations de *D. primitivus* comme étant intermédiaire et dès lors, peut-on s'attendre chez lui à plus d'adaptation locale ? La question reste encore sans réponse actuellement et des expériences d'infectivité (capacité d'infecter) permettraient de compléter ces premières données (cf. Greischar & Koskella 2007). Deux approches croisées sont envisageables: (i) Comparer l'infectivité d'une même population de crabes sur deux populations d'oursins (oursins du même site que les crabes *vs* des oursins issus d'un site distinct) ; (ii) Comparer sur une même population d'oursins, l'infectivité de crabes issus du même site que les oursins à celle de crabes issus de sites distincts. Au regard des résultats, les expériences d'infectivité semblent particulièrement pertinentes dans deux situations : (1) à partir de populations voisines, issues par exemple de deux îles des Petites Antilles (ex : Guadeloupe et Antigua) où un flux de gènes modéré est noté pour les crabes, (2) à partir de populations fortement isolées (géographiquement et génétiquement), comme par exemple celles situées au Panama et à la Barbade.

A l'échelle locale du lagon jamaïcain de Discovery Bay, les résultats **(Chapitre 3)** indiquent l'existence d'un flux de gènes important entre les infra-populations de crabes respectivement installées sur *M. ventricosa* et *P. grandis*, une situation tributaire de la mobilité des crabes adultes qui voyagent régulièrement d'une espèce d'hôte à l'autre, ce qui a été observé par De Bruyn (2010). Ajoutons que la mobilité des adultes (mâles et/ou femelles) est également soutenue par l'étude de leur mode de reproduction **(Chapitre 4)**. En effet, les mâles peuvent s'accoupler avec des femelles issues des deux espèces hôtes. Nos résultats indiquent que ces accouplements inter-hôtes sont cependant assez rares et correspondent à moins de 10% des fécondations. Ce phénomène pourrait limiter l'adaptation locale d'une partie de la population à l'hôte *P. grandis* (Johnston et al. 2012).

Importance du mode de reproduction du crabe

D. primitivus présente un mode de reproduction de type polygame prédit par le modèle théorique de Baeza & Thiel (2007) (Bruyn et al. 2009). Ce comportement est confirmé sur la base de critères génétiques dans ce travail **(Chapitre 4)**, ce qui n'avait jamais été réalisé dans le cadre du modèle de Baeza & Thiel. Il a été détecté que le nombre d'accouplements chez *D. primitivus* était élevé (jusqu'à six pères différents dans une seule ponte). Si ce comportement se confirme à l'échelle de la distribution de l'espèce, cette multiplication des partenaires sexuels pourrait expliquer l'absence de déficit en hétérozygotes dans les populations de *D. primitivus* **(cf. Chapitre 2)**. Certaines populations indiquent même un excès d'hétérozygotes ce qui correspondrait à un accouplement préférentiel avec des individus non-apparentés, ce qui n'a cependant pas été détecté dans la population du lagon jamaïcain étudiée dans le **Chapitre 4** (Jamaïque, Western Lagoon). Toutefois, l'environnement local (ex : risque de prédation, abondance des hôtes) peut également influencer le système de reproduction qui pourrait dès lors fluctuer en fonction de ces paramètres (Baeza & Thiel 2007). Ces fluctuations pourraient être cernées dans le futur en caractérisant le mode de reproduction des crabes rencontrant des situations environnementales différentes.

Le couple symbiotique *D. primitivus - M. ventricosa* comme modèle

Le couple symbiotique *D. primitivus/M. ventricosa* est-il un modèle généralisable? Répondre à cette question demanderait d'envisager d'autres cas de symbioses au sein du biota caribéen. Des symbioses où l'un des partenaires serait proche taxonomiquement et/ou comportementalement de l'oursin *M. ventricosa* ou du crabe *D. primitivus*, pourraient constituer un point de départ à ces comparaisons. Parmi celles-ci, on pourrait envisager la symbiose entre *M. ventricosa* et le polychète Hesionidae *Ophiodromus obscurus,* ou encore la symbiose entre l'oursin irrégulier *Clypeaster rosaceus* et le crabe Pinnotheridae *Clypeasterophilus rugatus*.

(1) *Meoma ventricosa* est l'hôte du polychète *O. obscurus* (Fig. 28). Ce polychète, sans doute commensal (Werding & Sanchez 1979), cotoie sur l'oursin le crabe *D. primitivus*. Tout comme *D. primitivus*, le polychète se rencontre aussi sur un second oursin hôte, dans son cas le clypéastéroïde *Clypeaster subdepressus*. Il présente une distribution géographique et bathymétrique semblables à celles de *M. ventricosa* bien que légèrement plus septentrionale (Nord des États-Unis ; Fauchald 1977).



Fig. 28. Extrémité antérieure du polychète commensal associé à *M. ventricosa* (photo : Q. Jossart)

Les traits d'histoire de vie relatifs à la dispersion d'*O. obscurus* restent méconnus. Cependant, on sait que la majorité des Hesionidae ont des larves planctotrophes de type trochophore (Giangrande 1997), considérées comme ayant une faible habilité à la nage (cf. larves de *M. ventricosa*) (Yearsley & Sigwart 2011). La fécondité, quant à elle, est probablement intermédiaire entre celles de *D. primitivus* et *M. ventricosa* puisqu'elle a été estimée à 20,000 œufs chez une autre espèce du genre *Ophiodromus* (Haalanda & Schram 1983). Ainsi, on peut s'attendre à plus de congruence (par comparaison à *D.*

primitivus) des structures génétiques entre ce polychète et *M. ventricosa*. En outre, cette symbiose, où le polychète est également associé à une autre espèce d'hôte, fournit une belle opportunité de vérifier si une spécialisation d'hôte s'est mise en place.

(2) L'oursin irrégulier *Clypeaster rosaceus* et son crabe symbiotique Pinnotheridae *Clypeasterophilus rugatus* sont des espèces sympatriques de *M. ventricosa* et de *D. primitivus* ; ces couples symbiotiques se côtoient en occupant les mêmes habitats de la Mer des Caraïbes (Hendler et al. 1995 ; obs. pers.). Précisons que *C. rugatus* est un symbiote spécifique qui n'est associé qu'avec *C. rosaceus* (Reeves & Brooks 2001 ; De Bruyn 2010). Concernant les traits d'histoire de vie de ces espèces, deux informations intéressantes sont à considérer. Tout d'abord, le crabe *C. rugatus* possède une durée de vie larvaire proche de celle de *D. primitivus* (8-18 jours *vs* 6-20 jours ; Marques & Pohle 1996) et sa fécondité est probablement d'un même ordre de grandeur (<300 œufs, De Bruyn 2010). Par contre, l'oursin *Clypeaster rosaceus* possède une durée de vie larvaire contrastée par rapport à *M. ventricosa*. En effet, celle-ci ne serait que de 5 à 7 jours chez *C. rosaceus* (larve planctotrophe facultative) ce qui pourrait réduire sa dispersion (Emlet 1986). Ainsi, on peut s'attendre à ce que les structures génétiques de ce couple soit plus congruentes que ne le sont celles de *D. primitivus* et *M. ventricosa*. Cependant, ceci reste à démontrer puisque des différences persistent au niveau de l'habilité à la nage ou encore dans la disponibilité des habitats.

Le mot de la fin…

Grâce à la caractérisation de marqueurs moléculaires nucléaires et mitochondriaux, ce travail a mis en évidence des structures génétiques contrastées au sein d'un couple symbiotique appartenant au biota caribéen tout en les reliant au contexte environnemental actuel et passé de ce biota. Les populations de *M. ventricosa* se sont révélées homogènes à l'échelle des Caraïbes alors que celles de *D. primitivus* sont structurées d'Ouest en Est. Précisions que *D. primitivus* présente de la différenciation génétique au sein des Petites Antilles ce qui, à notre connaissance, n'avait jamais été détecté chez une autre espèce animale.

Au vu de ces données, des différences dans l'adaptation locale des partenaires symbiotiques sont attendues mais de nouvelles approches (mesures d'infectivité) sont nécessaires pour les documenter et en mesurer l'étendue.

Enfin, les résultats ont permis de caractériser génétiquement le mode de reproduction polygame de *D. primitivus*, une caractérisation qui constitue aussi un atout dans la compréhension de la biologie et de l'écologie de ce crabe symbiotique.

Le travail qui a été mené dans cette thèse permet de proposer des perspectives de recherche diverses, et ce, depuis l'échelle locale à celle, régionale, de la Mer des Caraïbes.

BIBLIOGRAPHIE

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- **Agrawal AA** (2001) Phenotypic Plasticity in the Interactions and Evolution of Species. *Science* 294: 321-326.
- **Alvarado JJ** (2011) Echinoderm diversity in the Caribbean Sea. *Marine Biodiversity* 41:261–285.
- **Anderson CM, Aparicio GJ, Atangana AR, Beaulieu J, Bruford MW, Cain F, Campos T, Cariani A, Carvalho MA, Chen N, et al.** (2010) Permanent Genetic Resources added to Molecular Ecology Resources Database 1 December 2009-31 January 2010. *Molecular Ecology Notes* 10(3): 576-579.
- **Andras JP, Rypien KL, Harvell CD** (2013) Range-wide population genetic structure of the Caribbean sea fan coral, *Gorgonia ventalina*. *Molecular Ecology* 22: 56-73.
- **Angeloni L, Bradbury JW, Burton RS** (2003) Multiple mating, paternity, and body size in a simultaneous hermaphrodite, *Aplysia californica*. *Behavioral Ecology* 14(4): 554-560.
- **Arnaud L** (1999) La compétition spermatique chez les insectes: les stratégies d'assurance de la paternité et la préséance du sperme. *Biotechnology, Agronomy, Society and Environment* 3(2):86-103.
- **Avise JC, Tatarenkov A, Liu JX (2011)** Multiple mating and clutch size in invertebrate brooders versus pregnant vertebrates. *Proceedings of the National Academy of Sciences of the United States of America* 108(28): 11512-11517.
- **Ayling AM** (1981) The Role of Biological Disturbance in Temperate Subtidal Encrusting Communities. *Ecology* 62(3): 830-847.
- **Baeza JA, Thiel M** (2007) The mating system of symbiotic crustaceans: a conceptual model based on optimality and ecological constraints. In: Duffy JE, Thiel M (eds). Evolutionary ecology of social and sexual systems. Crustaceans as model organisms. Oxford University Press, Oxford: 249–267.
- **Baeza JA** (2008) Social monogamy in the shrimp *Pontonia margarita*, a symbiont of *Pinctada mazatlanica*, off the pacific coast of Panama. *Marine Biology* 153(3): 387-395.
- **Baeza JA, Ritson-Williams R, Fuentes MS** (2013) Sexual and mating system in a caridean shrimp symbiotic with the winged pearl oyster in the Coral Triangle. *Journal of Zoology* 289: 172-181.
- **Baggio R, Pil MW, Boeger W, Patella L, Ostrensky A, Pie MR** (2011) Genetic evidence for multiple paternity in the mangrove land crab *Ucides cordatus* (Decapoda: Ocypodidae). *Marine Biology Research* 7(5): 520-524.
- **Bailey RC, Burns J** (1990) A New, Old Method for Assessing Measurement Error in Both Univariate and Multivariate Morphometric Studies. *Systematic Zoology* 39: 124-130.

- **Bailie D, Hynes R, Prodöhl P** (2011) Genetic parentage in the squat lobsters *Munida rugosa* and *M. sarsi* (Crustacea, Anomura, Galatheidae). *Marine Ecology Progress Series* 421: 173–182.
- **Baums IB, Miller MW, Hellberg ME** (2005) Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. *Molecular Ecology* 14: 1377-1390.
- **Becker C, Brandis D, Storch V** (2011) Morphology of the female reproductive system of European pea crabs (Crustacea, Decapoda, Brachyura, Pinnotheridae). *Journal of Morphology* 272(1): 12-26.
- **Beerli P, Felsenstein J** (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences USA* 98: 4563-4568.
- **Bell JL, Stancyk SE** (1983) Population dynamics and reproduction of *Dissodactylus mellitae* (Brachyura: Pinnotheridae) on its sand dollar host *Mellita quinquiesperforata* (Echinodermata). *Marine Ecology Progress Series* 13: 141-149.
- **Beninger PG, Boldina I, Katsanevakis S** (2012) Strengthening statistical usage in marine ecology. *Journal of Experimental Marine Biology and Ecology* 426-427: 97-108
- **Berthet J** (2006) Dictionnaire de Biologie. Éditions De Boeck, Bruxelles : 1034p.
- **Blanquart F, Kaltz O, Nuismer SL, Gandon S** (2013) A practical guide to measuring local adaptation. *Ecology Letters* 16: 1195-1205.
- **Bohonak AJ** (1999) Dispersal, Gene Flow, and Population Structure. *The Quarterly Review of Biology* 74: 21-45.
- **Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF** (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques* 31: 24-28.
- **Bouzid W, Stefka J, Hypsa V, Lek S, Scholz T, Legal L, Ben Hassine OK, Loot G** (2008) Geography and host specificity: Two forces behind the genetic structure of the freshwater fish parasite *Ligula intestinalis* (Cestoda: Diphyllobothriidae). *International Journal for Parasitology* 38: 1465-1479.
- **Bowen BW, Bass AL, Muss A, Carlin J, Robertson DR** (2006) Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. *Marine Biology* 149(4) : 899-913.
- **Bradbury IR, Snelgrove PVR** (2001) Contrasting larval transport in demersal fish and benthic invertebrates: the roles of behaviour and advective processes in determining spatial pattern. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 811-823.

- **Bretman A, Wedell N, Tregenza T** (2004) Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. *Proceedings of the Royal Society B: Biological Sciences* 271: 159-164.
- **Brookfield JFY** (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* 5: 453-455.
- **Bush GL** (1969) Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). *Evolution* 23: 237-251.
- **Campbell NA, Reece JB** (2004) Biologie. Éditions De Boeck, Bruxelles : 1364p.
- **Cassone BJ, Boulding EG** (2006) Genetic structure and phylogeography of the lined shore crab, *Pachygrapsus crassipes*, along the northeastern and western Pacific coasts. *Marine Biology* 149: 213-226.
- **Caulier G, Parmentier E, Lepoint G, Van Nedervelde F, Eeckhaut I** (2012) Characterization of a population of the Harlequin crab, *Lissocarcinus orbicularis* Dana, 1852, an obligate symbiont of holothuroids, in Toliara bay (Madagascar). In: Kroh A, Reich M. Echinoderm Research 2010: Proceedings of the Seventh European Conference on Echinoderms; 2010/10/2-9; Göttingen. Germany: *Zoosymposia* 7 : 177-183.
- **Chao A, Shen TJ** (2010) Program SPADE (Species Prediction And Diversity Estimation): http://chao.stat.nthu.edu.tw.
- **Chapuis MP, Estoup A** (2007) Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* 24(3): 621-631.
- **Chesher RH** (1969) Contributions to the biology of *Meoma ventricosa* (Echinoidea: Spatangoida). *Bulletin of Marine Science* 19: 72-110.
- **Chesher RH** (1970) Evolution in the Genus *Meoma* (Echinoidea: Spatangoida) and a Description of a New Species from Panama. *Bulletin of Marine Science* 20(3): 731-761.
- **Christensen AM, McDermott JJ** (1958) Life-history and biology of the oyster crab *Pinnotheres ostreum*. Say. *Biological Bulletin* 114: 146-179.
- **Clark PU, Dyke AS, Shakun JD, Carlson AE, Clark J, Wohlfarth B, Mitrovica JX, Hostetler SW, McCabe AM** (2009) The last glacial maximum. *Science* 325(5941): 710-714.
- **Combes C** (1997) Fitness of Parasites: Pathology and Selection. *International Journal of Parasitology* 27(1): 1-10.
- **Combes C** (2001) Parasitism: the ecology and evolution of intimate interactions. The University of Chicago Press, Chicago: 728p.
- **Combes C** (2001) Les associations du vivant. L'art d'être parasite. Flammarion, Paris : 348p.
- **Corander J, Marttinen P** (2006) Bayesian identification of admixture events using multi-locus molecular markers. *Molecular Ecology* 15: 2833-2843.

- **Cowen RK, Paris CB, Srinivasan A** (2006) Scaling of Connectivity in Marine Populations. *Science* 311: 522-527.
- **Cowen RK, Sponaugle S** (2009) Larval dispersal and marine population connectivity. *Annual Review of Marine Science* 1: 443-466.
- **Crandall ED, Jones ME, Muñoz MM, Akinronbi B, Erdmann MV, Barber PH** (2008) Comparative phylogeography of two seastars and their ectosymbionts within the Coral Triangle. *Molecular Ecology* 17: 5276–5290.
- **Crandall ED, Treml EA, Liggins L, Gleeson L, Yasuda N, Barber PH, Wörheide G, Riginos C** (2014) Return of the ghosts of dispersal past: historical spread and contemporary gene flow in the blue sea star *Linckia laevigata*. *Bulletin of Marine Science* 90(1): 399-425.
- **Crawford NG** (2010) SMOGD: software for the measurement of genetic diversity. *Molecular Ecology Resources* 10: 556-557.
- **Criscione CD, Blouin MS** (2007) Parasite phylogeographical congruence with salmon host evolutionarily significant units: implications for salmon conservation. *Molecular Ecology* 16: 993–1005.
- **Criscione CD** (2008) Parasite co-structure: broad and local scale approaches. *Parasite* 15: 439-443.
- **De Bruyn C, Rigaud T, David B, De Ridder C** (2009) Symbiosis between the pea crab *Dissodactylus primitivus* and its echinoid host *Meoma ventricosa*: potential consequences for the crab mating system. *Marine Ecology Progress Series* 375: 173-183.
- **De Bruyn C** (2010) Modalités fonctionnelles et évolutives des parasitoses développées par les crabes Pinnotheridae aux dépens des échinides fouisseurs. Thèse de doctorat, Université Libre de Bruxelles, Université de Bourgogne: 125p.
- **De Bruyn C, David B, De Ridder C, Rigaud T** (2010) Asymmetric exploitation of two echinoid host species by a parasitic pea crab and its consequences for the parasitic life cycle. *Marine Ecology Progress Series* 398: 183–191.
- **De Bruyn C, De Ridder C, Rigaud T, David B** (2011) Chemical host detection and differential attraction in a parasitic pea crab infecting two echinoids. *Journal of Experimental Marine Biology and Ecology* 397: 173-178.
- **Delmotte F, Bucheli E, Shykoff JA** (1999) Host and parasite population structure in a natural plant-pathogen system. *Heredity* 82: 300–308.
- **de Meeûs T, Marin R, Renaud F** (1992) Genetic heterogeneity within populations of *Lepeophtheirus europaensis* (Copepoda: Caligidae) parasitic on two host species. *International Journal of Parasitology* 22: 1179-1181.
- **de Meeûs T, Renaud F** (2002) Parasites within the new phylogeny of eukaryotes. *Trends in Parasitology* 18(6): 247-251.

- **de Meeûs T, McCoy KD, Prugnolle F, Chevillon C, Durand P, Hurtrez-Bousses S, Renaud F** (2007) Population genetics and molecular epidemiology or how to "débusquer la bête". *Infection, Genetics and Evolution* 7: 308-332.
- **De Ridder C, Jangoux M, Van Impe E** (1985) Food selection and absorption efficiency in the spatangoid echinoid, *Echinocardium cordatum* (Echinodermata). *Proceedings of 5th International Echinoderm Conference*: 245-251.
- **De Ridder C** (1986) La nutrition chez les échinodermes psammivores. Etude particulière du spatangide fouisseur, *Echinocardium cordatum* (Pennant) (Echinodermata, Echinoidea). Thèse de doctorat, Université Libre de Bruxelles: 275 p.
- **Diáz-Ferguson E, Haney RA, Wares JP, Silliman BR** (2010) Population Genetics of a Trochid Gastropod Broadens Picture of Caribbean Sea Connectivity. *PLoS ONE* 5(9): e12675.
- **Dommergues CH, Dommergues J-L, Verrecchia EP** (2007) The Discrete Cosine Transform, a Fourier-related Method for Morphometric Analysis of Open Contour. *Mathematical Geology* 39: 749-763.
- **Donovan SK** (2005) The geology of Barbados: a field guide. *Caribbean Journal of Earth Science* 38: 21-33.
- **Douglas AE** (2010) The symbiotic habit. Princeton University Press, Princeton: 202p.
- **Drès M, Mallet J** (2002) Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society B* 357:471-492
- **Drummond AJ, Rambaut A** (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionnary Biology* 7: 214.
- **Dufva R** (1996) Sympatric and allopatric combinations of hen fleas and great tits: a test of the local adaptation hypothesis. *Journal of Evolutionary Biology* 9: 505–510.
- **Earl DA, vonHoldt BM** (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4(2): 359-361.
- **Eberhard WG** (1991) Copulatory courtship and cryptic female choice in insects. *Biological Reviews* 66: 1-31.
- **Egea E** (2011) Histoire évolutive, structures génétique, morphologique et écologique comparées dans un complexe d'espèces jumelles : *Echinocardium cordatum* (Echinoidea, Irregularia). Thèse de Doctorat, Université de la Méditerranée: 321p.
- **Emery AR** (1972) Eddy formation from an oceanic island: ecological effects. *Caribbean Journal of Science* 12: 121-128.
- **Emlen ST, Oring LW** (1977) Ecology, sexual selection and the evolution of mating systems. *Science* 197: 215-223.

- **Emlet RB** (1986) Facultative planktotrophy in the tropical echinoid *Clypeaster rosaceus* (Linnaeus) and a comparison with obligate planktotrophy in *Clypeaster subdepressus* (Gray) (Clypeasteroida: Echinoidea). *Journal of Experimental Marine Biology and Ecology* 95: 183-202.
- **Emlet RB, McEdward LR, Strathmann RR** (1987) Echinoderm larval ecology viewed from the egg. In: Jangoux M, Lawrence JM (eds) Echinoderm studies 2. Balkema, Rotterdam: 55-136.
- **Emlet RB, McEdward LR, Strathmann R** (1997) Echinoderm larval ecology viewed from the egg in Jangoux M, Lawrence JM (eds) Echinoderm Studies 2, Balkema Publisher, Rotterdam: 285p.
- **Evanno G, Regnaut S, Goudet J** (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611-2620.
- **Excoffier L, Smouse PE** (1994) Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: molecular variance parsimony. *Genetics* 136: 343-359.
- **Excoffier L, Lischer HEL** (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564-567.
- **Faircloth BC** (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* 8: 92-94.
- **Fauchald K** (1977) Polychaetes from Inter tidal Areas in Panama, with a Review of Previous Shallow-Water Records. *Smithsonian Contributions to Zoology* 221: 1-81.
- **Faurby S Barber PH** (2012) Theoretical limits to the correlation between pelagic larval duration and population genetic structure. *Molecular Ecology* 21: 3419-3432.
- **Feder JL, Berlocher SJ, Roethele JB, Dambroski H, Smith JJ, Perry WL, Gavrilovic V, Filchak KE, Rull J, Aluja M** (2003) Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis*. *Proceedings of the National Academy of Sciences USA* 100: 10314-10319.
- **Fernandez M, Iribarne OO, Armstrong DA** (1994) Swimming Behavior of Dungeness Crab, *Cancer magister* Dana, Megalopae in Still and Moving Water. *Estuaries* 17(1): 271-275.
- **Fleming K, Johnston P, Zwartz D, Yokoyama Y, Lambeck K, Chappell J** (1998) Refining the eustatic sea-level curve since the Last Glacial Maximum using far-and intermediate-field sites. *Earth and Planetary Science Letters* 163(1): 327-342.
- **Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R** (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3(5): 294-299.

- **Foster NL, Paris CB, Kool JT, Baums IB, Stevens JR, Sanchez JA, Bastidas C, Agudelo C, Bush P, Day O, Ferrari R, Gonzalez P, Gore S, Guppy R, McCartney MA, McCoy C, Mendes J, Srinivasan A, Steiner S, Vermeij MJA, Weil E, Mumby PJ** (2012) Connectivity of Caribbean coral populations: complementary insights from empirical and modelled gene flow. *Molecular Ecology* 21: 1143-1157.
- **Fratini S, Vannini M** (2002) Genetic differentiation in the mud crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. *Journal of Experimental Marine Biology and Ecology* 272: 103-116.
- **Fratini S, Schubart CD, Ragionieiri L** (2011) Population genetics in the rocky shore crab *Pachygrapsus marmoratus* from the western Mediterranean and eastern Atlantic: complementary results from mtDNA and microsatellites at different geographic scales. In Held C, Koenemann S, Schubart CD (eds) Phylogeography and Population Genetics in Crustacea. CRC Press, Boca Raton: 191-213.
- **Froeschke G, von der Heyden S** (2014) A review of molecular approaches for investigating patterns of coevolution in marine host-parasite relationships. *Advances in Parasitology* 84: 209-252.
- **Fu Y** (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147: 915–925.
- **Gandon S, Capowiez Y, Dubois Y, Michalakis Y, Olivieri I** (1996) Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proceedings of the Royal Society B: Biological Sciences* 263: 1003–1009.
- **Gandon S** (2002) Local adaptation and the geometry of host-parasite coevolution. *Ecology Letters* 5: 246-256.
- **Gandon S, Michalakis Y** (2002) Local adaptation, evolutionary potential and host-parasite coevolution: interactions between migration, mutation, population size and generation time. *Journal of Evolutionary Biology* 15(3): 451–462.
- **Garnier S, Magniez-Jannin F, Rasplus J-Y, Alibert P** (2005) When morphometry meets genetics: inferring the phylogeography of *Carabus solieri* using Fourier analyses of pronotum and male genitalia. *Journal of Evolutionary Biology* 18: 269-280.
- **Gayle PMH, Woodley JD** (1998) Discovery Bay, Jamaica. In: Kjerfve B (ed) CARICOMP - Caribbean coral reef, seagrass and mangrove sites. *Coastal region and small island papers*, No. 3, UNESCO, Paris
- **Gerlach G, Jueterbock A, Kraemer P, Deppermann J, Harmand P** (2010) Calculations of population differentiation based on GST and D: forget GST but not all of statistics! *Molecular Ecology* 19: 3845-3852.

- **Germa A** (2008) Evolution volcano-tectonique de l'île de la Martinique (arc insulaire des Petites Antilles): nouvelles contraintes géochronologiques et géomorphologiques. Thèse de doctorat, Université Paris XI: 350 p.
- **Giangrande A** (1997) Polychaete reproductive patterns, life cycles and life histories: an overview. *Oceanography and Marine Biology* 35: 323-386.
- **Gómez-Díaz E, González-Solís J, Peinado MA, Page RDM** (2007) Lack of host-dependent genetic structure in ectoparasites of *Calonectris* shearwaters. *Molecular Ecology* 16: 5204-5215.
- **Gopurenko D (2002)** Genetic structure within the distribution of the Indo-West Pacific mud crab *Scylla serrata* (Forskål, 1775). Thèse de doctorat, Griffith University: 131p.
- **Goudet J** (1995) FSTAT (vers. 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86: 485-486.
- **Goudet J, Raymond M, de Meeüs T, Rousset F** (1996) Testing differentiation in diploid populations. *Genetics* 144(4): 1933-1940.
- **Greischar MA, Koskella B** (2007) A synthesis of experimental work on parasite local adaptation. *Ecology Letters* 10: 418–434.
- **Griffith H** (1987) Phylogenetic relationships and evolution in the genus *Dissodactylus* Smith, 1870 (Crustacea: Brachyura: Pinnotheridae). *Canadian Journal of Zoology* 65(9): 2292-2310.
- **Guichoux E, Lagache L, Wagner S, Chaumeil P, Léger P, Lepais O, Lepoittevin C, Malausa T, Revardel E, Salin F, et al.** (2011) Current trends in microsatellite genotyping. *Molecular Ecology Resources* 11: 591-611.
- **Gyory J, Mariano AJ, Ryan EH** (2013a) The Caribbean Current. *Ocean Surface Currents*: http://oceancurrents.rsmas.miami.edu/caribbean/caribbean.html.
- **Gyory J, Mariano AJ, Ryan EH** (2013b) The Loop Current. *Ocean Surface Currents*: http://oceancurrents.rsmas.miami.edu/atlantic/loop-current.html
- **Haaland B, Schram TA** (1983) Larval development and metamorphosis of *Ophiodromus flexuosus* (Delle Chiaje) (Hesionidae, Polychaeta). *Sarsia* 68(2): 85-96.
- **Hammond LS** (1982) Patterns of Feeding and Activity in Deposit-Feeding Holothurians and Echinoids (Echinodermata) from a Shallow Back-Reef Lagoon, Discovery Bay, Jamaica. *Bulletin of Marine Science* 32: 549-571.
- **Hardy OJ, Vekemans X** (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2: 618-620.
- **Harrison JS** (2004) Evolution, biogeography, and the utility of mitochondrial 16S and COI genes in phylogenetic analysis of the crab genus *Austinixa* (Decapoda: Pinnotheridae). *Molecular Phylogenetics and Evolution* 30: 743-754.

- **Hellberg ME** (2009) Gene flow and isolation among populations of marine animals. *Annual Review of Ecology, Evolution, and Systematics* 40: 291-310.
- **Hendler G, Miller JE, Pawson DL, Kier PM** (1995) Sea stars, sea urchins, and allies: Echinoderms of Florida and the Caribbean. Smithsonian Institution Press, Washington: 390 p.
- **Herborg L-M, Weetman D, Van Oosterhout C, Hänfling B** (2007) Genetic population structure and contemporary dispersal patterns of a recent European invader, the Chinese mitten crab, *Eriocheir sinensis*. *Molecular Ecology* 16:231-242.
- **Houghton DR** (1963) The Relationship between Tidal Level and the Occurrence of *Pinnotheres pisum* (Pennant) in *Mytilus edulis*. L. *Journal of Animal Ecology* 32(2): 253-257.
- **Jangoux M, Lawrence JM** (1982) Echinoderm Nutrition. CRC Press; Boca Raton: 700p.
- **Jensen PC, Orensanz JM, Armstrong DA** (1996) Structure of the female reproductive tract in the dungeness crab (*Cancer magister*) and implications for the mating system. *Biological Bulletin* 190(3): 336-349.
- **Johnston L, Miller MW, Baums IB** (2012) Assessment of Host-Associated Genetic Differentiation among Phenotypically Divergent Populations of a Coral-Eating Gastropod across the Caribbean. *PLoS ONE* 7(11): e47630.
- **Jones CG, Lawton JH, Shachak M** (1994) Organisms as ecosystems engineers. *Oikos* 69: 373-386.
- **Jones AG** (2005) GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. *Molecular Ecology Notes* 5: 708-711.
- **Jones OR, Wang J** (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* 10(3): 551-5.
- **Jossart Q, David B, De Bruyn C, De Ridder C, Rigaud T, Wattier RA** (2013) No evidence of host specialization in a parasitic pea-crab exploiting two echinoid hosts. *Marine Ecology Progress Series* 475: 167-176.
- **Jossart Q, Wattier RA, Kastally C, Aron S. David B, De Ridder C, Rigaud T** (2014) Genetic Evidence Confirms Polygamous Mating System in a Crustacean Parasite with Multiple Hosts. *PLoS ONE,* 9(3): e90680.
- **Jossart Q, Geyer LB, Lessios HA** (submitted to *Journal of the Marine Biological Association of the United Kingdom*) Characterization of eight microsatellite loci for the sea-urchin *Meoma ventricosa* (Spatangoida, Brissidae) through Next Generation Sequencing.
- **Jost L** (2008) GST and its relatives do not measure differentiation. *Molecular Ecology* 17(18): 4015-4026.

- **Karaket T, Poompuang S** (2012) CERVUS vs. COLONY for successful parentage and sibship determinations in freshwater prawn *Macrobrachium rosenbergii* de Man. *Aquaculture* 324-325: 307-311.
- **Kashenko SD** (2007) Adaptive responses of embryos and larvae of the heart-shaped sea urchin *Echinocardium cordatum* to temperature and salinity changes. *Russian Journal of Marine Biology* 33(6): 381-390.
- **Kawecki TJ, Ebert D** (2004) Conceptual issues in local adaptation. *Ecology Letters* 7: 1225–1241.
- **Kempf F, Boulinier T, de Meeûs T, Arnathau C, McCoy KD** (2009) Recent evolution of host-associated divergence in the seabird tick *Ixodes uriae*. *Molecular Ecology* 18: 4450-4462.
- **Kier PM, Grant RE** (1965) Echinoid distribution and habits, Key Largo coral reef preserve, Florida. *Smithsonian miscellaneous collections* 149: 1-68.
- **Kochzius M, Seidel C, Hauschild J, Kirchhoff S, Mester P, Meyer-Wachsmuth I, Nuryanto A, Timm J** (2009) Genetic population structures of the blue starfish *Linckia laevigata* and its gastropod ectoparasite *Thyca crystallina. Marine Ecology Progress Series* 396: 211-219.
- **Kroh A** (2014). *Meoma ventricosa* (Lamarck, 1816) In: Kroh A, Mooi R (2014) World Echinoidea Database: http://www.marinespecies.org/aphia.php?p=taxdetails&id=367846 (02-05-2014)
- **Laffont R, Firmat C, Alibert P, David B, Montuire S, Saucède T** (2011) Biodiversity and evolution in the light of morphometrics: From patterns to processes. *Comptes Rendus Palevol* 10: 133-142.
- **Lawrence JM**. Sea Urchins: Biology and Ecology. *Developments in Aquaculture and Fisheries Science* 38: 1-509.
- **Lenormand T** (2002) Gene flow and the limits to natural selection. *Trends in Ecology & Evolution* 17(4): 183-189.
- **Lessios HA, Robertson DR, Cubit JD** (1984) Spread of *Diadema* mass mortality through the Caribbean. *Scienc*e 226: 335-337.
- **Lessios HA, Kessing B, Pearse J** (2001) Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. *Evolution* 55(5): 955-975.
- **Lessios HA, Kane J, Robertson DR** (2003) Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. *Evolution*, 57(9): 2026-2036.

- **Lessios HA** (2008) The Great American Schism: Divergence of Marine Organisms After the Rise of the Central American Isthmus. *Annual Review of Ecology, Evolution, and Systematics* 39(1): 63-91.
- **Luckenbach MW, Orth RJ** (1992) Swimming velocities and behavior of blue crab (*Callinectes sapidus* Rathbun) megalopae in still and flowing water. *Estuaries* 15(2): 186-192.
- **Magniez-Jannin F, David B, Dommergues J-L, Su ZH, Okada TS, Osawa S** (2000) Analysing disparity by applying combined morphological and molecular approaches to French and Japanese carabid beetles. *Biological Journal of the Linnean Society* 71: 343-358.
- **Mantelatto FLM, Fransozo A** (1997) Fecundity of the crab *Callinectes ornatus* Ordway, 1863 (Decapoda, Brachyura, Portunidae) from the Ubatuba region, São Paulo, Brazil. *Crustaceana* 70: 214-226.
- **Marques F, Pohle G** (1996) Complete larval development of *Clypeasterophilus stebbingi* (Decapoda: Brachyura: Pinnotheridae) and a comparison with other species within the *Dissodactylus*. *Bulletin of Marine Science* 58(1): 165-185.
- **Marshall TC, Slate J, Kruuk L, Pemberton JM** (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7(5): 639-665.
- **McCoy KD** (2003) Sympatric speciation in parasites – what is sympatry? *Trends in Parasitology* 19: 400-404.
- **McCoy KD, Boulinier T, Tirard C** (2005) Comparative host–parasite population structures: disentangling prospecting and dispersal in the black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology* 14: 2825–2838.
- **McCoy KD** (2009) Adaptations et contraintes dans l'évolution des tiques et leurs microparasites : effets de l'hôte en cascade et feedbacks co-évolutifs. Diplôme d'Habilitation à Dirider des Recherches, Université Montpellier II: 55p.
- **McDonald JH** (2009) Handbook of Biological Statistics. Sparky House Publishing, Baltimore: 319 p.
- **McKeown N, Shaw P** (2008) Single paternity within broods of the brown crab *Cancer pagurus*: a highly fecund species with long-term sperm storage. *Marine Ecology Progress Series* 368: 209-215.
- **Metaxas A** (2013) Larval Ecology of Echinoids. In: Lawrence JM (ed) Sea Urchins: Biology and Ecology. *Developments in Aquaculture and Fisheries Science* 38: 69-81.
- **Miller KG, Kominz MA, Browning JV, Wright JD, Mountain GS, Katz ME, Pekar SF** (2005) The Phanerozoic record of global sea-level change. Science 310(5752): 1293-1298.
- **Moellering H, Raynor JN** (1981) The harmonic analysis of spatial shapes using dual axis Fourier shape analysis (DAFSA). *Geographical Analysis* 13: 64-77.

- **Molinari RL, Spillane M, Brooks I, Atwood D, Duckett C** (1981) Surface currents in the Caribbean Sea as deduced from Lagrangian observations. *Journal of Geophysical Research* 86(C7): 6537–6542.
- **Monceau K, Cézilly F, Moreau J, Motreuil S, Wattier R** (2013) Colonisation and diversification of the zenaida dove (*Zenaida aurita*) in the Antilles: phylogeography, contemporary gene flow and morphological divergence. *PLoS ONE* 8(12): e82189.
- **Moreau J, Seguin S, Caubet Y, Rigaud T** (2002) Female remating and sperm competition patterns in a terrestrial crustacean. *Animal Behaviour* 64: 569-577.
- **Mortensen T** (1921) Studies of the development and larval forms of echinoderms. G.E.C. GAD, Copenhague: 252p.
- **Mulvey M, Aho JM, Lydeard C, Leberg PL, Smith MH** (1991) Comparative population genetic structure of a parasite (*Fascioloides magna*) and its definitive host. *Evolution* 45: 1628–1640.
- **Nagelkerken I, Smith GW, Snelders E, Karel M, James S** (1999) Sea urchin *Meoma ventricosa* die-off in Curaçao (Netherlands Antilles) associated with a pathogenic bacterium. *Diseases of Aquatic Organisms* 38: 71-74
- **Narum SR** (2006) Beyond Bonferroni: Less conservative analyses for conservation genetics. *Conservation Genetics* 7(5) : 783-787.
- **Navarro N, Zatarain X, Montuire S** (2004) Effects of morphometric descriptor changes on statistical classification and morphospaces. *Biological Journal of the Linnean Society* 83: 243-260.
- **Neff B, Pitcher T** (2002) Assessing the statistical power of genetic analyses to detect multiple mating in fishes. *Journal of Fish Biology* 61(3): 739-750.
- **Nei M, Li WH** (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences USA* 76: 5269-5273.
- **Ni L, Li Q, Kong L** (2010) Microsatellites reveal fine-scale genetic structure of the Chinese surf clam *Mactra chinensis* (Mollusca, Bivalvia, Mactridae) in Northern China. *Marine Ecology* 32: 488-497.
- **Nice CC, Shapiro AM** (1999) Molecular and morphological divergence in the butterfly genus *Lycaeides* (Lepidoptera: Lycaenidae) in North America: evidence of recent speciation. *Journal of Evolutionary Biology* 12: 936-950.
- **Nichols D** (1962) Differential selection in populations of a heart-urchin. *Systematics Association Publication* 4: 105-118.
- **Nieberding C, Olivieri I** (2007) Parasites: proxies for host history and ecology? *Trends in Ecology and Evolution* 22(3): 156-165.

- **OBIS** (2014) Ocean Biogeographic Information System: www.iobis.org (02-05-2014)
- **Ocampo EH, Nuñez JD, Cledón M, Baeza JA** (2012) Host-specific reproductive benefits, host selection behavior and host use pattern of the pinnotherid crab *Calyptrae otheresgarthi*. *Journal of Experimental Marine Biology and Ecology* 429: 36-46.
- **Olsson J, Florin AB, Mo K, Aho T, Ryman N** (2012) Genetic structure of whitefish (Coregonus maraena) in the Baltic Sea. *Estuarine, Coastal and Shelf Science*: 104-113.
- **Onogi A, Nurimoto M, Morita M** (2011) Characterization of a Bayesian genetic clustering algorithm based on a Dirichlet process prior and comparison among Bayesian clustering methods. *BMC Bioinformatics* 12: 263.
- **Oppliger A, Vernet R, Baez M** (1999) Parasite local maladaptation in the Canarian lizard *Gallotia galloti* (Reptilia: Lacertidae) parasitized by haemogregarian blood parasite. *Journal of Evolutionary Biology* 12: 951–955.
- **Palacios-Theil EP, Cuesta JS, Campos E, Felder DL** (2009) Molecular Genetic Re-Examination of Subfamilies and Polyphyly in the Family Pinnotheridae (Crustacea: Decapoda). In: Martin JW, Crandall KA, Felder DL Decapod (eds) Crustacean Phylogenetics. CRC press, Boca Raton: 620 p.
- **Palumbi SR** (1994) Genetic Divergence, Reproductive Isolation, and Marine Speciation. *Annual Review of Ecology and Systematics* 25(1): 547-572.
- **Pamilo P, Crozier RH** (1996) Reproductive skew simplified. *Oikos* 75: 533-535.
- **Parker GA** (1970) Sperm competition and its evolutionary consequences in the insects. *Biological Reviews* 45: 525-567.
- **Paterson S, Fisher MC, Viney ME** (2000) Inferring infection processes of a parasitic nematode using population genetics. *Parasitology* 120: 185-194.
- **Pearcy M, Timmermans I, Allard D, Aron S** (2009) Multiple mating in the ant *Cataglyphis cursor*: testing the sperm limitation and the diploid male load hypotheses. *Insectes Sociaux* 56(1): 94-102.
- **Peltier WR** (2002) On eustatic sea level history: Last Glacial Maximum to Holocene. *Quaternary Science Reviews* 21: 377–396.
- **Peltier WR, Fairbanks RG** (2006) Global glacial ice volume and Last Glacial Maximum duration from an extended Barbados sea level record. *Quaternary Science Reviews* 25: 3322-3337.
- **Phillips WJ, Cannon LRG** (1978) Ecological observations on the commercial sand crab, *Portunus pelagicus* (L.), and its parasite, *Sacculina granifera* Boschma, 1973 (Cirripedia: Rhizocephala). *Journal of Fish Diseases* 1: 137–149.
- **Pillans B, Gibbard P** (2012) The Quaternary Period. In: Gradstein F, Ogg, J, Schmitz M, Ogg G (eds) The Geological Time Scale 2012. Elsevier, Amsterdam: 979-1010.

- **Pinceel J, Jordaens K, Van Houtte N, De Winter AJ, Backeljau T** (2004) Molecular and morphological data reveal cryptic taxonomic diversity in the terrestrial slug complex *Arion subfuscus/fuscus* (Mollusca, Pulmonata, Arionidae) in continental north-west Europe. *Biological Journal of the Linnean Society* 83: 23-38.
- **Pohle G, Telford M** (1981) The larval development of *Dissodactylus crinitichelis* Moreira, 1901 (Brachyura: Pinnotheridae) in laboratory culture. *Bulletin of Marine Science* 31: 753–773.
- **Pohle G, Telford M** (1983) The larval development of *Dissodactylus primitivus* Bouvier 1917 (Brachyura: Pinnotheridae) reared in the laboratory. *Bulletin of Marine Science* 33(2): 257-273.
- **Poulin R** (2007) Evolutionary ecology of parasites. Princeton University Press, Princeton: 332p.
- **Price PW** (1980) Evolutionary Biology of Parasites. Princeton University Press, Princeton: 237p.
- **Pritchard JK, Stephens M, Donnelly P** (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.
- **Przeslawski R, Ahyong S, Byrne M, Wörheide G, Hutchings P** (2008) Beyond corals and fish: the effects of climate change on noncoral benthic invertebrates of tropical reefs. *Global Change Biology* 14: 2773-2795.
- **Puebla O, Bermingham E, Guichard F** (2009) Estimating dispersal from genetic isolation by distance in a coral reef fish (Hypoplectrus puella). *Ecology* 90(11): 3087-3098.
- **Purcell JFH, Cowen RK, Hugues CR, Williams DA** (2006) Weak genetic structure indicates strong dispersal limits: a tale of two coral reef fish. ? *Proceedings of the Royal Society B: Biological Sciences* 273: 1483-1490.
- **Ramos-Onsins SE, Rozas J** (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution* 19: 2092-2100.
- **Ray N, Currat M, Excoffier L** (2003) Intra-deme molecular diversity in spatially expanding populations. *Molecular Biology and Evolution* 20: 76-86.
- **Raymond M, Rousset F** (1995) An exact test for population differentiation. *Evolution* 49: 1280-1283.
- **Reeves M, Brooks W** (2001) Host selection, chemical detection, and protection of the symbiotic pinnotherid crabs *Dissodactylus crinitichelis* and *Clypeasterophilus rugatus* associated with echinoderms. *Symbiosis* 30: 239-256.
- **Reichard M, Bryja, J, Polačik M, Smith C** (2011) No evidence for host specialization or host-race formation in the European bitterling (*Rhodeus amarus*), a fish that parasitizes freshwater mussels. *Molecular Ecology* 20(17): 3631–3643.

- **Rice WR** (1989) Analysing tables of statistical tests. *Evolution* 43: 223-225.
- **Roberts CM** (1997) Connectivity and management of Caribbean coral reefs. *Science* 278: 1454-1457.
- **Rosenberg NA** (2004) Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes* 4: 137-138.
- **Ross DM** (1983) Symbiotic relations. In: Vernberg SJ, Vernberg WB (eds) The biology of Crustacea 7. Academic Press, Walham: 314 p.
- **Rousset F** (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8(1): 103-106.
- **Rozen S, Skaletsky HJ** (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, New York: 365-386.
- **Rullman JP** (2010) *Gammarus pulex* et *Gammarus fossarum* : confrontation de données morphologiques, génétiques et comportementales dans un contexte d'espèces cryptiques. Mémoire de Master 2, Université de Bourgogne : 36p.
- **Ruppert EE, Fox RS, Barnes RD** (2004) Invertebrate Zoology, a functional evolutionary approach. Thomson Learning, Belmont: 963p.
- **Ryman N, Palm S** (2006) POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. Molecular Ecology 6: 600-602.
- **Sainte-Marie B** (2007) Sperm Demand and Allocation in Decapod Crustaceans. In: Duffy JE, Thiel M (eds) Evolutionary ecology of social and sexual systems. Crustaceans as model organisms. Oxford University Press, Oxford: 191-210.
- **Sainte-Marie G, Sainte-Marie B, Sevigny JM** (2000) Ejaculate-storage patterns and the site of fertilization in female snow crabs (*Chionoecetes opilio*; Brachyura, Majidae). *Canadian Journal of Zoology* 78: 1902-1917.
- **Salas E, Molina-Ureña H, Walter RP, Heath DD** (2010) Local and regional genetic connectivity in a Caribbean coral reef fish. *Marine Biology* 157(2): 437-445.
- **Sapp J** (1994) Evolution by Association. A history of symbiosis. Oxford University Press, Oxford: 255p.
- **Schneider S, Excoffier L** (1999) Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: Application to human mitochondrial DNA. *Genetics* 152: 1079-1089.
- **Schuelke M** (2000) An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18: 233-234.
- **Schultz H** (2009) Sea urchins II: worldwide irregular deep water species. Heinke & Peter Schultz Partner, Hemdingen: 849p.

- **Selkoe KA, Toonen RJ** (2011) Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. *Marine Ecology Progress Series* 436: 291-305.
- **Seutin G, White BN, Boag PT** (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* 69: 82-90.
- **Shulman MJ, Bermingham E** (1995) Early-life histories, ocean currents, and the population-genetics of Caribbean reef fishes? *Evolution* 49: 897-910.
- **Shuster SM, Wade MJ** (2003) Mating Systems and Strategies. Princeton University Press, Princeton: 552 p.
- **Shuster SM, Briggs WR, Dennis PA** (2013) How multiple mating by females affects sexual selection. *Philosophical Transactions B* 368: 20120046.
- **Silberman JD, Sarver SK, Walsh PJ** (1994) Mitochondrial DNA variation and population structure in the spiny lobster *Panulirus argus*. *Marine Biology* 120: 601-608.
- **Silva IC, Mesquita N, Schubart CD, Alves MJ, Paula J** (2009) Genetic patchiness of the shore crab *Pachygrapsus marmoratus* along the Portuguese coast. *Journal of Experimental Marine Biology and Ecology* 378: 50-57.
- **Silva IC, Mesquita N, Paula J** (2010a) Genetic and morphological differentiation of the mangrove crab *Perisesarma guttatum* (Brachyura: Sesarmidae) along an East African latitudinal gradient. *Biological Journal of the Linnean Society* 99: 28-46.
- **Silva IC, Alves MJ, Paula J, Hawkins SJ (**2010b) Population differentiation of the shore crab *Carcinus maenas* (Brachyura: Portunidae) on the southwest English coast based on genetic and morphometric analyses. *Scientia Marina* 74: 435-444.
- **Sotka EE** (2005) Local adaptation in host use among marine invertebrates. *Ecology Letters* 8: 448–459.
- **Stevens PM** (1990a) A genetic analysis of the pea crabs (Decapoda:Pinnotheridae) of New Zealand. I. Patterns of spatial and host- associated genetic structuring in *Pinnotheres novaezelandiae* Filhol. *Journal of Experimental Marine Biology and Ecology* 141:195-212.
- **Stevens PM** (1990b) A genetic analysis of the pea crabs (Decapoda:Pinnotheridae) of New Zealand. II. Patterns and intensity of spatial population structure in *Pinnotheres atrinicola*. *Marine Biology* 108(3): 403-410.
- **Stockley B, Smith AB, Littlewood T, Lessios HA, Mackenzie-Dodds JA** (2005) Phylogenetic relationships of spatangoid sea urchins (Echinoidea): taxon sampling density and congruence between morphological and molecular estimates. *Zoologica Scripta* 34(5): 447-468.
- **Storfer A** (1999) Gene flow and local adaptation in a sunfish-salamander system. *Behavioral Ecology and Sociobiology* 46(4): 273-279.

- **Streiff R, Mira S, Castro M, Cancela ML** (2004) Multiple paternity in Norway lobster (*Nephrops norvegicus* L.) assessed with microsatellite markers. *Marine Biotechnology* 6(1): 60-66.
- **Sturmbauer C, Levinton JS, Christy JH** (1996) Molecular phylogeny analysis of fiddler crabs: Test of the hypothesis of increasing behavioral complexity in evolution. *Proceedings of the National Academy of Sciences USA* 93(20): 10855-10857.
- **Tajima F, Nei M** (1984) Estimation of evolutionary distance between nucleotide sequences. *Molecular Biology and Evolution* 1: 269-285.
- **Takami Y** (2007) Spermatophore displacement and male fertilization success in the ground beetle *Carabus insulicola*. *Behavorial Ecology* 18(3): 628-634.
- **Takeda S, Tamura S, Washio M** (1997) Relationship between the pea crab *Pinnixa tumida* and its endobenthic holothurian host *Paracaudina chilensis*. *Marine Ecology Progress Series* 149: 143-154.
- **Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S** (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731-2739.
- **Taylor MS, Hellberg ME** (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. *Science* 299: 107-109.
- **Teacher GF, Griffiths DJ** (2011). HapStar: automated haplotype network layout and visualization. *Molecular Ecology Resources* 11(1): 151–153.
- **Telford M** (1978) Post-larval growth in two species of *Dissodactylus* (Brachyura: Pinnotheridae). *Bulletin of Marine Science* 28: 645-650.
- **Telford M** (1982) Echinoderm spine structure, feeding and host relationship of four species of *Dissodactylus* (Brachyura: Pinnotheridae). *Bulletin of Marine Science* 32: 584-594
- **Tellier A, Moreno-Gámez S, Stephan W** (2014) Speed of adaptation and genomic footprints of host–parasite coevolution under arms race and trench warfare dynamics. *Evolution* 68: 1-14.
- **Thiel M, Baeza JA** (2001) Factors affecting the social behaviour of symbiotic Crustacea: a modelling approach. *Symbiosis* 30: 163-190.
- **Thomas F, Guégan JF, Renaud F** (2007) Écologie et évolution des systèmes parasités. Éditions De Boeck, Bruxelles: 427p.
- **Tixier A, Gaillard JM** (1969) Anatomie animale et dissection. Editions Vigot Frères, Paris : 375 p.
- **Treml EA, Halpin PN, Urban DL, Pratson LF** (2008) Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. *Landscape Ecology* 23 : 19-36.

- **Tsang LM, Chan BKK, Shih F-L, Chu KH, Allen Chen C** (2009) Host-associated speciation in the coral barnacle *Wanella milleporae* (Cirripedia: Pyrgomatidae) inhabiting the *Millepora* coral. *Molecular Ecology* 18: 1463-1475.
- **Urbani N, Sainte-Marie B, Sévigny JM, Zadworny D, Kuhnlein U** (1998) Sperm competition and paternity assurance during the first breeding period of female snow crab (*Chionoecetes opilio*) (Brachyura: Majidae). *Canadian Journal of Fisheries and Aquatic Sciences* 55(5): 1104-1113.
- **Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P** (2004) Micro-Checker: Software for identifying and correcting genotyping errors in microsatellite Data. *Molecular Ecology Notes* 4(3): 535-538.
- **Van Valen** L (1973) A new evolutionary law. *Evolutionary Theory* 1: 1-30.
- **Via S** (2001) Sympatric speciation in animals: the ugly duckling grows up. *Trends in Ecology and Evolution* 16: 381–390.
- **Vogt G** (2013) Abbreviation of larval development and extension of brood care as key features of the evolution of freshwater Decapoda. *Biological Reviews* 88(1): 81-116.
- **Waelbroeck C, Labeyrie L, Michel E, Duplessy JC, McManus J, Lambeck K, Balbon E, Labracherie M** (2002) Sea-level and deep water temperature changes derived from benthic foraminifera isotopic records. *Quaternary Science Reviews* 21: 295-305.
- **Walsh PS, Metzger DA, Higuchi R** (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10: 506-513.
- **Waples RS, Gaggiotti O** (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity *Molecular Ecology* 15: 1419-1439.
- **Weber LI, Hartnoll RG, Thorpe JP** (2000) Genetic divergence and larval dispersal in two spider crabs (Crustacea: Decapoda). *Hydrobiologia* 420:211-219
- **Weir BS** (1996) Genetic Data Analysis II: Methods for discrete population genetic data. Sinauer Associates, Sunderland: 376p.
- **Weir BS, Cockerham CC** (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.
- **Werding B, Sanchez H** (1989) Pinnotherid crabs of the genus *Dissodactylus* Smith, 1870, associated with irregular sea urchins at the Caribbean coast of Colombia (Crustacea: Decapoda: Pinnotheridae). *Zoologische Mededelingen* 63(4): 35-42.
- **White C, Selkoe KA, Watson J, Siegel DA, Zacherl DC, Toonen RJ** (2010) Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B: Biological Sciences* 277: 1685-1694.

- **Wirtz P, de Melo G, De Grave S** (2009) Symbioses of decapod crustaceans along the coast of Espírito Santo, Brazil. *Marine Biodiversity Records* 2(e162): 1-9.
- **Yearsley JM, Sigwart JD** (2011) Larval transport modeling of deep-sea invertebrates can aid the search for undiscovered populations. *PLoS ONE* 6(8): e23063.
- **Yednock BK, Neigel JE** (2011) Rethinking the mechanisms that shape marine decapod population structure. In Held C., Koenemann S. and Schubart C.D. (eds) Phylogeography and Population Genetics in Crustacea. CRC Press, Boca Raton: 57-73.
- **Yue GH, Chang A** (2010) Molecular evidence for high frequency of multiple paternity in a freshwater shrimp species *Caridina ensifera*. *PLoS ONE* 5(9): e12721.
- **Yue GH, Li JL, Wang CM, Xia JH, Wang GL, Feng JB** (2010) High prevalence of multiple paternity in the invasive crayfish species, *Procambarus clarkii*. *International Journal of Biological Sciences* 6(1): 107-15.

ANNEXES

ANNEXES

	PAN	JAM-WL	JAM-PTB	SCRO-TB	SCRO-KB	SBAR	ANT	GUAD-PL	GUAD-BB	GUAD-SAI	MAR	BEQ	CAN	BARB
PAN		-0.063	-0.000	0.105	0.087	0.040	**0.582**	0.074	0.057	0.052	**1.000**	**1.000**	**1.000**	**1.000**
JAM-WL	0.057		-0.071	0.115	0.094	0.015	**0.547**	0.048	0.052	0.032	**1.000**	**1.000**	**1.000**	**1.000**
JAM-PTB	-	-		0.141	0.118	0.009	**0.516**	0.040	0.065	0.030	**1.000**	**1.000**	**1.000**	**1.000**
SCRO-TB	-0.062	0.379	-		-0.012	0.063	**0.651**	0.104	0.016	0.061	**1.000**	**1.000**	**1.000**	**1.000**
SCRO-KB	0.181	0.276	-	-0.050		0.043	**0.637**	0.084	0.003	0.043	**1.000**	**1.000**	**1.000**	**1.000**
SBAR	-0.037	0.297	-	-0.080	-0.028		**0.442**	-0.010	-0.007	-0.019	**0.877**	**0.894**	**0.809**	**0.957**
ANT	-0.044	0.219	-	-0.090	0.032	-0.065		**0.419**	**0.532**	**0.512**	**0.487**	**0.583**	**0.434**	**0.787**
GUAD-PL	0.028	0.078	-	0.252	**0.447**	0.202	0.254		0.010	-0.039	**0.780**	**0.916**	**0.804**	**0.845**
GUAD-BB	-0.038	0.187	-	-0.061	-0.016	-0.077	0.087	0.009		-0.017	**0.890**	**0.919**	**0.906**	**0.966**
GUAD-SAI	0.060	0.401	-	0.039	0.024	-0.137	0.106	0.211	-0.117		**0.875**	**0.952**	**0.894**	**0.845**
MAR	-0.064	0.386	-	-0.155	-0.142	-0.118	-0.135	0.355	0.003	-0.010		0.043	0.095	**0.925**
BEQ	0.072	-0.446	-	0.225	0.110	0.153	0.144	0.144	0.006	0.228	0.174		0.276	**1.000**
CAN	-0.088	0.201	-	-0.060	0.036	-0.105	0.066	0.028	-0.222	-0.139	-0.028	-0.019		**0.848**
BARB	-0.047	0.070	-	0.200	0.335	0.183	0.254	-0.054	-0.032	0.178	0.178	0.102	-0.021	

Annexe 1. Pairwise D of Jost values (COI analysis) for *D. primitivus* (above diagonal) and *M. ventricosa* (bellow diagonal). Bold values differ significantly from zero (confidence interval created with 10,000 bootstrap replications).

	PAN	JAM-WL	SCRO-TB	SCRO-KB	BAR	ANT	GUAD-PL	GUAD-BB	GUAD-SAI	MAR	BEQ	CAN	BARB
PAN		0.002	0.0026	0.017	0.0009	0.0133	0.0081	0.013	-0.0038	0.0113	0.0067	0.0123	0.0158
JAM-WL	0.0008		-0.005	0.0007	0.0032	-0.0008	0.0021	0.0048	-0.0069	0.0117	0.0006	-0.0013	0.0181
SCRO-TB	0.0013	-0.0076		-0.0022	-0.0008	0.0034	-0.0059	0.0022	0.0036	-0.0041	0.0081	0.005	0.0118
SCRO-KB	0.0116	0.0023	-0.0022		0.0029	-0.0019	-0.007	-0.0056	0.0089	0.0137	0.0028	0.0103	-0.001
BAR	-0.0019	0.0059	-0.0008	0.0032		0.0008	-0.0047	0.003	0.0059	0.0128	0.0026	0.012	0.0013
ANT	0.0123	-0.0023	0.0022	-0.0017	0.0006		-0.0058	-0.0041	0.0077	0.02	-0.0023	0.0141	0.0004
GUAD-PL	0.0049	0.0034	-0.0073	-0.0092	-0.0066	-0.0075		-0.0051	0.0029	0.0044	-0.0046	0.0004	-0.0043
GUAD-BB	0.0116	0.0079	0.0051	-0.007	0.0036	-0.0036	-0.0055		0.0135	0.0085	-0.0032	0.0071	-0.0034
GUAD-SAI	-0.0053	-0.0056	0.0046	0.0093	0.0046	0.0074	0.0012	0.0173		0.0196	-0.0004	0.0036	0.0117
MAR	0.0062	0.0086	-0.005	0.0105	0.0109	0.0173	0.0025	0.0094	0.0186		0.0174	0.0124	0.0324
BEQ	0.0046	0.0000	0.0077	0.0006	0.0013	-0.0053	-0.0076	-0.003	-0.0029	0.0147		-0.0017	0.0026
CAN	0.0111	-0.0027	0.0047	0.0089	0.0136	0.0124	-0.0006	0.0075	0.004	0.0112	-0.002		0.0148
BARB	0.0126	0.0241	0.0162	0.0005	0.0033	0.004	-0.0036	-0.0044	0.0131	0.0321	0.0028	0.0151	

Annexe 2. Pairwise F_{ST} values (Weir 1996) adjusted (above diagonal) and non-adjusted (bellow diagonal) for null alleles in *M. ventricosa*.

	PAN	JAM-WL	JAM-PTB	SCRO-TB	SCRO-KB	SBAR	ANT	GUAD-PL	GUAD-BB	GUAD-SAI	MAR	BEQ	CAN	BARB
PAN		**0.0586**	**0.0916**	**0.1282**	**0.1366**	**0.1994**	**0.2476**	**0.2436**	**0.2856**	**0.2849**	**0.3202**	**0.2826**	**0.3536**	**0.324**
JAM-WL	-0.0007		0.1124	**0.1219**	**0.1694**	**0.2631**	**0.2015**	**0.2208**	**0.2052**	**0.2837**	**0.2724**	**0.299**	**0.2976**	**0.2976**
JAM-PTB	-	-		**0.1205**	**0.1754**	**0.2533**	**0.2432**	**0.2221**	**0.2373**	**0.3053**	**0.246**	**0.2999**	**0.3173**	**0.3173**
SCRO-TB	0.0158	-0.0283	-		0.0678	**0.1286**	**0.0582**	**0.094**	**0.0978**	**0.2221**	**0.1482**	**0.2124**	**0.2483**	**0.2483**
SCRO-KB	**0.0868**	0.0345	-	-0.0027		**0.1705**	**0.1115**	**0.1299**	**0.1465**	**0.257**	**0.2142**	**0.2851**	**0.3229**	**0.3229**
SBAR	0.0141	0.0132	-	-0.0178	0.0049		**0.0837**	**0.0717**	**0.0727**	**0.1654**	**0.0874**	**0.154**	**0.2055**	**0.2055**
ANT	0.0616	0.0017	-	-0.0004	0.0162	0.0227		0.0831	0.0999	**0.1564**	**0.0991**	**0.1522**	**0.2085**	**0.2085**
GUAD-PL	0.0705	0.0882	-	-0.0056	-0.0089	-0.0042	-0.0164		0.0015	**0.1483**	**0.107**	**0.1137**	**0.1752**	**0.1752**
GUAD-BB	0.0708	0.0411	-	0.0161	-0.0133	0.0108	-0.0098	-0.0084		**0.0959**	**0.0521**	0.0815	**0.1647**	**0.1647**
GUAD-SAI	-0.0012	-0.0165	-	-0.0163	0.0374	0.011	0.0263	0.0334	0.0596		**0.1178**	**0.1499**	**0.2069**	**0.2069**
MAR	0.0285	0.0166	-	-0.0152	0.0399	0.036	0.0401	0.0252	0.0101	0.0299		0.01	0.1573	**0.1573**
BEQ	0.0421	0.0569	-	0.0217	0.0236	0.0055	-0.0078	0.0003	-0.0062	0.0106	0.0346		0.1713	**0.1713**
CAN	0.0753	0.0192	-	0.0085	0.0375	0.0392	0.0392	0.0153	0.0223	0.0165	0.0275	-0.0064		**0.1582**
BARB	0.0517	0.0894	-	0.045	0.0135	0.01	0.03	0.0054	-0.0085	0.0368	0.0764	0.0126	0.0472	

Annexe 3. Pairwise D of Jost values (microsatellites analysis) for *D. primitivus* (above diagonal) and *M. ventricosa* (bellow diagonal). Bold values differ significantly from zero (permutation tests with 1000 permutations). The p-threshold was corrected by Benjamini-Yekutieli method (Narum 2006) and were 0.0094 for *D. primitivus* and 0.0099 for *M. ventricosa*.

Mother's (clutch) ID	Relative contributions of each father	Mp	SSq	Me,p	S (skew)
M12	0.978 : 0.022	2	0.96	1.04	0.96
M16	0.636 : 0.364	2	0.54	1.86	0.14
M49	0.838 : 0.162	2	0.73	1.37	0.63
M64	0.833 : 0.056 : 0.028 : 0.028 : 0.028 : 0.028	6	0.70	1.43	0.91
M69	0.886 : 0.086 : 0.029	3	0.79	1.26	0.87
M70	0.844 : 0.133 : 0.022	3	0.73	1.37	0.82
P3	0.976 : 0.024	2	0.95	1.05	0.95
P5	0.867 : 0.089 : 0.044	3	0.76	1.31	0.84
P11	0.951 : 0.049	2	0.91	1.10	0.90
P59	0.857 : 0.143	2	0.75	1.32	0.68
P69	0.867 : 0.067 : 0.044 : 0.022	4	0.76	1.32	0.89
P70	0.636 : 0.205 : 0.159	3	0.47	2.12	0.44

Annexe 4. Summary of the calculation of the mating skew in multipaternal clutches. Mp = total number of fathers. SSq = sum of squares of the relative contributions. Me,p = effective number of fathers (=1/SSq). S = (Mp – Me,p)/(Mp -1)

Male's ID	Offspring tested in clutch 1	Offspring fertilized in clutch 1	Total offspring fertilized in clutch 1	Offspring tested in clutch 2	Offspring fertilized in clutch 2	Total offspring fertilized in clutch 2	Total offspring produced
M15	46	45	198.59				198.59
M24	31	31	203				203
M31	39	39	203				203
M38	47	47	203				203
M63	36	30	169.17				169.17
M71	45	6	27.07				27.07
P27	37	37	203				203
P45	37	37	203				203
M18	36	2	11.28				11.28
M84	36	1	5.64	35	31	179.80	185.44
1	46	1	4.41				4.41
2	44	28	129.18	35	3	17.40	146.58
3	44	16	73.82				73.82
4	37	31	170.08	44	7	32.30	202.38
5	37	6	32.92				32.92
6	36	1	5.64	42	41	198.17	203.81
7	36	1	5.64	35	1	5.80	11.44
8	36	1	5.64	42	1	4.83	10.47
9	45	38	171.42				171.42
10	45	1	4.51				4.51
11	45	39	175.93				175.93
12	45	2	9.02				9.02
13	45	4	18.04	45	39	175.93	193.98
14	41	39	193.10				193.10
15	41	2	9.90				9.90
16	43	43	203	35	30	174.00	377
17	35	5	29				29
18	45	3	13.53				13.53
19	45	2	9.02				9.02
20	45	1	4.51				4.51
21	44	28	129.18				129.18
22	44	9	41.52				41.52
Mean (variance)							114.19 (9677.30)

Annexe 5. Estimate of the average number of offspring produced per male (and variance). Total brood size of 203 was considered, because it is the average brood size calculated from female data.

RÉSUMÉ

Comparer les structures génétiques des populations de deux espèces permet d'évaluer les facteurs environnementaux et les traits d'histoire de vie qui façonnent la dispersion des individus. Dans le cas d'un couple hôte-parasite, cette approche permet aussi de mettre en évidence l'adaptation locale de ces espèces. Le modèle étudié ici est le crabe ectoparasite *Dissodactylus primitivus* et son oursin-hôte *Meoma ventricosa*, deux espèces endémiques des Caraïbes et des côtes américaines voisines. Plusieurs outils moléculaires ont été utilisés à savoir des microsatellites ainsi qu'un marqueur mitochondrial (cytochrome oxydase I). De plus, des analyses morphométriques (analyse de contour) ont également été réalisées.

En étudiant des populations le long de l'arc antillais et de la côte panaméenne, ce travail a mis en évidence que la structure génétique des populations du parasite *D. primitivus* diffère fortement de celle de son hôte *M. ventricosa*. En effet, alors que les populations du parasite présentent une différenciation au sein de cette région, celles de l'hôte sont génétiquement homogènes. Ce contraste peut être expliqué par des caractères biologiques et écologiques propres à chacune des deux espèces (fécondité, habilité à la nage, disponibilité de l'habitat) et suggère des potentialités d'adaptation locale distinctes selon l'espèce considérée. La distance géographique semble être un facteur important dans la structuration génétique des populations du crabe parasite mais la courantologie actuelle ou encore des évènements passés (glaciations) jouent également un rôle. A l'échelle locale d'une même île, les crabes ne présentent pas de différenciations génétique et morphologique entre des sites côtiers distincts. En outre, à cette même échelle (lagon jamaïcain), nous avons pu montrer que des crabes issus d'hôtes d'espèces différentes ne sont pas différenciés génétiquement. Cette absence de différenciation est liée au moins en partie à la mobilité des crabes adultes. Par des analyses de paternité, nous avons souligné cette mobilité et démontré non seulement que le mode de reproduction du crabe correspond à de la polygamie mais aussi que des accouplements pouvaient avoir lieu entre des crabes issus d'espèces d'hôtes distinctes.

ABSTRACT

Comparing the population genetic structures of two species documents on the environmental factors and life history traits that shape the dispersal of the individuals. For host-parasite couple, this approach also permits to predict local adaptation of these species. The investigated species in this work are the ectoparasitic crab *Dissodactylus primitivus* and its sea urchin host *Meoma ventricosa*, both species being endemic to the Caribbean and neighboring American coasts. Several molecular markers were used, namely microsatellites and cytochrome oxidase I (mitochondrial). Moreover, morphometric analyses (shape) were also done.

By studying populations across the Antilles arc and along the Panamanian coast, this work have shown that the genetic structure of the crab populations deeply differ from that of its host. Indeed, while the parasite populations are differentiated within this region, host populations are genetically homogeneous. This contrast can be explained by biological and ecological features (fecundity, swimming capacities, suitability of habitat) that are species specific and it suggests a distinct potentiality of local adaptation between host and parasite. Geographical distance seems to be a key parameter in the observed patterns but marine currents and historical events (glaciations) also play a role. At the local scale of a single island, crabs lack genetic and morphological differentiations when sites are compared along the coast. In addition, at the same scale (Jamaican lagoon), we demonstrated that crabs from different host species are not genetically differentiated. This lack of differentiation is at least partly explained by the mobility of adult crabs. Through paternity analyses, we underlined this mobility and we demonstrated that the crabs have a polygamous behavior but also that mating can occur among crabs from different host species.