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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) 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) 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.

Keywords: Host specialization, Spatial scale, Ectoparasite, Population genetic structure, Microsatellite, Morphometry, Brachyuran decapods, Echinoid

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 (intrapopulations), 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 and 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 novaezealandiae* 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 Meeüs 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 Meeüs 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. 1). 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.1). Discovery Bay (18°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.

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 m²). 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 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 1). Each reaction in multiplex (15 μl) includes 7.5 μl of Master Mix Qiagen (Taq Polymerase, nucleotides),

1 μl of DNA, 0.3 μl (10 μM) 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).

Statistical analyses of population genetics data

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 ($1-\beta$) 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.

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 and 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 2). Allelic richness per locus ranged from 5

to 10.6, with an average of 7.5 (Table 2), 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 2). 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 (Table 2; 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 $p = 0.0011$).

Genetic differentiation of crabs among hosts

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 ($1-\beta$) 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 3A). Other posterior probabilities ($K = 2, 3, 4$) were close to 0. Results from DPART (data not shown) and STRUCTURE were congruent.

Population genetic differentiation among sites

At WL, the samples from the two hosts were pooled since they showed no differentiation (see above). The statistical power ($1-\beta$) 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 4). Fisher tests of differentiation were all non significant (Table 4). 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 3B). 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.2). Female crabs from *P. grandis* tend to have a more rounded margin than females from *M. ventricosa* which have a more angular margin (Fig. 2).

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). Indeed, 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 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 *Pachygrapsus crassipes* in the North Pacific (Cassone & Boulding 2006) and in *Perisesarma guttatum* from the African mangrove (Silva et al. 2010b). 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, Faubry & 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 from 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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Tables and Figures

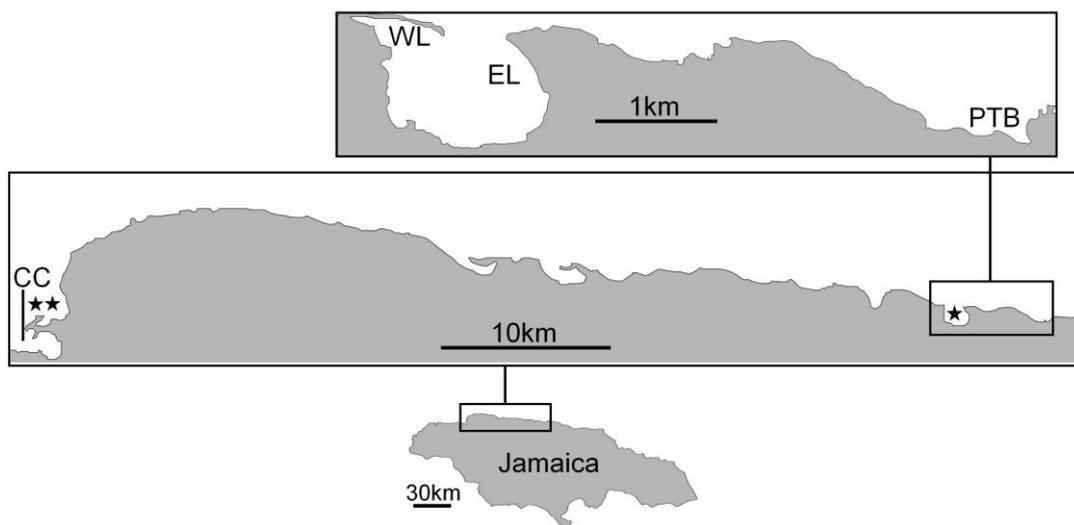


Fig. 1. Sampling sites on the North coast of Jamaica. WL: Western Lagoon, EL: Eastern Lagoon, PTB: Pear Tree Bottom, CC: Chalet Caribe. Simple black star indicates Discovery Bay and double black stars indicate Montego Bay

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 1. 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.

Locus	A					AR					F_{IS}				
	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 2. 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.

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 x 10 ⁻²¹
	3	-5080.8	182.2	7 x 10 ⁻²³
	4	-5049.7	119.8	2 x 10 ⁻⁹
B	1	-12242.5	55.5	1
	2	-12324	284.1	4 x 10 ⁻³⁶
	3	-12344.4	337.9	5 x 10 ⁻⁴⁵
	4	-12360.9	373.7	4 x 10 ⁻⁵²

Table 3. 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 4. 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).

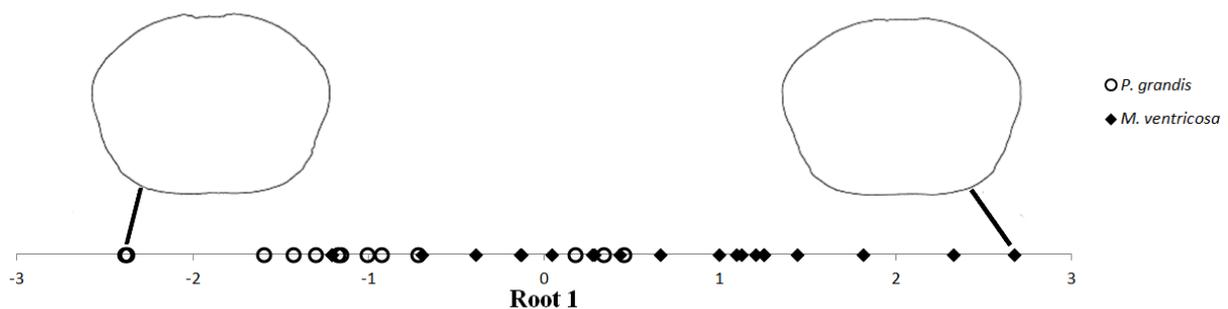


Fig. 2. Females distribution on the canonical axis (Root 1) of a multiple discriminant analyses (MDA). Crabs coming from *P. grandis* are represented by open circles while crabs from *M. ventricosa* are labeled by black diamonds. True outlines of extreme individuals showed the deformation along the axis.

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