Biophysical connectivity explains population genetic structure in a highly dispersive marine species

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Abstract

Connectivity, the exchange of individuals among locations, is a fundamental ecological process that explains how otherwise disparate populations interact. For most marine organisms, dispersal occurs primarily during a pelagic larval phase that connects populations. We paired population structure from comprehensive genetic sampling and biophysical larval transport modeling to describe how spiny lobster (*Panulirus argus*) population differentiation is related to biological oceanography. A total of 581 lobsters were genotyped with 11 microsatellites from ten locations around the greater Caribbean. The overall F_{ST} of 0.0016 ($P = 0.005$) suggested low yet significant levels of structuring among sites. An isolation by geographic distance model did not explain spatial patterns of genetic differentiation in *P. argus* ($P = 0.19$; Mantel $r = 0.18$), whereas a biophysical connectivity model provided a significant explanation of population differentiation ($P = 0.04$; Mantel $r = 0.47$). Thus, even for a widely dispersing species, dispersal occurs over a continuum where basin-wide larval retention creates genetic structure. Our study provides a framework for future explorations of wide-scale larval dispersal and marine connectivity by integrating empirical genetic research and probabilistic modeling.

Introduction

Marine population genetics studies often try to identify ecological and physical processes responsible for shaping spatial patterns of genetic variation among populations (Selkoe et al. 2010). Ocean currents play an important role because the dispersal of many marine species occurs during a pelagic larval phase (reviewed in Cowen and Sponaugle 2009). Oceanographic features such as persistent offshore gyres and counter currents can prevent the mixing and

dispersal of larvae and can significantly increase the retention of larvae (Cowen et al. 2006). Diverse approaches have detected larval retention at spatial scales ranging from tens to hundreds of kilometers (reviewed in Jones et al. 2009). In contrast, strong advective currents may disperse larvae thousands of kilometers from their natal source connecting distant populations (e.g., Banks et al. 2007). Many larvae migrate vertically through the water column and develop stronger swimming abilities as they grow allowing them to remain in retentive currents or swim toward the coast (Kingsford et al. 2002; Paris and Cowen 2004; Staaterman et al. 2012). Interactions between marine larvae and the complex oceanographic environment they inhabit can produce nonlinear patterns of genetic differentiation (i.e., genetic patchiness) that may result from the decoupling of geographic distance from larval dispersal distance (Weersing and Toonen 2009; White et al. 2010).

The seascape genetics approach, which incorporates environmental, physical, and behavioral parameters into marine population genetics studies, provides novel models for explaining how genetic patchiness is correlated with specific features of the seascape environment. Seascape genetics studies have identified genetic structure associated with large-scale oceanographic features such as fronts, semi-permanent gyres, strong boundary currents, and upwelling (Iacchei et al. 2013; Galarza et al. 2009). Since the life history characteristics and behaviors of many marine organisms can greatly influence their dispersal potential, coupled biological–physical models (biophysical models) that incorporate ocean circulation data with larval behavior to describe probabilistic connectivity have become an important component of seascape genetics research by demonstrating that complex genetic structure can form in the marine environment despite the high dispersal potential of larvae (Baums et al. 2006; Galindo et al. 2006; Foster et al. 2012). For example, seascape genetics studies revealed that reproductive

timing, larval behavior, and small-scale oceanographic features acted in concert to limit gene flow among populations of the reef building coral, *Acropora palmata*, separated by the Mona Passage (Baums et al. 2006; Hellberg 2009). Studies of the Caribbean reef fish *Elacatinus lori* suggested that the interaction between seascape continuity and the larval dispersal kernel were the most important drivers of spatial genetic structure, whereas isolation by distance (IBD) was a poor predictor (D'Aloia et al. 2014). An isolation by larval resistance to connectivity model, which used biophysical modeling to identify barriers to gene flow, was a more informative predictor of spatial genetic structure than IBD for the broadcast-spawning coral *A. spicifera* (Thomas et al. 2015). Thomas et al. (2015) hypothesized that isolation by larval resistance to connectivity may be particularly robust in marine species with a high capacity for dispersal that are subjected to complex oceanographic environments.

Seascape genetic analyses of spiny lobsters provide evidence that barriers to gene flow in the marine environment can explain spatial genetic structure in species with high dispersal potential. A study of the California spiny lobster (*Panulirus interruptus*) found that spatial genetic structure was correlated with high upwelling intensity, despite the species' long pelagic larval duration (PLD) of 240–330 d (Iaccei et al. 2013). A genetic and oceanographic modeling approach also identified a region of high self-recruitment that was most likely responsible for the genetic structure observed in *Jasus edwardsii* (Thomas and Bell 2013), which has a PLD >2 yr. Consequently, seascape genetics studies of species with long PLDs, such as spiny lobsters, may further our understanding of the nature of marine dispersal.

Here, we characterize the drivers of spatial genetic structure in the Caribbean spiny lobster (*P. argus*) across the greater Caribbean seascape. This ecologically and commercially important marine species has a prolonged PLD and its range extends throughout shallow seas

and coral reefs in the tropical west Atlantic. Adults inhabit coral reefs and are known to migrate across wide oceanic shelves to reach forereef spawning sites near ocean currents (Bertelsen and Hornbeck 2009). Spawning is seasonal in the northern Caribbean and Florida but year-round in the southern Caribbean (Cruz and Bertelesen 2009). *Panulirus argus* produce pelagic larvae that undergo diel and ontogenetic vertical migration throughout their 5–7-month PLD (Goldstein et al. 2008). The larvae of *P. argus* have the potential to disperse throughout the Caribbean given their long PLD and the strong flow of the Caribbean Current. But that is likely to be an over-simplification. Biophysical modeling studies predict that ontogenetic vertical migration of spiny lobster larvae coupled with retentive ocean currents (i.e., meso- and basin-scale gyres) may increase the retention of larvae, whereas advective environments appear to broadcast larvae more widely (Butler et al. 2011; Kough et al. 2013).

Thus far, it has proven difficult to detect consistent patterns of spatial genetic structure in *P. argus*. There is no evidence of genetic differentiation or IBD using mitochondrial DNA (mtDNA) markers in *P. argus* (Silberman et al. 1994), which has led to the widely accepted hypothesis that there is a single, panmictic population throughout the Caribbean Sea. The only strong divergences in mtDNA sequences reported were between populations from the Caribbean Sea and Brazil, which was attributed to a barrier to larval connectivity created by the Amazon and Orinoco river plumes (Sarver et al. 1998). More recent phylogenetic analyses suggest that Caribbean and Brazilian spiny lobster populations are most likely different species that have been isolated for ~16 million yr (Tourinho et al. 2012). Studies of the population structure of *P. argus* using microsatellite DNA (msDNA) markers suggest that the complex oceanographic environment of the Mesoamerican Barrier Reef (Chérubin et al. 2008) may be an important driver of spatial and temporal patterns of genetic structure (Truelove et al. 2014, 2015).

However, comparing studies that use mtDNA and msDNA markers is difficult due to the different types of information each type of marker provides (Luikart and England 1999).

The seascape genetics approach may help to improve our understanding of the processes driving gene flow and spatial genetic structure in *P. argus* across its range. The larvae of *P. argus* disperse among localities via the prevailing Caribbean Current, which is largely continuous and unidirectional (Fig. 1a). The current originates near the southern Windward Islands, and flows west-northwest through South and Central America into the Gulf of Mexico and Straits of Florida (Florida Current) then emerges from the Caribbean and into the western Atlantic between Florida and The Bahamas to join the Gulf Stream. Persistent gyres, large systems of rotating ocean currents that have a circular pattern of flow, are located in the Gulf of Honduras, Panama–Colombia coast, off the southwest coast of Cuba, and the north of The Bahamas. These gyres are important oceanographic mechanisms that promote local retention of larvae. Coastal topography, particularly the large shallow banks of the Nicaraguan rise and Bahamas, may also create regions of reduced exchange of water from the outside where larval retention is also likely (Fig. 1b).

In this study, we used 17 microsatellites and a comprehensive sampling effort to perform a detailed study of genetic population structure in *P. argus* as related to Caribbean oceanographic conditions. We used patterns of *P. argus* larval dispersal predicted by a biophysical model to identify oceanographic regions associated with low (advective) and high (retentive) levels of larval local retention within the Caribbean seascape. We then genotyped spiny lobsters from these specific oceanographic environments and integrated population genetics and biophysical modeling datasets to explore associations between genetic population structure and potential barriers to larval lobster dispersal. Our sampling strategy included sites within: (1) retentive

oceanographic environments located in offshore gyres; (2) advective oceanographic environments located in the Caribbean Current; and (3) Bermuda, an isolated island archipelago far to the north of the primary Caribbean distribution of *P. argus*. We addressed the following questions: (1) is there evidence for population differentiation in *P. argus* within the greater Caribbean Sea; and (2) how well do spatial patterns of genetic variation correlate with IBD and biophysical modeling estimates of larval connectivity? This paper describes how the complex oceanographic environment of the greater Caribbean seascape acts to reduce gene flow and drive genetic differentiation among Caribbean spiny lobster populations.

Methods

Biophysical modeling

Lagrangian stochastic models of larval transport couple oceanographic circulation models with adult reproductive strategy and larval traits to describe dispersal. Here we use the open-source connectivity modeling system (CMS) that probabilistically describes linkages between locations by moving particles through a virtual ocean, and variability prescribed by distributions of biological traits and resulting from subscale turbulent diffusion (Paris et al. 2013). The CMS parameterized for *P. argus* has been described in detail elsewhere (Kough et al. 2013), but here we summarize its basic structure and present the terms used.

CMS parameterization

We coupled CMS to the data-assimilated 3D Global Hybrid Coordinate Ocean Model 1/25° (~4 km horizontal resolution) (HYCOM; Chassignet et al. 2007) nested within the data-assimilated Global HYCOM 1/12° (~7 km horizontal resolution) from 2004 to 2008 to examine

connectivity in the greater Caribbean. This combination of models has been validated in previous works investigating predicted oil transport in the subsea and at the surface (Le Hénaff et al. 2012), larval damselfish settlement verified with light trap catch (Sponaugle et al. 2012), Pacific coral planula transport verified with genetics (Wood et al. 2016) and lobster larval dispersal verified with post-larval arrival (Kough et al. 2013). The biophysical parameterization here (Electronic Supplementary Material, ESM, Table S1) is specific to *P. argus* and includes larval traits (PLD, competency, mortality, ontogenetic vertical migration), as well as phenology and population structure synthesized from field research and fishery data. This is used to examine connectivity among 261 reef polygons (ca. 50 km x 36 km) representing coral reef habitat in the Caribbean and *P. argus* post-larval sensory zone. Model results were sensitive to perturbations in the biological parameters, thus we used a previously verified configuration (Kough et al. 2013).

Sampling strategy

From September 2010 through October 2011 Caribbean spiny lobsters were sampled from nine locations throughout the greater Caribbean and Bermuda (n = 581). The tissue sampling methodology has been described elsewhere (Moss et al. 2013). The CMS was used to identify a subset of sites from the Moss et al. (2013) study located specifically in retentive or advective oceanographic environments. The CMS was coupled to the Global HYCOM 1/12° bounded to a domain (8–32°N, 55–100°W) and was used to release particles throughout the greater Caribbean to select sites on the extreme ends of the retentive-advective continuum. This analysis identified four retentive sites, located in persistent offshore gyres, and five advective sites in close proximity to the Caribbean Current (Fig. 1; ESM Table S2). Bermuda, located outside the target domain of the CMS, was selected as an outlier site based on its geographic

distance from all other sites. The CMS predicted that retentive sites had at least 70% of the larval imports derived locally and advective sites had a maximum of 30% of locally derived larval imports.

Genotyping

Spiny lobsters were genotyped using 17 polymorphic microsatellite loci (Diniz et al. 2004, 2005; Tringali et al. 2008). Genotyping was performed using an ABI 3730xl automatic DNA sequencer (Applied Biosystems) at the University of Manchester DNA Sequencing Facility. Microsatellite alleles were scored manually with GeneMapper v3.7 (Applied Biosystems). Microsatellite data quality checks are described in the ESM.

Genetic diversity and differentiation

Allelic richness (A_R) was corrected for sample size using rarefaction at each sample site with the R-package HIERFSTAT using 50,000 permutations (Goudet 2005). Microsatellite locus characteristics, departures from Hardy–Weinberg Equilibrium (HWE), and summary statistics are reported in the ESM. A hierarchical AMOVA was run in GENODIVE (Meirmans and Van Tienderen 2004) to identify differences between advective and retentive environments (F_{CT}), among sites within advective and retentive environments (F_{SC}), and among sites irrespective of advective and retentive oceanographic environments (F_{ST}). The AMOVA analysis used 11 rather than 17 loci due to departures from HWE (ESM Table S3). An infinite allele model was used based on Weir and Cockerham's (1984) calculations of F_{ST}. The level of significance was tested using 50,000 permutations. For the AMOVA it should be noted that GENODIVE requires that missing data at any locus be replaced with randomly drawn alleles based on the overall allele

frequencies. This data replacement occurred only for the AMOVA analysis in GENODIVE. We also estimated Hedrick's G'_{ST} in GENODIVE, which can be a more appropriate measure of differentiation when heterozygosity is high because it corrects mathematically for the tendency of F_{ST} to decline as polymorphism increases (Hedrick 2005). The *P*-values for all pairwise comparisons of population differentiation were calculated in GENODIVE with the log-likelihood G-statistic using 50,000 permutations. The sequential goodness of fit (SGoF) multi-test correction was used as a correction against type I errors for all statistical analyses that included multiple comparisons (Carvajal-Rodríguez 2009).

Isolation by genetic distance and biophysical connectivity

We tested for correlations of genetic distance (F_{ST} and G'_{ST}) with geographic distance (IBD) and by modeled biophysical connectivity (isolation by biophysical connectivity) in the R-package ADEGENET (Jombart 2008) using a Mantel test with 10,000 permutations. The R function mantel.randtest was used to perform a Mantel test on matrices of genetic distance and larval connectivity. Probabilities based on 5 yr of model simulations for larval dispersal were used to create a pairwise matrix of biophysical connectivity among all study sites. We did not include biophysical modeling data for Bermuda since its northern location is outside the model domain for this simulation. We used a simple graph theory approach to create a measure of larval connectivity directly related to the probabilities obtained from the biophysical model. However, the spatial and temporal scales of the simulation and the complex oceanography of the Caribbean output created a network in which some nodes did not directly exchange larvae, hence we identified connections based on a stepping-stone approach. The connectivity metric was the shortest loop between pairs of sites (the shortest path from $Node_1 \rightarrow Node_2$ + the shortest path

from $Node_2 \rightarrow Node_1$). Our rationale for using a loop rather than a path is to account for probabilistic exchange from both sites. The biophysical model generates a full matrix with two measures of distance for each pair that we combine as a loop for comparison with the single measure of differentiation given by the genetics. Larval connectivity calculations were made using the shortest path function in the BGL toolbox for MATLAB (Gleich 2015). Edges between nodes were weighted by one minus the probability of larval export, thus the most probable connections between nodes have the lowest values and are selected by the shortest path algorithm as pathways with the least distance. The diagonal (same-site retention) was ignored and only connections between sites were considered. The exact origins of the samples from The Bahamas, Nicaragua, Puerto Rico, Grand Cayman, and Panama were uncertain as they were obtained from fishermen or markets. However, in each of these cases fishing logistics restricted the potential harvest locations and we are confident that the lobsters originated within national waters. To account for origin uncertainty in these cases we took the average shortest loop between each pair. For the three sites in Belize and the Venezuela site, where we knew the definitive sample collection location was a single habitat site, each of these sites was compared to a single habitat site. However, in the sites with origin uncertainty the average of the shortest loop between all of the habitat sites within the country of collection was compared against a single habitat site (or all of the habitat sites within the other country of collection if comparing two locations with origin uncertainty).

Geographical structure of genetic variation

The Bayesian model-based software STRUCTURE v.2.3.3 was used to infer the number of genetically homogeneous clusters (Pritchard et al. 2000). Considering the high levels of gene

flow reported in previous studies of *P. argus* (Silberman et al. 1994; Naro-Maciel et al. 2011; Tourinho et al. 2012; Truelove et al. 2014), we used sampling locations as prior information, which allows structure to be detected at lower levels of divergence than the original STRUCTURE model (Hubisz et al. 2009). We then applied the same model parameters used for marine species with high levels of gene flow (Vandamme et al. 2014). Briefly, we ran 10^5 burn-in iterations followed by 10^6 MCMC (Markov chain Monte Carlo) iterations. A total of three independent replicates were carried out in order to calculate the likely number of clusters (K) ranging from two through eight. The Evanno method (Evanno et al. 2005) was then used to infer K using the online version of STRUCTURE Harvester Web v0.693 (Earl and vonHoldt 2012).

Kinship analysis

A non-random pattern of elevated relatedness due to self-recruitment of larvae was an important driver of spatial genetic structure in the California spiny lobster, *P. interruptus* (Iacchei et al. 2013). We used kinship analysis to test the hypothesis that local retention of larvae within retentive Caribbean oceanographic environments may cause elevated numbers of full siblings in *P. argus* populations. The R-package DEMERELATE was used to calculate the relatedness of individuals within sampling sites following the methods of Truelove et al. (2014), who previously found evidence for elevated numbers of full siblings in *P. argus* populations from the Mesoamerican Barrier Reef.

Results

Population structure

The AMOVA detected no significant structure between advective and retentive environments (F_{CT} = -0.0004; P = 0.712), but there was significant structure among sites nested within advective and retentive environments (F_{SC} = 0.0020; P = 0.0013), and among sites irrespective of advective and retentive environment (F_{ST} = 0.0016; P = 0.005). Likewise, Hedrick's measure of genetic differentiation provided further evidence of significant population structure (G'_{ST} = 0.008; P = 0.0026). Pairwise comparisons of F_{ST} and G'_{ST} among sites provided additional evidence of significant levels of genetic structuring after corrections for multiple tests (ESM Table S5); the two metrics of population structure were also highly correlated ($P < 2.2\ e^{-16}$; $R^2 = 0.98$). Levels of F_{ST} and G'_{ST} were significant for 13 and 9 of the 45 pairwise comparisons, respectively. Panama had the highest number of significant pairwise comparisons (n = 7) of F_{ST} and G'_{ST}, followed by Puerto Rico (n = 5) and Nicaragua (n = 4).

Bayesian cluster analysis in STRUCTURE identified three unique clusters (K = 3; Fig. S1). We assigned sampling sites with a mean membership probability of >0.6 to one of the three unique clusters. Nicaragua (98%), Bermuda (96%), Venezuela (93%), Caye Caulker in Belize (93%), Grand Cayman Island (92%), Bahamas (87%), Glover's Reef Atoll in Belize (85%), Puerto Rico (74%), and Sapodilla Cayes in Belize (67%) were assigned to cluster 1. Panama (65%) was assigned to cluster 2. Individuals assigned to cluster 3 were less frequently observed and were present at levels >5% only in Belize and The Bahamas. (Fig. 1, 2; ESM Table S4).

The kinship analysis suggested that all sampling sites with the exception of Puerto Rico had significantly higher levels of half siblings than expected ($P < 0.05$). Half of the sampling sites [Caye Caulker (Belize), Nicaragua, Panama, Sapodilla Cayes (Belize), and Venezuela] had significantly higher than expected levels of full siblings ($P < 0.05$; Fig. 3). However, levels of kinship were not correlated with biophysical estimates of local retention (P = 0.37; $R^2 = 0.11$), or

the two measures of genetic differentiation, F_{ST} ($P = 0.57$; $R^2 = 0.05$), or G'_{ST} ($P = 0.20$; $R^2 = 0.19$).

Isolation by biophysical connectivity

Isolation by biophysical connectivity revealed a significant correlation between the two measures of genetic differentiation and biophysical modeling estimates of larval connectivity (Fig. 4). A positive relationship between genetic differentiation and larval connectivity was identified for both F_{ST} ($P = 0.04$; Mantel $r = 0.47$) and G'_{ST} ($P = 0.04$; Mantel $r = 0.46$). In contrast, the IBD analysis revealed no relationship between genetic differentiation and geographic distance for F_{ST} ($P = 0.19$; Mantel $r = 0.18$) or G'_{ST} ($P = 0.20$; Mantel $r = 0.22$).

Larval imports based on genetic structure

Following the results of the Bayesian clustering analysis in STRUCTURE (K = 3; Fig. S1) habitat throughout the greater Caribbean seascape was split into three regions: Mesoamerica; the northern Caribbean; and the central and eastern Caribbean. The CMS was used to simulate levels of larval retention within these three regions (Fig. 5). The CMS results suggested that sites within each region tend to receive the majority of their larvae from other sites within the same region.

Discussion

This study integrated two established techniques, microsatellite genetics and biophysical larval transport modeling, to investigate the connectivity of spiny lobster populations throughout the greater Caribbean. The microsatellite genetics identified genetic patchiness among Caribbean

basins that was best explained by biophysical modeling of larval connectivity. Gene flow across Caribbean basins was not constrained by geographic distance, but rather by larval retention within Caribbean basins, which is influenced by the complex oceanographic environment of the Caribbean. Isolation by biophysical connectivity (IBC) explained 21% of the genetic structure among sites and was primarily driven by high levels of IBC between Panama and The Bahamas. Incorporating biophysical modeling into interpretations of genetic structure improved our understanding of the processes driving the connectivity of a widely dispersing marine species and corroborates the utility of this framework for studying gene flow in marine species (Thomas et al 2015; Kendrick et al. 2016; Wood et al. 2016).

Our study found that *P. argus* does not form a single panmictic population in the Caribbean. Microsatellite genetics identified significant pairwise levels of genetic differentiation between neighboring basins (e.g., Panama and Nicaragua), within basins (e.g., Caye Caulker and Sapodilla Cayes in Belize), but not between the most geographically distant basins separated by >2,000 km (e.g., Venezuela and Bermuda). These results indicate that the genetic structure of *P. argus* is more complex than a simple stepping-stone model of IBD. Whereas IBD has explained population structure in other widely dispersing species of spiny lobster (Thomas and Bell 2013), our IBC analysis supports the hypothesis that larval biology coupled with complex oceanographic circulation may isolate *P. argus* populations residing in retentive basins sufficiently to result in genetic differentiation.

The majority of the significant pairwise between-site genetic divergences occurred in Panama, Nicaragua, and Puerto Rico despite very different levels of local retention (0.00, 0.98, and 0.02, respectively). Each of these locations had its highest between-site level of IBC with The Bahamas and the highest levels occurred between The Bahamas and Panama. Bayesian

cluster analysis found similar evidence for limited gene flow between The Bahamas and Panama, assigning >60% of individuals at each site to distinct genetic clusters. High levels of basin-scale larval retention in Panama and The Bahamas coupled with limited larval connectivity between these basins may have isolated each area sufficiently to result in population subdivision. At the same time, stochastic long-distance dispersal events appear to have maintained connectivity across a broad range of populations (i.e., leptokurtic gene flow).

Despite the continuum between long-distance dispersal and basin-scale retention, our results revealed that *P. argus* exhibits population structure. When we examined the probabilistic larval imports from each model habitat location to each cluster identified by the Bayesian genetic analyses the approach identified segregation among the three clusters that was driven by basin-scale retention (Fig. 4). Despite the variable oceanographic environment in the Caribbean and its influence on connectivity (Cowen et al. 2006; Qian et al. 2015) our study supports the hypothesis that larval imports may become constrained within retentive Caribbean basins (Paris and Cowen 2004; Paris et al. 2007; Butler et al. 2011).

Mesoscale oceanographic features may influence the continuum between long-distance dispersal and local retention, which could explain the genetic discontinuity observed in our pairwise comparisons of genetic differentiation among sites in Belize (ESM Table S5). These findings concord with physical oceanographic research (Ezer et al. 2005; Chérubin et al. 2008) suggesting that mesoscale oceanographic features create a strong oceanographic boundary that divides Belize into two provinces: a southern province dominated by recirculation; and a northern province influenced more by offshore currents that flow northward (Lindo-Atichati et al. 2016). This boundary makes it far more likely for sites in northern Belize to receive larvae from distant downstream sites, rather than from local sources, as supported by genetic (Truelove

et al. 2014), oceanographic (Briones-Fourzán et al. 2008), and biophysical modeling (Butler et al. 2011; Kough et al. 2013) studies.

Spatial genetic patchiness may also arise from a variety of processes due to the stochastic nature of larval dispersal. Self-recruitment, sweepstakes recruitment, or behavioral and physical mechanisms that allow for coordinated larval transport may prevent the mixing of siblings throughout the larval pool. These mechanisms may lead to an elevated frequency of siblings within sites, which may help explain genetic patchiness (Christie et al. 2010; Iacchei et al. 2013). Kinship analysis suggested that half of the sites in our study had significantly more full siblings than expected. These findings are in agreement with Iacchei et al. (2013), who found significantly more full siblings than expected in populations of *P. interruptus* along the southwest coast of North America. Levels of kinship in this study were highest in locations of persistent upwelling that may act as a barrier to larval recruitment from outside the local system (Iacchei et al. 2013). As a consequence, populations associated with regions of persistent upwelling were the most genetically differentiated. In contrast, we found no correlation between local retention and elevated frequencies of full siblings in *P. argus*. Our findings suggest that connectivity among many locations in the Caribbean is sufficient to maintain high levels of gene flow, despite the potential for local retention. Studies of population connectivity on other Caribbean coral reef species indicate that even though retentive oceanographic environments combined with larval behavior may substantially increase the likelihood of local retention (Paris et al. 2007), they are by no means 'closed' systems with respect to larval dispersal (Cowen et al. 2006; Christie et al. 2010). The levels of gene flow for the larvae that 'leak out' of retentive oceanographic environments may be sufficient to mask F_{ST}-based signals of local retention (Christie et al. 2010). These hypotheses may explain our findings that spiny lobster populations

in Venezuela and Sapodilla Cayes (Belize) were genetically similar to several other populations over broad spatial scales, despite evidence for local retention at these locations.

Even though we found a significant correspondence between IBC and genetic structure, these results should be interpreted with caution considering that the time scales covered by the genetics and biophysical modeling are not synchronized. While the model tracked lobster larval dispersal over 5 yr, the microsatellite markers may detect signals spanning hundreds to thousands of past generations, depending on the rate of gene flow and migration among lobster populations (Hellberg 2009). Isolation-with-migration (IM) models have been developed to tease apart historical from contemporary signals (Marko and Hart 2011), but this approach would require a much more comprehensive sampling design than employed in our study since unsampled populations can have an unpredictable effect on IM models (Crandall et al. 2012). Likewise, complex mutational processes of microsatellites may prevent IM model convergence (Putman and Carbon 2014). The timescale of genetic parentage analysis should synchronize well with single generation biophysical modeling; however, sampling parents and offspring of spiny lobster requires a labor-intensive and costly multinational sampling regime.

Future studies that employ genomic techniques capable of genotyping spiny lobsters with thousands of single nucleotide polymorphisms (SNPs) may resolve historical from contemporary levels of gene flow without the use of parentage analysis. Currently, the results of the biophysical modeling are likely to have the greatest relevance to fishery management in terms of the potential ebbs and flows and volatility in larval supply that matter on timescales of years to decades. We expect that the next generation of higher resolution genomic and biophysical modeling techniques will continue to find that lobster populations throughout the greater Caribbean are inter-linked by larval supply in complex ways, so that the maintenance of

sustainable lobster fisheries will require a careful mix of both local and international management.

Implications for management

The high inter-annual variability of Caribbean currents and long PLD suggest that managing *P. argus* is likely to remain a formidable challenge. Most prior genetic evidence suggested that population structure of *P. argus* on a Caribbean scale is weak or absent (Silberman et al. 1994; Naro-Maciel et al. 2011; Tourinho et al. 2012), although studies similar to ours have identified biophysical mechanisms (Butler et al. 2011; Kough et al. 2013) that limit larval connectivity among Caribbean populations leading to reduced gene flow and genetic differentiation among sites (Truelove et al. 2014). Biophysical modeling suggests that larvae can disperse across the Caribbean, but also that larval behavior promotes regional retention, especially in areas and seasons where retentive hydrodynamic circumstances dominate (Karnauskas et al. 2011; Sponaugle et al. 2012; Snyder et al. 2014). Thus, despite the potential for uniformly high dispersal by long-lived larvae, the connectivity of spiny lobster populations in the greater Caribbean appears to be complex and spatiotemporally dynamic (Truelove et al. 2015). Our results suggest that management of *P. argus* stocks in the greater Caribbean should be tailored to the regional conditions based on patterns of connectivity to ensure sustainability. Where localized stock structure is evident and associated with obvious retentive oceanographic features, those countries will benefit the most from their local conservation measures to sustain and conserve breeding stock biomass (Kough et al. 2013). However, some larvae travel across political boundaries even in relatively retentive environments, thus Caribbean nations would benefit from targeted and cooperative cross-boundary management schemes that recognize

basin-scale connectivity and the international links among stocks in a larger metapopulation. Integration between biophysical modeling and genetics provides a framework for future research to achieve this goal.

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Figure captions

Fig. 1 (a) Map of the Caribbean Sea and Bermuda showing the locations of the *Panulirus argus* sampling sites (•). The three sites in Belize are abbreviated (CC = Caye Caulker, GR = Glover's Reef, and SC = Sapodilla Cayes). (b) Pie charts indicate the proportions of each of the three discrete genetic clusters identified by the population genetics program STRUCTURE. The white arrow indicates the direction of flow the Caribbean current and gyres. Note that Bermuda was placed inside the panel to maintain the scale of the map but is located at approximately 32° North latitude, 64° West longitude (c) Advective (red) and retentive (blue) environments for modeled spiny lobster larvae in the Caribbean based on the distance from the origin to the endpoints of larvae (N= 16,502,752) to settlement in a Lagrangian model parameterized for spiny lobster. Distances (km) were averaged for all endpoints falling within a 0.1° × 0.1° cell, and gridded across the Caribbean. The scale bar on the right assigns a unique color for the endpoints of larvae with blues indicating low dispersal distances and reds indicating high dispersal distance.

Fig. 2 Graphical summary of Bayesian clustering results in the population genetics program STRUCTURE. (a) Average cluster assignments for each sampling location. The proportions of each location colored red, green, and blue represents the proportion of spiny lobsters (*Panulirus argus*) assigned to discrete clusters 1, 2, and 3, respectively. (b) Cluster assignments for individual spiny lobsters, where each thin vertical line represents an individual spiny lobster. The Y-axis represents the total proportion of each discrete cluster. Black lines separate the sampling locations with names located above panel (a).

Fig. 3 The proportion of full-siblings (gray bar) and half-siblings (hatched bar) for *Panulirus argus* at each sampling site that are greater than levels expected by chance. The expected levels of kinship were calculated using 1000 pairs of randomized populations at each sampling site. Asterisks next to the grey and hatched portions of the histograms indicate significant differences ($P < 0.05$) between observed and expected percentages of siblings for full – and half-siblings, respectively.

Fig. 4 (a – b) Scatterplots indicating a significant relationship (in bold) between two pairwise measures of genetic differentiation (F_{ST} and Hedrick's G'_{ST}) and by biophysical connectivity determined by biophysical modeling of *Panulirus argus* larvae. Red points indicated pairwise comparison between Andros, Bahamas and 1) Panama, 2) Puerto Rico, 3) Venezuela, and 4) Nicaragua. Biophysical connectivity was calculated using the shortest loop between pairs of locations: the sum of the shortest paths connecting two locations, starting at each location. Edges between nodes were weighted by one minus the probability of larval export, thus the most probable connections between nodes have the lowest values on the x-axis and the highest levels of biophysical connectivity. Consequently, as the values on the x-axis increase the likelihood of biophysical connectivity decreases. (c – d) No significant relationships were found between the two pairwise measures genetic differentiation and geographic distance between Panulirus argus sampling sites. The shaded areas indicate confidence intervals of the blue trend line.

Fig. 5 (a) Following the apparent grouping from the genetic relatedness (Figures 1a, 2a, and 2b), habitat around the Caribbean was split into three groups: the northern Caribbean (cyan), the central Caribbean (blue), and the southwestern Caribbean (magenta). Divisions are shown on the

map between the northern and central with a solid line, and the central and southwestern with a dashed line. A biophysical model of *Panulirus argus* larval transport simulated larval exchange among habitat locations. A stacked barplot (b) shows the origin of settling larvae within each location that were imported from each respective group on the Y-axis. Origins are shown as a proportion of the total settling larvae to each site to account for differences in between-site settlement magnitude. The grayscale shade filling the circles on the map corresponds to the location of the habitat along the X-axis of the panel. Four example, sites are shown with Roman numerals: I) Andros Island in the northern Caribbean receive mostly larvae from the northern Caribbean, II) the Cay Sal banks receive from diverse upstream sources, III) Puerto Rico mainly receives larvae from the Eastern Caribbean, IV) Barbados is isolated from the northern and southwestern Caribbean, V) San Blas, Panama and IV) Corn Islands, Nicaragua are both situated at the edge of a gyre causing them to receive larvae from the northern and central Caribbean as well as from more local southwestern sites.

Figure 1

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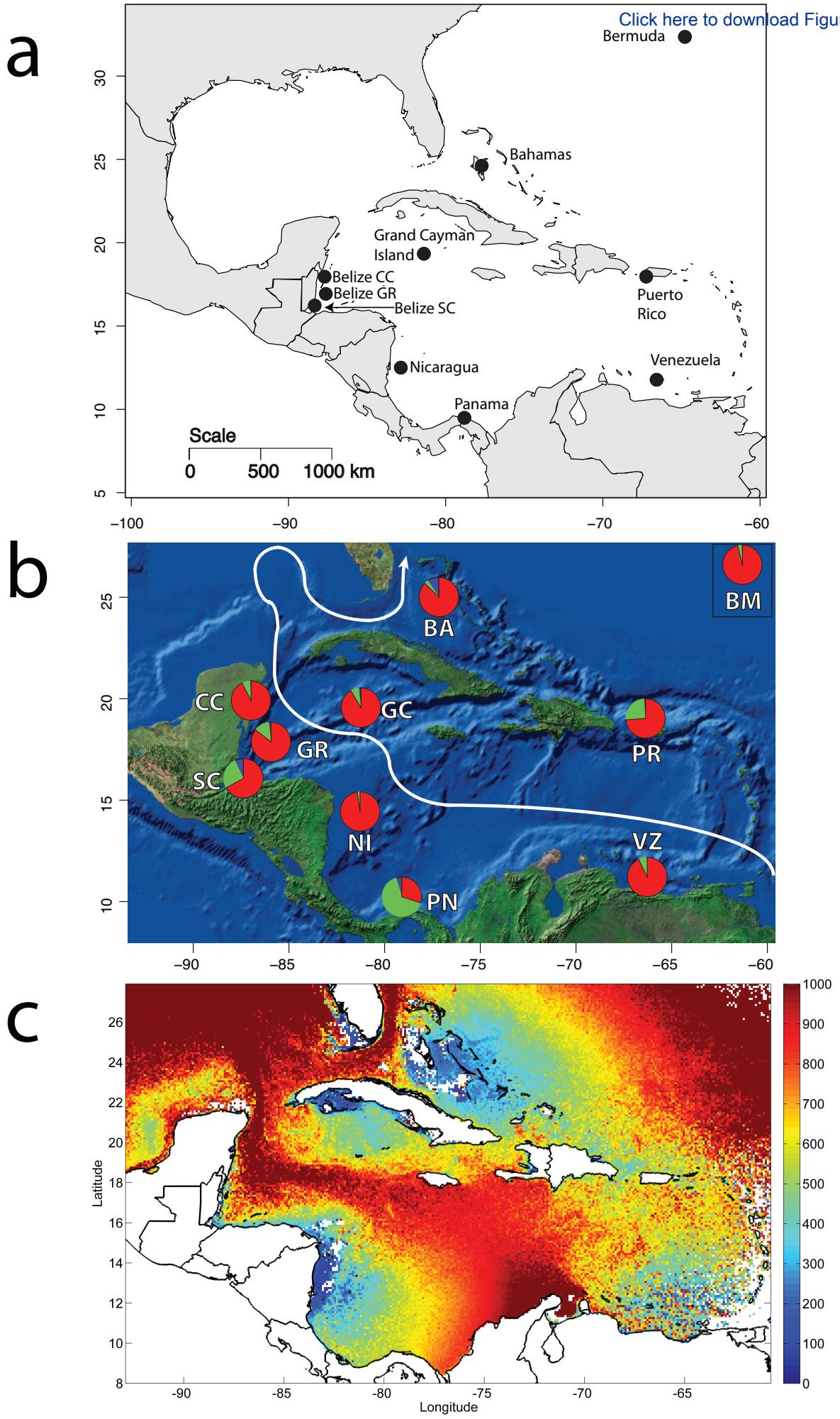

Figure 2

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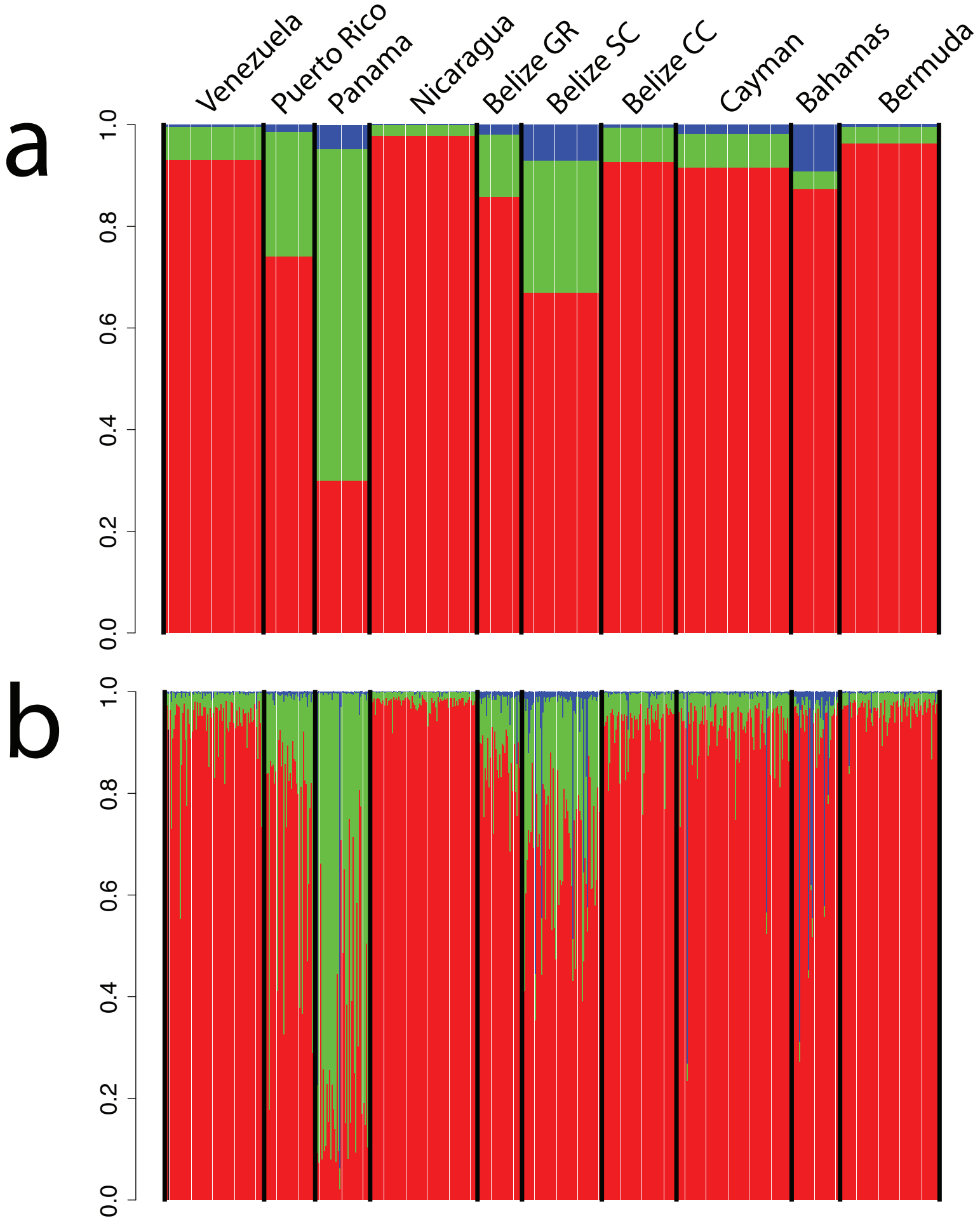

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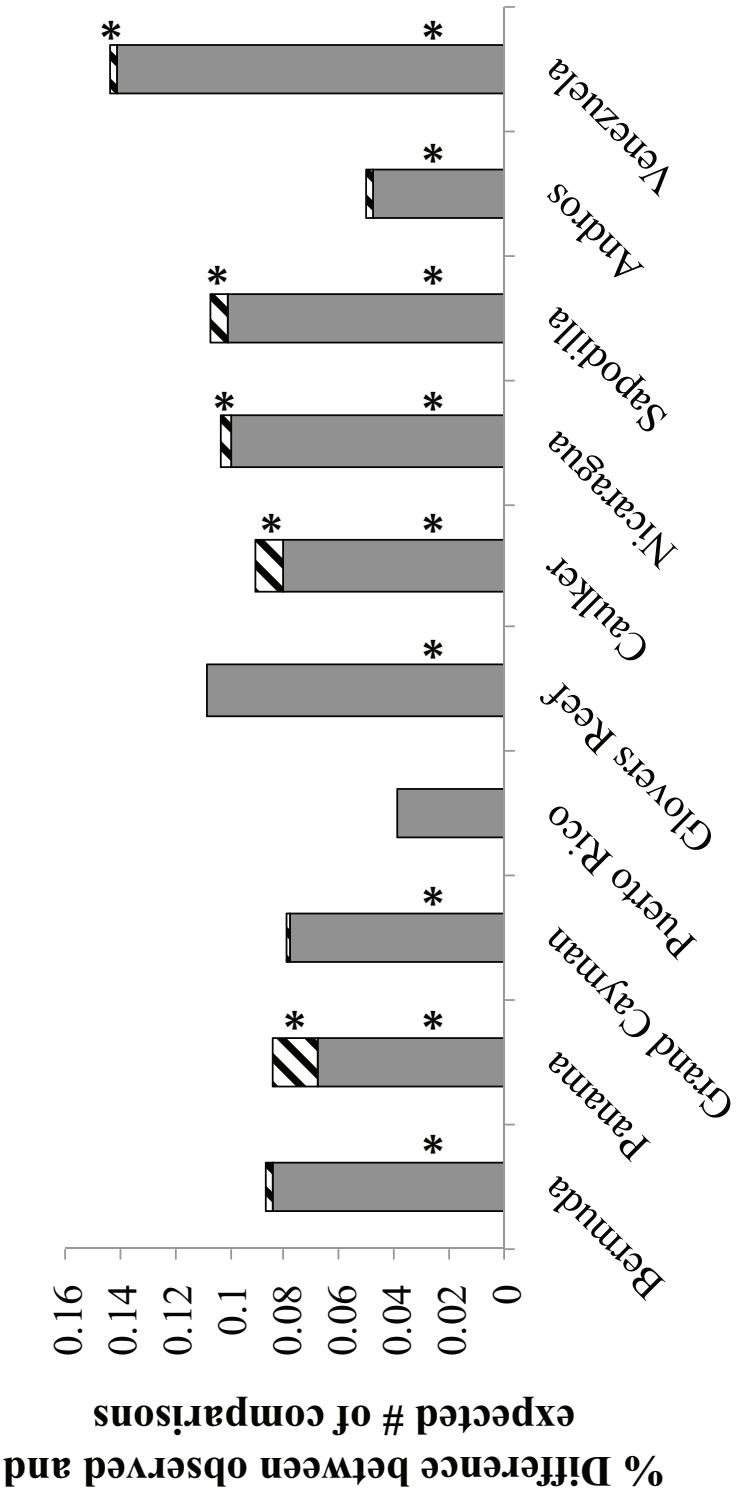

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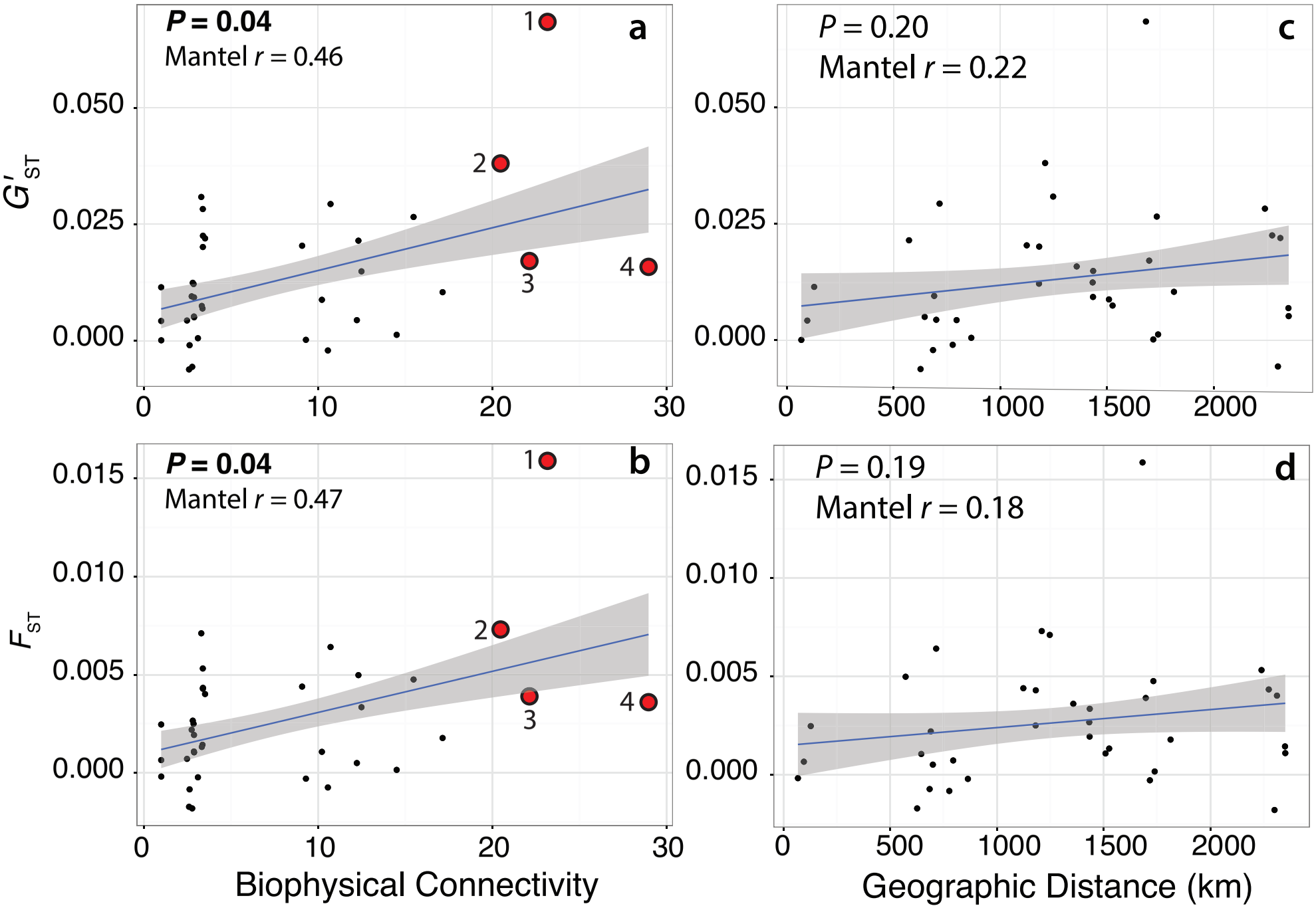

Figure 5

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