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ORIGINAL PAPER

The role of genes in understanding the evolutionary ecology of reef building corals

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Abstract A key tool in evolutionary ecology is information about the temporal dynamics of species over time. Paleontology has long been the major source of this information, however, a very different source of temporal data resides in the variation of genes within and between species. These data provide an independent way to date species divergence but can also uniquely reveal processes such as gene introgression between species and demographic isolation within species. Genetic tools are particularly useful for understanding genera with closely related species that can potentially hybridize, such as reef building corals. Here we use genetic data from four loci (3 introns and 1 mitochondrial) to assay divergence and gene flow in Caribbean corals. The data show that there is persistent gene flow between species in the genus *Acropora*, but that this gene flow is unidirectional and highly variable among loci. Selection against introgressed alleles is high enough at one locus, Mini-collagen, to prevent gene flow between species. By contrast, selection against mitochondrial introgression appears much weaker, with 40–80 times higher rates of inter-specific gene flow than for any nuclear locus we examined. The same loci also show that gene flow among locations within species is locally restricted, but is nevertheless much higher between populations than between species. Interpretation of population data is complicated by the variable nature of selection on introgressed alleles, and some patterns of genetic differentiation might be driven by local introgression and selection. The combination of inter-specific and intra-specific data using the same loci treated in a

In honor of Jeremy B. C. Jackson.

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genealogical framework helps resolve complications due to introgression and helps paint a picture of the evolution and maintenance of species in a complex spatial and temporal framework.

Keywords Coral · Genetics · FST · *Acropora*

Introduction

Information about the temporal history of species is key to evolutionary ecology and has been approached in many different ways. Paleontology has long used the concept of species as morphological entities sailing through time (Jackson and Cheetham 1994) cataloguing the stable occurrence of distinct morphospecies or their rapid evolution (Cheetham et al. 1993). Phylogenetics places a species within the historical framework of its evolutionary relationships to other species (Hillis et al. 1996), and the relative timing of lineage splitting. These reconstructions depend at some level on understanding the temporal framework of species, using the lens of paleontological dating (Jackson and Cheetham 1994), climate records, human historical records (Jackson et al. 2001) or other links to the past (Jackson and Johnson 2000; Jackson and Erwin 2006).

In recent decades, genetic and genomic data have augmented these morphological and paleontological investigations of species and have provided a different temporal window. Analyzed and interpreted in an historical context, molecular data have been valuable in informing at least four main areas of species research that also depend on time. First, molecular data now form the backbone of phylogenetic interpretation of species relationships, and when properly calibrated with fossil information, can supply an idea about the time scale of species divergence. For reef building corals, as an example, many existing families have roots hundreds of millions of years in the past (Romano and Palumbi 1996; van Oppen et al. 1999).

Second, molecular data allow characterization of cryptic species. For coral communities, this includes not only corals but also the symbionts that provide an important layer of ecological diversity in functioning ecosystems (Knowlton and Jackson 1994).

Third, molecular data from many loci can provide insight into the stability and identity of species in the face of hybridization. Through coalescent models of gene exchange, these data can shed light on the genetic and temporal processes that maintain morphological integrity of species. Hybridization as an evolutionary force in reef building corals has been a topic of much discussion (Veron 1995; Willis et al. 2006). Many colonies in some genera show morphological features intermediate between co-occurring species (Veron 1981), and gene trees are often not monophyletic among species (van Oppen et al. 2000, 2001, 2004). However, few tests of genetic introgression have been performed (Vollmer and Palumbi 2002). Genetic data for corals are particularly needed to assay the potential for introgression of genes between species, and to understand the role of hybridization in species evolution.

Fourth, data on genetic variants can be used to infer patterns and rates of gene flow among sub-populations. In this case, the time spans are likely to be shorter than for other analyses. Analysis of gene flow patterns can suggest the degree to which populations are open to immigration, and provide a measure of dispersal capacity over long distances. For species groups with introgression, there is also the opportunity to compare rates of gene flow between species at one location with rates between locations within species.

Here, we present new data and analyses on patterns of genetic diversity within and between species of Caribbean branching corals in the genus *Acropora*. These species have helped form the backbone of modern Caribbean reefs, where they have been some of the most abundant and ecologically important species. They are morphologically distinct but form viable hybrids (Vollmer and Palumbi 2002) that occur throughout the Caribbean. Some evidence suggests limited dispersal capacity around the Caribbean (Baums et al. 2005; Vollmer and Palumbi 2007). However no study has compared the intrinsic patterns of gene flow between species and among populations.

In order to address questions of dispersal scale and genetic exchange between species with the same temporal metric, we sampled populations of *A. cervicornis* along a transect from Florida to Puerto Rico, analyzing genetic patterns at short scales within islands and longer scales among islands and island groups. We also provide data at the same loci for the co-occurring species *A. palmata*, examine the rates of movement of alleles between species using coalescent simulations, and contrast the rates of intraspecific, inter-population gene flow with the rates of exchange between species. These combined analyses within and between species paint a picture of the complex ecology of coral genetics at the species and population level. Our aim is to show how analysis of genetic diversity within and between species adds a dynamic temporal context.

Methods and materials

Multilocus sequence data were obtained for 297 colonies of *Acropora cervicornis*, and 84 colonies of *A. palmata* from eleven locations in the wider Caribbean (Tables 1, 2). This data set is an expansion of the data set in Vollmer and Palumbi (2007), including new data from *A. palmata*, and an expanded data set from *A. cervicornis* that includes many more closely spaced populations. DNA was isolated, amplified and sequenced for the mitochondrial (mtDNA) control region and three introns from single copy nuclear genes—Mini-collagen, Calmodulin, and PaxC—using coral-specific primers (see basic methods in Vollmer and Palumbi 2002, 2007). Heterozygous base calls were confirmed by sequencing in both directions. In most cases, there were few individuals with multiple heterozygous DNA positions. These were converted to allelic haplotypes using PHASE implemented in DnaSP (Librado and Rozas 2009). Allele sequences were also used to generate haplotype networks within and between species to identify alleles found only within *A. cervicornis* (native) and alleles shared with *A. palmata* (potentially introgressed). Different alleles were coded by number and multilocus genetic data for *A. cervicornis* and *A. palmata* were used to genotype corals and identify clones produced by asexual fragmentation using GenAlEx 6.4 (Peakall and Smouse 2006). Putative clones were excluded from all analyses.

A two-population Bayesian coalescent modeling approach (Nielsen and Wakeley 2001; Hey and Nielsen 2004) was used to estimate the rate of gene flow between *A. cervicornis* and *A. palmata* and test the null hypothesis that shared lineages between the species are due to ancestral polymorphism (i.e. migration rate between species has been zero since divergence). We used the program IM (Hey and Nielsen 2004), an extension of the original model (Nielsen and Wakeley 2001), which allows us to estimate the rate of gene flow between the species in both directions. IM estimates six population genetic parameters—three θ ($4N_e\mu$) parameters (one for each species and their ancestor), migration in both directions between the species, a divergence time (t), and calculates the time to the most recent common ancestor (TMRCA), (Marko and Hart 2011). The IM model estimates migration rates scaled to the mutation rate (migration per mutation event, or m/μ), where m is the fraction of migrants between

Table 1 Sample sizes per species used in the introgression analysis

Population	*A. cervicornis*		*A. palmata*
	Samples	Genets	Samples
Panama	38	25	11
Belize	51	21	5
Yucatan	3	3	3
Florida	15	5	–
Bahamas	52	33	19
Turks & Caicos	51	33	–
Jamaica	9	4	3
Puerto Rico	48	26	22
St. Croix	–	–	12
Curacao	30	19	9
Total	297	169	84

All samples from *A. palmata* represented different genotypes. Locations are described in Vollmer and Palumbi (2007)

Table 2 Sample sites and sample sizes for *Acropora cervicornis* collected in the Northern Caribbean and allele counts for the three nuclear intron loci

Location	*Ng*	Calmodulin			Mimi-collagen			PaxC				
		n1	n2	i1	n1	n2	n3	n1	i1	i2	i3	i6
Florida keys[a]	6	9	0	3	1	5	0	9	1	0	0	0
Andros	41											
North Andros[a]	8	10	0	6	5	5	0	12	3	0	1	0
North Mid Andros	16	18	0	14	20	12	0	26	1	0	4	1
Bastiane point	11	16	0	6	15	6	1	14	2	0	5	1
Lee stocking Island[a]	6	9	0	3	7	3	0	7	3	2	0	0
San Salvador	19											
N. San Salvador[a]	8	5	0	8	7	9	0	8	5	0	1	0
S. San Salvador[a]	11	15	0	7	12	8	0	17	1	0	4	0
Turks and Caicos	70											
South Caicos[a]	21	24	2	14	27	13	0	38	4	0	0	0
Southern Patch Reefs[a]	10	13	0	7	11	3	0	15	5	0	0	0
West Grand Turk	18	24	0	12	21	15	0	32	2	0	2	0
North Grand Turk	11	16	0	6	15	7	0	21	0	0	1	0
Southern Grand Turk	10	8	0	8	8	12	0	15	4	0	1	0
Puerto Rico[a]	20	33	0	7	24	0	0	22	7	1	0	0
Total	156											

Ng number of genotypes. Allele notations from Vollmer and Palumbi (2007) with n and i denoting native versus introgressed alleles, respectively.

[a] Sequences from Vollmer and Palumbi (2007)

populations per generation and μ is the per-generation mutation rate. Using the estimated θ ($4N_e\mu$) of the species, the estimated migration rate can be converted into the rate of diploid gene flow per generation ($M = 2N_em$) using the relationship $m/\mu \times \theta/2$.

Bayesian coalescent simulations were conducted for each gene separately in order to determine how strongly interspecific gene flow differed among genes. Coalescent estimates were first obtained for the complete Caribbean-wide dataset comparing *A. cervicornis* and *A. palmata* as two panmictic populations. Multiple simulations ($n = 5$ or more) were run for each gene to set the range of the parameter priors and then confirm that the model converged on similar parameter estimates across independent runs. Simulation results presented here used minimum burn-in periods of 2×10^5 steps followed by greater than 10^9 Markov chain simulation steps. All three nuclear loci used the infinite sites mutation model. A HKY mutation model and an inheritance scalar of 0.25 were used for the mtDNA data.

Moderate to high levels of population structure observed in *A. cervicornis* across the greater Caribbean also allowed us to use the coalescent simulations to compare interspecific gene flow across multiple regions within the Caribbean. To assess if the rates of interspecific gene flow varied geographically, independent coalescent simulations were conducted for four regions—Panama, Puerto Rico, Curacao, and the Bahamas Bank (the Bahamas and the Turks and Caicos)—for which we had good sampling (ca. 20+ genets) and which we have previously shown (Vollmer and Palumbi 2007) to be highly genetically distinct [$F_{ST} > 0.20$ or less than one migrant per generation after Wright (1951)]. Data were combined for the Bahamas and Turks & Caicos for these analyses because pairwise genetic differentiation (Φ_{ST}) between the locations was not greater than 0.20 and because of their geographic proximity. In these independent regional comparisons, *Acropora palmata* was treated as a single panmictic population to increase our sample sizes and because significant genetic structure was not detected at the four genes that we surveyed (e.g. mtDNA $\Phi_{ST} = 0.01$).

Standard analyses of population genetic structure were based on analysis of molecular variance (AMOVA) in Arlequin v2.0 (Schneider et al. 2000) and standard F_{ST} estimates [GenePop v 1.2, (Raymond and Rousset 1995)]. Hierarchical AMOVA was used to estimate levels of genetic differentiation among locations (Φ_{ST}), among locations on an island (Φ_{ct}), and between populations on different islands (Φ_{sc}) for the mtDNA dataset and the combined nuclear SNPs. All populations were in Hardy–Weinberg and linkage equilibrium. Assignment tests were conducted according to the methods of GeneClass2 as implemented in GenAlEx6.1 (Peakall and Smouse 2006).

Spatial autocorrelation was conducted over several different spatial scales based on the intron allele data at all three nuclear loci and at each locus separately. Patterns at all alleles were combined using the autocorrelation methods in GenAlEx and tested against Monte Carlo simulations of correlation confidence intervals under conditions of no spatial structure. To visualize the geographic areas over which spatial autocorrelation might exist in the data set, we used Local 2D Spatial Autocorrelation analysis (Double et al. 2005) to graph areas within which ten nearest neighbor samples consistently showed higher than expected genetic correlations. Results did not differ when the number of nearest neighbors analyzed varied up to the average population sample size of 20. We report areas that showed significant local spatial correlation coefficients ($P < 0.05$) graphically in order to estimate whether the global autocorrelation analysis is driven by local patterns at a single or multiple localities.

Results

Genealogical evidence of one-way gene flow in corals

Statistical parsimony networks for all four genes show two distinct clades of alleles (Fig. 1). Three genes—Calmodulin, PaxC, and the mtDNA control region—show similar

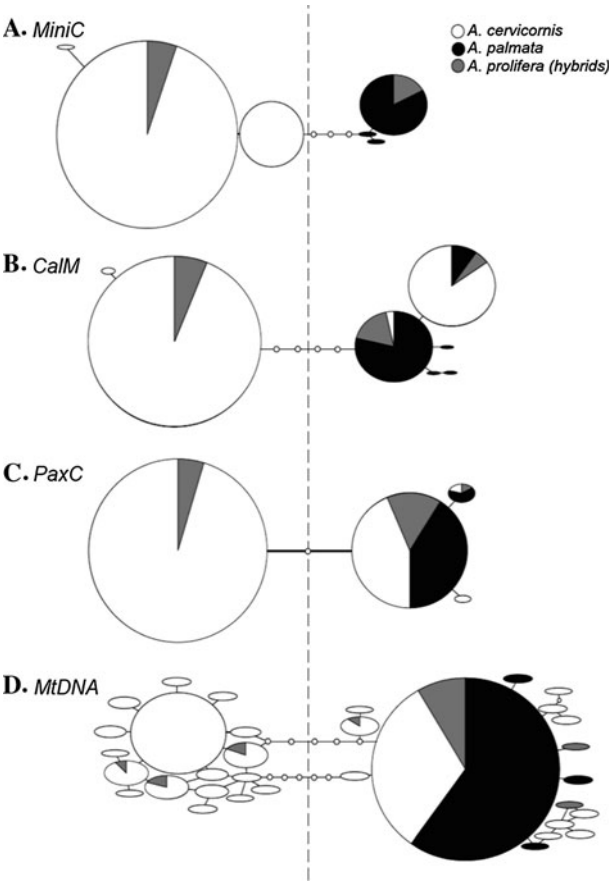

Fig. 1 Minimum spanning networks for **a** Mini-collagen, **b** Calmodulin, **c** PaxC, and **d** the Mitochondrial Control Region showing the distribution of alleles from the species *Acropora cervicornis* (*white*), *A. palmata* (*black*), and their hybrids *A. prolifera* (*gray*). Each gene genealogy is comprised of two clades of alleles—one clade of alleles native to *A. cervicornis*, i.e. 'cervicornis' alleles (*left*) and a clade of 'palmata' alleles that reside in *A. palmata* but also cross the species boundary into *A. cervicornis* via one-way backcrossing with hybrid *A. prolifera*. Networks are drawn proportionally to the number of alleles observed in each species. Sequences are available on GeneBank (Accession numbers JN871685-JN871694)

patterns in which one clade of alleles is found exclusively in *A. cervicornis* (Fig. 1, left), and in the second clade identical and/or related alleles are found in both morphospecies (Fig. 1, right). This pattern is consistent with one-way gene flow from *A. palmata* into *A. cervicornis.* The allele clade found exclusively in *A. cervicornis* is interpreted to contain only native '*cervicornis*' alleles. The clade with alleles shared between the species contains both *A. palmata* native alleles and alleles that crossed the species boundary (i.e. introgressed) into *A. cervicornis* via one-way backcrossing with hybrid *A. prolifera*. However, it is possible that these phylogenetic patterns result from ancestral shared alleles being present in modern populations of the two coral species. At Mini-collagen, both species are monophyletic and possess their own divergent set of alleles, suggesting little or no gene flow or no ancestral polymorphism at this locus.

Genetic differentiation among species

Despite the evidence for gene flow between species, AMOVA results indicate that there is strong and significant genetic differentiation between *A. cervicornis* and *A. palmata* at each of the four genes. Highest genetic differentiation was detected at Mini-collagen ($\Phi_{ST} = 0.942$) where no interspecific gene flow was observed. Large genetic differences were also detected in Calmodulin, PaxC, and mtDNA ($\Phi_{ST} = 0.548$, 0.608, and 0.519, respectively). These high Φ_{ST}'s confirm that the rates of interspecific gene flow are low and not sufficient to homogenize the genetic differences between the species.

Divergence time between species

The timing of species divergence is estimated as the number of observed mutation events between species corrected for inter-specific gene flow in the IM program, (t in Table 3), divided by the rate of mutation per million years. Because corals have a low rate of mtDNA and nuclear mutation (Romano and Palumbi 1996; van Oppen et al. 1999), the small number of differences among species nevertheless requires a long time to accumulate. Coral mtDNA appears to evolve at about a rate of 0.1% per million years (Romano and Palumbi 1996; van Oppen et al. 1999). Part of the explanation of this low rate is the possible occurrence of mismatch repair in cnidarians (Hellberg 2006), making mtDNA and nuclear evolutionary rates more similar.

Voolstra et al. (2011) surveyed 3295 orthologous genes between *A. palmata* and the Pacific species *A. millepora*. They found an overall divergence of 4.3% over the 40–50 million years separating these taxa (Wallace 1999), suggesting a genome-wide divergence rate of 0.09–0.11% per million years. In our data set, we surveyed 334–516 bp of nuclear sequences per locus. At the nuclear rates above, we expect a mean number of mutations of 0.3–0.6 per nuclear locus per million years. For mtDNA, we would expect 0.9 mutations per million years (across 941 bp). These analyses suggest a splitting time of 3.2 and 6.9 million years based on the polyphyletic sequence data and IM analyses from mtDNA and PaxC (Table 3). For the non-introgressing MiniCollagen, the major alleles between species show one fixed base pair difference with an estimated origination time of 2.9 million years (Table 3).

Estimated rates of interspecific gene flow

Results of the IM analyses (Table 3) for the complete Caribbean-wide dataset comparing *A. cervicornis* and *A. palmata* confirm that shared polymorphisms between the species are due to one-way gene flow from *A. palmata* to *A. cervicornis* (m_c) and not due to ancestral polymorphism (in which case m would be indistinguishable from zero). Estimated rates of gene flow into *A. cervicornis* were highest for mtDNA ($N_f m = 12.5$), which is equivalent to 8 introgressed haplotypes crossing the species boundary per generation. Much lower rates of gene flow were estimated for the two introgressed nuclear genes—Calmodulin and PaxC. The estimated rate of gene flow at Calmodulin ($2N_e m = 0.15$) is equivalent to one diploid introgression event every 6 generations. Estimated gene flow at PaxC was slightly higher ($2N_e m = 0.26$) or roughly one diploid introgression event every four generations. Nuclear introgression was absent at Mini-collagen.

IM analyses comparing the four distinct *A. cervicornis* populations—Panama, Bahamas and the Turks & Caicos, Puerto Rico, and Curacao—separately did not show strong differences in the estimated rates of interspecific gene flow (Table S1). Instead, estimated

Table 3 Bayesian coalescent estimates of the rate of gene flow (i.e. genetic introgression) between *A. cervicornis* and *A. palmata*, their effective population sizes (θ), and divergence times (t)

Gene	N_C	N_P	bp	Θ_C	Θ_P	Θ_A	t	TMRCA	m_C	m_P	M_C	M_P
Mini-collagen	222	46	373	0.07 0.02–0.73	0.27 0.09–1.6	–	10.48 2.2–48.8	2.9	0 0–4.1	0 0–2.3	0	0
Calmodulin	302	84	334	0.16 0.08–0.87	0.88 0.36–2.45	–	–		1.08 0.38–7.9	0 0–2.2	0.15	0
PaxC	300	70	516	0.16 0.06–1.07	0.07 0.04–1.06	–	1.58 0.7–48.5	3.1	2.85 1.05–37.3	0 0–8.5	0.26	0
mtDNA	168	66	941	31.74 21.1–67.1	1.49 0.49–6.47	–	6.25 4.9–94.2	6.9	0.63 0.21–3.15	0 0–6.45	12.5	0

Migration parameter (m) is scaled to the mutation rate (m/μ), which can be converted to the population migration rate (M, or $2N_em = m/\mu \times \theta/2$). Time to most recent common ancestor (TMRCA) is in millions of years, based on mutation rates discussed in the text. We report mean values with 95% confidence limits listed underneath each estimate

N number of alleles sampled for *A. cervicornis* (N_c) and *A. palmata* (N_p), Length in base pairs of sequence, Subscripts are *A. cervicornis* (C), *A. palmata* (P), and common ancestor (A), Dashed cells indicate parameters that can not be accurately estimated

rates of interspecific gene flow (m_c and M_c) for each of the three introgressed loci (Calmodulin, PaxC and the mtDNA) were similar between the four populations. The only possible exception was the elevated rate of mtDNA gene flow in Panama, which was at least four times higher than in the other three populations.

Intraspecific genetic differentiation among islands for *A. cervicornis*

Nuclear and mitochondrial genetic variation within *A. cervicornis* was significantly partitioned among island groups (Florida, Bahamas, Turks and Caicos and Puerto Rico) separated by 100–1,000 s of km. AMOVA of nuclear loci showed significant differences across loci among all populations ($\Phi_{ST} = 0.024$, $P = 0.02$) and among island groups ($\Phi_{CT} = 0.02$, $P = 0.04$). For the AMOVA, inspection of pairwise differences showed that Puerto Rico samples were highly differentiated from the Turks and Caicos, Bahama and Florida populations ($P < 0.018$ in all cases, Fig. 2; Table 4). Exact tests show nuclear differentiation in 12 of 20 comparisons among islands (Table 4). Pairwise comparisons to Puerto Rico are significant in five of six tests. Grand Turk populations are significantly different from all other island groups ($P < 0.001$ in all cases). Pairwise comparisons to Florida are different in 4 of 6 cases.

Inspecting the nuclear allele data (Table 2) shows some of the allele frequency differences responsible for these patterns. For example, Puerto Rico shows no variation at Mini-collagen, and is nearly fixed for the native allele 1 at Calmodulin, unlike the other islands. Puerto Rico and Turks and Caicos have a higher frequency of the native PaxC allele 1 (generally above 80%) compared to lower frequencies of this allele in the other island groups.

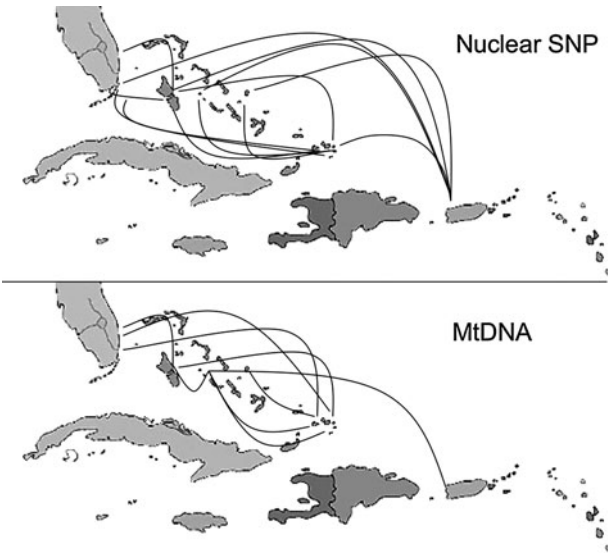

Fig. 2 Sample locations for coral collections and significant population differences. *Upper* Concave down curves denote significant differences in nuclear allele frequencies between pairs of populations based on Φ_{ST}'s. Concave up curves denote significant pairwise Fisher's Exact tests (note that Puerto Rico is different in Exact tests from all other populations except Lee Stocking Island but that these curves are omitted for figure clarity). *Lower* curves represent significant pairwise population differences for mtDNA control region sequences

Table 4 Broad scale nuclear differentiation across the northern Caribbean for *A. cervicornis*

Island		1	2	3	4	5	6	7
Florida	1		–	++	+++	–	+	++
Lee Stocking	2	0.0150		–	+++	–	–	–
		−0.0036						
Andros Island	3	**0.057**	−0.020		+++	–	–	++
		0.195	**0.333**					
Grand Turk	4	0.0661	0.045	0.041		+++	+++	+++
		0.1654	***0.286***	−0.006				
San Salvador	5	0.0213	−0.015	−0.004	***0.029***		–	++
		0.1019	0.215	0.009	0.005			
South Caicos	6	0.0454	−0.008	−0.003	0.027	0.000		+
		0.3214	***0.467***	−0.007	0.007	**0.055**		
Puerto Rico	7	***0.1783***	**0.043**	**0.037**	***0.071***	***0.076***	**0.031**	
		0.1062	**0.262**	−0.020	−0.013	−0.010	0.017	

Lower values include pairwise Φ_{ST} between populations from intron allele frequencies across all three loci as the top value and Φ_{ST} values for mtDNA as the lower value. Bold values are significant at $P < 0.05$. Values in bold italics show $P < 0.01$. Upper data triangle shows results from Fisher's Exact tests on nuclear alleles: + signifies a significant difference at the $P < 0.05$ level; ++, $P < 0.005$; +++, $P < 0.0001$

For mtDNA, AMOVA also shows regional differentiation across island groups ($\Phi_{CT} = 0.058$, $P = 0.004$). Florida is distinct in three of six comparisons (Fig. 2). The population on South Caicos in the Turks and Caicos Islands is substantially differentiated from most populations to the Northwest (Florida, Lee Stocking and San Salvador—only Andros is an exception, Table 2). This is because 17 of the 20 genets from South Caicos share just two mtDNA haplotypes.

Intraspecific genetic differentiation within islands

Average F_{ST} for nuclear or mitochondrial loci in *A. cervicornis* was highly variable between populations within islands. These populations typically were found within 50 km of each other, and for the 14 within-island pairwise comparisons we could make, F_{ST} averaged 0.04 for mtDNA and 0.02 among nuclear genes. Seven of 14 comparisons showed a combined P value for genetic differentiation of $P < 0.05$, but for the other seven, P values indicated no significant differentiation. Because these values are small, we explored three additional methods of evaluating short scale population structure.

Spatial autocorrelation estimates the similarity of multilocus genotypes based on individual position. It tests whether individual genotypes are more similar to one another when the individuals are sampled in close proximity than when they are sampled from further away. This analysis shows significant clustering of nuclear genotypes at the smallest spatial scales (0–20 km) across all three nuclear loci (Fig. 3, inset). All spatial comparisons within this distance are for populations on the same islands. Correlation coefficients tend to be zero or negative at higher spatial scales. For example, samples collected 20–40 km apart (inset, Fig. 3) show no significant spatial autocorrelation, and samples collected further than 60 km apart tend to show negative correlation, though not significantly so. Separate analysis of each of the three intron loci shows significant spatial autocorrelation at the scale of 20 km or less for both Mini-collagen and PaxC, but not Calmodulin (data not shown).

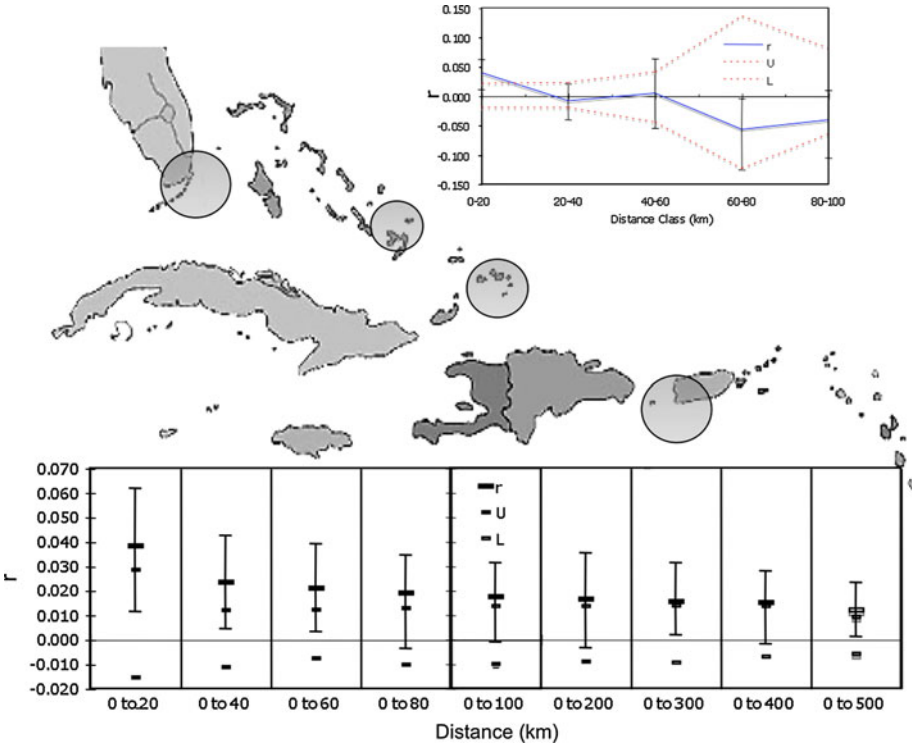

Fig. 3 *Inset* Spatial autocorrelation measured at three nuclear loci from sampling distances of 0–20 km, 20–40 km, 40–60 km, 60–80 km and 80–100 km. Individuals sampled at the smallest distance class (within 20 km) show higher levels of similarity than expected by chance. The *dotted lines* denote 95% confidence limits around spatial autocorrelation coefficients generated by permutation. *Map* A two-dimensional estimate of the spatial pattern of genetic autocorrelation shows autocorrelation hotspots in Florida ($P < 0.01$), San Salvador ($P < 0.04$), the Turks and Caicos ($P < 0.03$) and Puerto Rico ($P < 0.01$). *Circles* are proportional in size to the local correlation coefficient for 10 nearest neighbors, which range from 0.23 to 0.37. *Lower panel* Levels of spatial autocorrelation at increasing scales in *A. cervicornis* show the signature of short scale-structure even when samples from increasingly distant areas are included (up to 500 km). The *wide rectangles* denote the calculated '*r*' along with the standard deviation. Permuted upper and lower bounds of '*r*' are shown by *narrow rectangles*. See Peakall and Smouse (2006) for analysis details

These patterns do not pinpoint the area or areas in which local genetic structure is found, but show across the entire data set that genotypes within 20 km are significantly more similar than expected. To explore the geography of local structure, we used local 2D spatial autocorrelation analysis (Double et al. 2005) and showed that there were four significant hot spots of local genetic structure: Florida, San Salvador, the Turks and Caicos and Puerto Rico (Fig. 3, map). The significant global autocorrelation is thus most likely derived from larger than expected genetic similarities in these four areas rather than being driven by strong local structure at a single location (Fig. 4).

Analyses of the spatial distribution of mtDNAs also show small scale structure, especially among rare mtDNA haplotypes. For example, for haplotypes only seen twice in the data set (haplotypes 5, 8, 14, and 22), all are private haplotypes found only within island groups. Of these, haplotype 5 is native to *A. cervicornis*, but the others are introgressed

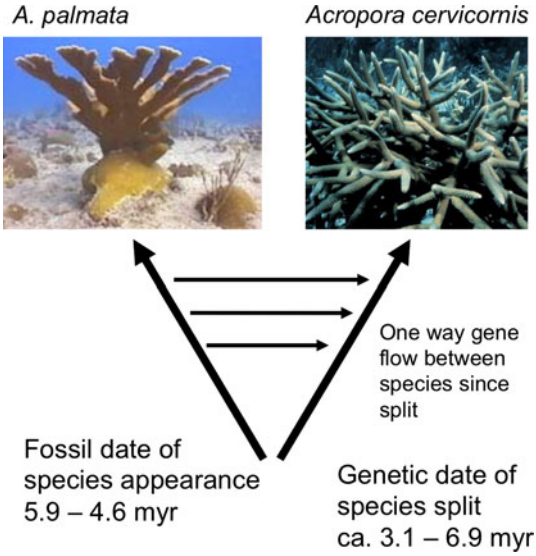

Fig. 4 Schematic of divergence and gene flow within Caribbean *Acropora* species based on coalescent analysis of introgression and on analysis of gene flow among conspecific populations. Despite the ongoing introgressive gene flow in these species, dispersive gene flow, even between distant island groups, is much higher. As a result, the populations within species are connected to one another by much more genetic exchange than there is introgressive gene flow from other species

from *A. palmata*. Haplotypes seen 3–7 times (haplotypes 3, 10, 16, 18, 20, 21 of which three are native and three introgressed) are only found in one or two locations. To test the statistical significance of spatial limitation of identical haplotypes more fully, we used a Spatial Analysis of Shared Alelles (SAShA) procedure recently developed to test the spatial extent of identical alleles (Kelly et al. 2010).This procedure establishes the spatial distribution of alleles in a data set and tests it against the distribution expected by chance. For *A. cervicornis*, mtDNA haplotype clumping is significant across the entire data set ($P < 0.01$). There are more observations of identical haplotypes among individuals within the same populations than expected (10 observations vs. 3 expected) and more among populations of the same island than expected (20 observations vs. 7 expected). By contrast there are fewer observations of identical haplotypes than expected at distances larger than about 50 km (chi square contingency table test $P < 0.01$).

Intraspecific population variation among loci

The IM analyses suggest that interspecific gene flow is curtailed in some loci, carrying the implication that alleles introgressed from *A. palmata* (hereafter termed *palmata* alleles) at these loci are under selection when in an *A. cervicornis* genetic background. Calculation of inter-population genetic differences for these loci also shows them to be slightly different from one another. The loci with the largest number of native, non-introgressed alleles (Mini-collagen and mtDNA) show the highest level of regional differentiation. The loci with only one native allele and one major introgressed allele (PaxC and Calmodulin) show lower differentiation at the regional level but higher differentiation at the island-level (Table 5). These differences may be driven by low power to detect structure in PaxC and Calmodulin because heterozygosity is low at these loci. However, Vollmer and Palumbi (2007) also showed these patterns in their Caribbean-wide survey of *A. cervicornis* populations, showing as well that native variants in mini-Collagen and mtDNA tended to have higher structure at regional scales than the introgressed variants.

Table 5 Summary of distribution of genetic differentiation (Φ_{ST}) among different loci at different spatial scales in *A. cervicornis*

Locus	Overall Φ_{ST}	Φ_{ST} Among regions	Φ_{ST} Among populations within regions	Selection against introgression
mtDNA	0.06**	0.04*	0.01	Low
Mini-collagen	0.09**	0.08**	0.02	High
PaxC	0.05	0.02	0.03	Moderate
Calmodulin	0.03	0	0.03	Moderate

Also shown is a qualitative summary of inferred selection against introgression at each locus, as discussed in the text. ** $P < 0.01$, * $P < 0.05$

Discussion

Our data take advantage of patterns of genetic variation among and within coral species at multiple loci to describe the timing and nature of species divergence, intra-specific gene flow and movement of alleles among populations. Analysis of genetic patterns between *A. cervicornis* and *A. palmata* shows that these species continue to exchange genes. However, despite this gene flow, a combined estimate of the date of divergence and the rate of gene flow between species suggests that the species have been distinct for millions of years and that gene flow is highly variable among loci.

Molecular data suggest a late Miocene divergence of *A. cervicornis* and *A. palmata.* The average estimate of time since last common ancestor is 4.3 million years (Table 4); however there is a large range in values derived from these different loci (2.9–6.9 million years). Data from Caribbean fossils for these species suggests them to have originated over a similar time frame, before 5.9–4.6 million years ago (Budd and Wallace 2008). Specimens of *A. cervicornis* were present in fossil deposits by this time. However, *A. palmata* does not appear until later, in the Pliocene of Curacao dated at 3.0–5.6 mya (Budd and Wallace 2008). Prior to the appearance of *A. cervicornis*, the Caribbean housed two other *Acropora* species, neither of which is likely to be the ancestor of *A. palmata* or *A. cervicornis* (Budd and Wallace 2008).

However, divergence of these two species did not prevent gene flow between them, and *A. palmata* and *A. cervicornis* continue to produce F1 hybrid offspring that are morphologically distinct enough to have been given their own species name, *A. prolifera* (Vollmer and Palumbi 2002). Our genetic evidence suggests that *A. prolifera* rarely breeds, but when it does, it backcrosses with *A. cervicornis,* thereby injecting genes into *A. cervicornis* from *A. palmata*. The rate of movement of *A. palmata* genes into *A. cervicornis* is highest for mtDNA and more moderate for two nuclear loci, occurring about 8 times a generation for mtDNA and about 40–80 times less than that for the nuclear genes. Such per-generation rates sum up gene movements throughout the species' ranges, and given the evidence that gene flow from *A. cervicornis* to *A. palmata* is virtually zero, this scale of gene flow across the entire Caribbean Sea can be considered a low rate.

Differentiation and selection between species and between populations

One of our goals was to compare rates of gene flow between species and between populations within species. Our results, show that populations of *A. cervicornis* on different islands show distinct allele frequencies indicative of genetic isolation, with average F_{ST}

values of 0.05 and 0.06 for nuclear and mtDNA sequences (Table 5). By contrast, genetic differentiation with these same markers is much higher between species—ranging as low as 0.52 for mtDNA and as high as 0.95 for the non-introgressing mini-collagen. Ten fold higher levels of genetic differentiation among species than among populations strongly suggests that gene flow among even distant locations of the same species far exceeds gene flow within locations between species.

These genetic patterns derive from loci under varying amounts of selection, and so it is important to consider the impact of this selection on the results. In this case, the nature of selection is different than in typical cases where alleles face different evolutionary pressures due to environmental variation. In our case, the selection is against an allele not in an unsuitable physical environment but against an allele in an unsuitable genetic background.

For Mini-collagen, we see no introgression, suggesting that *A. palmata* alleles (i.e. those alleles that appear from the genealogy (Fig. 1) to have originated in *A. palmata* but then introgressed to *A. cervicornis*) are strongly deleterious in an *A. cervicornis* background. However, there is no reason to suspect that native *cervicornis* alleles are deleterious in a *cervicornis* background. It is more likely that these alleles evolved to reside entirely within *A. cervicornis* and that their geographic patterns reflect the typical balance between genetic drift and dispersal. On the opposite spectrum, mtDNA appears to introgress quickly—not only in this system, but also in Pacific acroporid corals (e.g. van Oppen et al. 2001). Although introgressed mtDNA can be under selection in the wrong genetic background (Burton and Lee 1994), the high rates of realized introgression of mtDNA into *A. cervicornis* argues for low selection against introgressed *palmata* mtDNAs in a *cervicornis* background. We suggest that Mini-collagen and mtDNA show similarly low impacts of selection at the intra-specific level for two very different reasons: mini-collagen because strong selection keeps introgressed alleles out, and mtDNA because selection on introgressed variants is low. These two loci share similar levels of genetic differentiation at regional and local scales (Table 5).

The remaining two loci are intermediate in introgression, showing a single native allele and one common introgressed allele. Moderate introgression suggests that *palmata* alleles in a *cervicornis* background are mildly deleterious and are under mild selection, but that introgression pressure is high enough to maintain introgressed alleles within *A. cervicornis* populations. These loci have lower regional genetic differentiation but higher local genetic differentiation (Table 5) a pattern also seen in Vollmer and Palumbi (2007).

Alleles under selection may have lower gene flow than strictly neutral alleles. This is because larvae and young colonies with deleterious alleles might survive more poorly, reducing the pool of new recruits and reducing the number of migrating larvae with such alleles. Alternatively, this form of selection may be too weak to have a substantial impact on gene flow. The realized rate of interspecies gene flow for Calmodulin and PaxC is about one gene copy per every 4–8 generations, about 40–80 times lower than mtDNA. The rate of selection that is needed to cause this introgression difference is slight on a per capita basis: selection would be approximately balanced by migration in these cases, with $N_e m = N_e s$ (see Hartl and Clark (1997) for a discussion of the balance between selection and migration). Because N_e (effective population size across the species range) is large for Caribbean *Acropora* corals with large ranges and large past population sizes, both the *per capita* migration and selection parameters (m and s) are probably very small.

As in previous work elsewhere in the Caribbean (Vollmer and Palumbi 2007), we observed lower genetic differentiation for introgressing loci than for mtDNA or mini-collagen over regional scales. Lower regional differentiation may occur because introgression of the same alleles into different populations may reduce inter-population gene

frequency differences caused by genetic drift. By contrast, higher local differences within islands could reflect ongoing local introgression in localities where *A. palmata* and *A. cervicornis* are sympatric, and selection against these alleles on neighboring reefs where only *A. cervicornis* occurs. As expected under this scenario, *A. cervicornis* occurring allopatrically without *A. palmata* are more genetically distinct from conspecifics on the same island living with *A. palmata* populations than they are to other allopatric populations. Sympatric populations have higher proportions of introgressed alleles in 5 of 6 comparisons. However, the number of comparisons is small (three populations on each of two islands) and can not be considered conclusive. Moreover, spatial autocorrelation analysis and SAShA both suggest significant spatial structure at local scales even in mini-collagen and mtDNA. Further research on gene frequencies of sympatric and allopatric populations may shed light on this potential mechanism balancing introgression and selection.

Island scale differentiation in the northern Caribbean for *A. cervicornis*

Colonies of *A. cervicornis* in Puerto Rico are strongly different from populations in the rest of the study sites (Fig. 2), consistent with Caribbean wide genetic surveys of this species (Vollmer and Palumbi 2007) and in the congener *A. palmata* (Baums et al. 2005). Other species that show strong genetic breaks between Puerto Rico and the Dominican Republic, across the Mono Passage include cleaner gobies (Taylor and Hellberg 2003), a damselfish and a wrasse (Shulman and Bermingham 1995). Mathematical models of current flow (Cowen et al. 2006) and seascape genetic models of population differentiation (Baums et al. 2005; Galindo et al. 2006) also point to constricted gene flow in this area. Not all species show differentiation across the Mono Passage (Shulman and Bermingham 1995), however, and thus the nature of the genetic and ecological barriers in this area requires further study.

Our data also are the first to show the isolation of coral populations in the Turks and Caicos Islands (Fig. 2). Few other direct comparisons of populations in the Turks and Caicos to other northern Caribbean populations have been made, but Taylor and Hellberg (2005) found significant differences in gobies between Grand Turk and the central Bahamas (*E. evelynae* and *E. louisae*) and between the Turks and Caicos and Puerto Rico (for *E. chancei*). Cowen et al. (2006) and Galindo et al. (2006) used oceanographic models to suggest that the Turks and Caicos were more highly connected to the Bahamas than they were to the islands of Hispaniola or Puerto Rico. Our data contradict this prediction by showing greater genetic similarity of populations between the Turks and Caicos and Puerto Rico than to the Bahamas (Fig. 2). Future studies of genetic shifts at the Mono Passage should include comparison to Turks and Caicos to discern if this is a general trend.

Differences within islands

Some populations from the same islands show genetic differentiation over scales of 20 km or less. A two-dimensional visualization of the local genetic correlation coefficient, as introduced by Double et al. (2005), suggests that significant spatial autocorrelation is due to local structure in at least four northern Caribbean localities. In addition, Vollmer and Palumbi (2007) show significant population structure among several local populations of *A. cervicornis* in Panama based on F_{ST}. Thus, the overall pattern we see in genetic clustering does not appear to be driven by results from one or two populations but is likely to be fairly characteristic (though not necessarily universal) of *A. cervicornis*. These

conclusions should be considered tentative however, because we did not explicitly sample populations with this 2D analysis in mind, and our sampling of populations closer than 20 km was sparse. In addition, the potential role of selection against introgressed alleles, and the possibility that *A. cervicornis* co-occurring with *A. palmata* may have locally enhanced frequencies of these alleles, may affect the apparent scale of genetic structure. Further work should sample coral populations in a more fine-scale geographically explicit fashion and test for these local genetic correlations at the seascape level.

Genic view of coral speciation

In general, the differential flow of genes across species boundaries argues for a genic or gene-based view (Wu 2001), where the genetic integrity of species can be maintained at gene regions under divergent selection, even though interspecific gene flow may be high elsewhere in the genome (Wu 2001; Rieseberg et al. 2004; Wu and Ting 2004). Taking a genic view of coral species provides a potential explanation for how corals have rapidly diversified without the evolution of complete barriers to prezygotic hybridization, and how these species are maintained despite interspecific hybridization. We show here that nuclear introgression in Caribbean *Acropora* corals varies across loci, probably because of natural selection against introgression at some loci in backcross individuals.

Differential gene flow across the genomes of nominal and incipient species has been documented in a wide variety of plant and animal taxa (Barton and Hewitt 1985; Harrison 1990; Arnold 1997; Wang et al. 1997) as a mechanism by which species can both diverge and be maintained in the presence of interspecific gene flow (Rieseberg et al. 2004; Wu and Ting 2004). Divergent selection on genes conferring reproductive isolation, such as genes involved in mate choice, reproductive timing or hybrid sterility/inviability, are thought to play a critical role in limiting gene flow across species at key gene regions (Rieseberg et al. 2004; Wu and Ting 2004; Ortiz-Barrientos and Noor 2005). Evidence also suggests that chromosomal rearrangements and inversions play a prominent role in species divergence (Noor et al. 2001; Rieseberg 2001; Navarro and Barton 2003a, b) by inhibiting recombination and genetic exchange among species' genomes and allowing for additional divergence among genes within these chromosomal segments (Noor et al. 2001; Navarro and Barton 2003a, b).

Differential selection across the genome of *A. cervicornis* is apparently sufficient to preserve its genetic integrity at key gene regions, despite introgressive hybridization with its sympatric hybrid partner *A. palmata*. In addition, gene flow between species is low compared to gene flow among populations within species, and so dispersal across the Caribbean appears able to maintain the genetic cohesion of species across large distances despite local introgression. More genetic data are required to ascertain how much of the genome of *A. cervicornis* is involved in maintaining these strong genetic differences and to identify genes involved in the divergence of these coral species.

A byproduct of the differential flow of genes across coral species boundaries is that it makes for confusing and misleading species phylogenies because different genes will have different histories of hybridization and gene flow. In this way, studying hybridization in the Caribbean *Acropora* corals has a number of advantages because there are only three morpho-species, the species are easily distinguished (Budd et al. 1994), have divergent sets of alleles, and exchange genes in only one direction. However, in other coral groups where hybridization is thought to be common and important, such as the speciose Pacific *Acropora* and *Montipora* corals (van Oppen et al. 2001, 2004) and the *Montastrea* sibling species in the Caribbean (Fukami et al. 2004), hybridizing corals species are sometimes harder to distinguish, there may be cryptic species that complicate analysis (Ladner et al.,

in prep.) and species may show less genetic differentiation. Thus, genetic signatures of interspecific gene flow are likely to be complex and overlaid on top of shared ancestral polymorphism in the process of lineage sorting.

Genes as a time machine

The steady mutation of DNA sequences provides a temporal scale for estimates of species divergence, population change and dispersal. In the case of corals, a mutation that arises in one population of a species has the potential to travel to other species via introgression and to other conspecific populations via dispersal. Following the rates and timing of these very different patterns of movement can help us understand the complex population biology of closely related corals as they originate and persist in broad scale sympatry. Such reconstructions of the past once relied strictly on paleontology, and depended on the vagaries of the fossil record and clear morphological divergence among species (Cheetham et al. 1994; Jackson and Johnson 2000).

Adding information from DNA sequences to these data sets has the power to greatly expand our view of the divergence process at the population level by establishing a common currency in terms of gene exchange per generation. Although estimates of introgression and gene flow are based on inference, estimates of mutation rate, and models of population structure that are sometimes unrealistic (Whitlock and McCauley 1999; Hey and Nielsen 2004), these approaches provide a source of information about the past that is independent of specific fossil assemblages or the stability of morphological differences among species (Cheetham et al. 1995).

In the case of corals, data from DNA and fossils agree substantially in the timing of diversification of two important *Acropora* species in the Caribbean. The DNA data in addition help reconcile observations of long-term species distinctiveness in the fossil record (Budd and Wallace 2008) with the potential for interspecific hybridization (Willis et al. 2006), the existence of F1 hybrids in the wild (Vollmer and Palumbi 2002), and complex molecular phylogenies among species (van Oppen et al. 2000). However, current genetic data provide very wide ranges for divergence parameters (Table 3), at least partly because the loci used probably have had slightly different divergence histories. Genome wide analysis of coral divergence patterns (Voolstra et al. 2011) may help resolve these issues in the future and provide genetic divergence estimates that have more precision.

In addition, these data help begin to reconcile the rapid diversification of *Acropora* species in the fossil record (Wallace 1999) with the blending inheritance implied by reticulate evolution (Veron 1995). Our discovery that introgression rates are vastly different from locus to locus and that gene flow is largely one-way shows that large scale potential for hybridization in these corals does not produce large-scale introgression, but that selection winnows gene flow between species. This opens the possibility of discovering some of the genes responsible for species differences through enumeration of the genes that natural selection prevents from flowing from one species to another. Such insight into the genetic basis of coral species may help forge more ties between classic paleontology, morphology, ecology and genetics and help paint a more complete picture of the evolutionary history of corals.

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